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(54) Title: JAFFA, A NOVEL FIBROBLAST GROWTH FACTOR FAMILY MEMBER AND USES THEREFOR

(57) Abstract: The invention provides isolated nucleic acids molecules, designated JAFFA nucleic acid molecules, which encode novel fibroblast growth factor family members. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing JAFFA nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a JAFFA gene has been introduced or disrupted. The invention still further provides isolated JAFFA proteins, fusion proteins, antigenic peptides and anti-JAFFA antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

JAFFA, A NOVEL FIBROBLAST GROWTH FACTOR FAMILY MEMBER AND USES THEREFOR

Background of the Invention

5 Fibroblast Growth Factors (FGFs) are members of a family of polypeptides that are potent regulators of a variety of cellular processes including proliferation, differentiation, migration, and morphogenesis (Burgess, W.H. and Maciag, T (1989) *Annu. Rev. Biochem.* 58: 575-606; Rifkin, D.B. and Moscatelli, D. (1989) *J. Cell Biol.* 109:1-6). These proteins play important roles in normal development (Yamaguchi, J.P. and Rossant, J. (1995) *Curr.* 10 *Opin. Genet. Dev.* 485-491; Kimmelman et al. (1988) *Science* 242:1053-1056; Slack et al. (1988) *Development* 103: 581-590), in the maintenance of tissue, and in wound healing and repair (Clarke et al. (1993) *J. Cell Sci.*106: 121-133; Cuevas et al. (1988) *Biochem. Biophys. Res. Commun.* 156: 611-618). FGF family members have also been implicated in a wide range of pathological conditions, including tumorigenesis and metastasis (Davies et al. 15 (1996) *Int. J. Cancer* 65: 104-111; Myoken et al. (1996) *Int. J. Cancer* 65: 650-657).

The FGF family currently includes at least 19 structurally and functionally related proteins, including acidic and basic FGF, FGF-1 and FGF-2 respectively. Several FGF family members are oncogene products, for example, *int*2 (FGF-3), *hst* (FGF-4), FGF-5, and *hst*2 (FGF-6) (Galzie, Z. et al. (1997) *Biochem. Cell. Biol.*, 75:669-685). Other members of 20 this family include keratinocyte growth factor (FGF-7), androgen-induced growth factor (FGF-8) and glia-activating factor (FGF-9) (Galzie, Z. et al. (1997) *Biochem. Cell. Biol.*, 75:669-685). FGF-10 is preferentially expressed in the adult lung (Yamasaki, M. et al. (1996) *J. Biol. Chem*, 271:15918-15921). FGFs 11-14, also referred to as FGF homologous factors (FHFs), appear to be involved in the development and function of the nervous 25 system (Smallwood, P.M. et al. (1996) *Proc. Natl. Acad. Sci* USA, 93:9850-9857). FGF-15 displays a regionally restricted and dynamic pattern of expression in the developing nervous system (McWhirter, J.R. et al. (1997) *Development,* 124:3221-3232). FGF-16 is predominantly expressed in rat embryonic brown adipose tissue and in the adult heart. FGF-embryonic brain (Hoshikawa, M. et al. (1998) *Biochem. Biophys. Res. Comm.*, 244:187- 30 191). FGF-18 is expressed primarily in the lungs and kidneys, and stimulates hepatic and intestinal proliferation (Hu, M.C.T. et al. (1998) *Mol. Cell. Biol.*, 18:6063-6074). FGF-19 is primarily expressed in the fetal brain (Nishimura, T. et al. (1999) *Biochim Biophy Acta*, 1444:148-151).

Target cell responses are mediated, in part, by the binding of FGF ligands to cognate FGF receptors (FGFR) that possess intrinsic tyrosine kinase activity. There are currently four known genes encoding FGF receptors (FGFR-1, FGFR-2, FGFR-3, and FGFR-4), which can give rise to a variety of protein isoforms via alternative RNA splicing (Galzie, Z. et al. (1997) *Biochem. Cell. Biol.*, 75:669-685). A given FGFR can bind different members of the FGF family with varying degrees of specificity. Common structural features of the known FGFRs include an extracellular region with three immunoglobulin-like domains, a transmembrane region, and a cytosolic tyrosine kinase domain that is activated upon ligand binding. FGF binding causes dimerization of the receptors, resulting in receptor autophosphorylation on tyrosine residues and the activation of intracellular signal transduction cascades. The action of FGF appears to depend on interactions with heparan sulfate proteoglycans in the extracellular matrix. Several proposed roles for proteoglycans in this context include protection from proteolysis, localization, storage, and internalization of growth factors (Faham, S. et al. (1998) *Curr. Opin. Struct. Biol.*, 8:578-586). Heparan sulfate proteoglycans may serve as low affinity FGF receptors that act to present FGF to its cognate FGFR, and/or to facilitate receptor oligomerization (Galzie, Z. et al. (1997) *Biochem. Cell. Biol.*, 75:669-685).

Summary of the Invention

The present invention is based, at least in part, on the discovery of a novel Fibroblast Growth Factor (FGF), referred to herein as "JAFFA". The nucleotide sequence of a cDNA encoding JAFFA is shown in SEQ ID NO:1, and the amino acid sequence of a JAFFA polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequences of the coding region as well as the genomic sequence of JAFFA are depicted in SEQ ID NO:3 and SEQ ID NO:5, respectively.

Accordingly, in one aspect the invention features nucleic acid molecules encoding JAFFA proteins or biologically active portions thereof, that are useful as targets and reagents in assays applicable to the treatment and diagnosis of JAFFA mediated or related disorders. In certain embodiments, the invention provides isolated nucleic acid molecules that encode a polypeptide having the amino acid sequence of SEQ ID NO:2, or a polypeptide having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention provides isolated JAFFA nucleic acid molecules having the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ

ID NO:5, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number ____. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or the sequence of the
5 DNA insert of the plasmid deposited with ATCC Accession Number ____.

In a related aspect, the invention further provides nucleic acid constructs comprising the nucleic acid molecules described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to regulatory sequences. Also included, are vectors and host cells containing the JAFFA nucleic acid molecules of the invention
10 which are suitable for producing JAFFA nucleic acid molecules and polypeptides.

In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of JAFFA-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to a JAFFA encoding nucleic acid molecule are provided.

15 In another aspect, the invention features JAFFA polypeptides, and biologically active or antigenic fragments thereof, that are useful as reagents or targets in assays applicable to treatment and diagnosis of JAFFA mediated or related disorders. In certain embodiments, the invention provides isolated or recombinant JAFFA polypeptides that contain at least one fibroblast growth factor domain, or at least one fibroblast growth factor
20 domain, at least one phosphorylation site and at least one N-myristoylation site. In other embodiments, the invention provides JAFFA polypeptides encoded by the nucleic acid molecules of the invention, as well as JAFFA polypeptides having the amino acid sequence shown in SEQ ID NO:2, the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number ___, or an amino acid sequence that is
25 sufficiently identical to the amino acid sequence shown in SEQ ID NO:2.

In a related aspect, the invention provides JAFFA polypeptides or fragments operatively linked to non-JAFFA polypeptides to form fusion proteins.

In another aspect, the invention features antibodies and antigen-binding fragments thereof, that specifically bind JAFFA polypeptides.

30 In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the JAFFA polypeptides or nucleic acids.

The still another aspect, the invention provides a process for modulating JAFFA polypeptide or nucleic acid expression or activity, e.g. using the screened compounds. In certain embodiments, the methods involve treatment of conditions related to aberrant activity or expression of the JAFFA polypeptides or nucleic acids, such as conditions
5 involving aberrant or deficient cellular proliferation or differentiation.

The invention also provides assays for determining the activity of or the presence or absence of JAFFA polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

In further aspect the invention provides assays for determining the presence or
10 absence of a genetic alteration in a JAFFA polypeptide or nucleic acid molecule, including for disease diagnosis.

Brief Description of the Drawings

Figure 1 depicts a cDNA sequence (SEQ ID NO:1) and predicted amino acid
15 sequence (SEQ ID NO:2) of human JAFFA. The methionine-initiated open reading frame of human JAFFA (without the 5' and 3' untranslated regions) starts at nucleotide 158 until the end of SEQ ID NO:1 (shown also as coding sequence (SEQ ID NO:3)).

Figure 2 depicts a hydropathy plot of human JAFFA. Relative hydrophobic residues are shown above the dashed horizintal line, and relative hydrophilic residues are below the
20 dashed horizontal line. The cysteine residues (cys) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence of human JAFFA are indicated. The amino acid sequence of the fibroblast growth factor domain is indicated by the underlining and the abbreviation "FGF".

Figure 3 depicts an alignment of the fibroblast growth factor domain (also referred
25 to herein as "FGF domain") of human JAFFA with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequence (SEQ ID NO:4), while the lower amino acid sequence corresponds to amino acids 60 to 140 of SEQ ID NO:2.

Figure 4 depicts an alignment of human JAFFA with FGF family members (FGF-1
30 (SEQ ID NO:12), -2 (SEQ ID NO:13), -3 (int-2) (SEQ ID NO:15), -8 (SEQ ID NO:14), -15 (SEQ ID NO:7), -16 (SEQ ID NO:11), -17 (SEQ ID NO:10), -18 (SEQ ID NO:9), and –19 (SEQ ID NO:8). All of the sequences shown are from human origin, except FGF-15 which is a mouse sequence. The uppermost sequence is a consensus FGF amino acid sequence

(shown as "majority") (SEQ ID NO:6). Residues that exactly match the consensus sequence are indicated with solid black.

Figure 5 depicts a phylogenetic tree of FGF family members using the Clustal method with PAM250 residue weight table.

5 *Figure 6* depicts a genomic sequence (SEQ ID NO:5) of human JAFFA. Four introns and three exons (i.e., Exons 1-3; boxed sequences) of the JAFFA gene are shown. The nucleotides corresponding to the exons are as follows: Exon 1, nucleotides 847-1083 of SEQ ID NO:5; Exon 2, nucleotides 1538-1639 of SEQ ID NO:5; and Exon 3, nucleotides 2505-2792 of SEQ ID NO:5. Nucleotides 1-168 correspond to exons 1 and 2 of the □(1,2)-

10 fucosyltransferase (FUT1) gene. The predicted amino acid sequence of human JAFFA (SEQ ID NO:2) is also shown.

Figure 7 depicts the nucleotide sequences of the top primer (SEQ ID NO:16), the bottom primer (SEQ ID NO:17) and the probe (SEQ ID NO:18) used for detecting endogenous human JAFFA expression using the Taq Man technology.

15

Detailed Description of the Invention

The human JAFFA sequence (Figure 1; SEQ ID NO:1), which is approximately 784 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 627 nucleotides (nucleotides 158-784 of SEQ ID NO:1; SEQ ID

20 NO:3) which encodes a 209 amino acid protein (SEQ ID NO:2). The human JAFFA protein of SEQ ID NO:2 and Figure 2, includes an amino-terminal hydrophobic amino acid sequence, consistent with a signal sequence, of about 28 amino acids (from amino acid 1 to about amino acid 28 of SEQ ID NO:2), which upon protease removal results in the production of the mature protein.

25 The mature protein is approximately 181 amino acid residues in length (from about amino acid 29 to amino acid 209 of SEQ ID NO:2). Human JAFFA contains a fibroblast growth factor domain (PFAM Accession PF00167) located at about amino acid residues 60-140 of SEQ ID NO:2; two predicted Protein Kinase C sites (PS00005) at about amino acids 98 to 100, and 122 to 124 of SEQ ID NO:2; two predicted Casein Kinase II sites (PS00006)

30 are located at about amino acids 6 to 9, and 122 to 125; and five predicted N-myristoylation sites (PS00008) from about amino acids 12 to17, 20 to 25, 67 to 72, 95 to 100, and 108 to 113 of SEQ ID NO:2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and http://www.psc.edu/general/ software/packages/pfam/pfam.html.

A plasmid containing the nucleotide sequence encoding human JAFFA was
5 deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number ______. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an
10 admission that a deposit is required under 35 U.S.C. §112.

The JAFFA molecules of the present invention are members of a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to
15 mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues
20 of non-human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

For example, sequence conservation among FGF family members indicates that these proteins are likely to include a beta trefoil structure. As used herein, the term "beta trefoil structure" includes a protein tertiary (i.e. three dimensional) structure that preferably
25 has twelve antiparallel beta strands linked to form a structure with three-fold internal symmetry. This structure consists of three copies of a basic four-stranded antiparallel beta sheet. Beta trefoil structures are described in, for example, Zhu, X. et al. (1991) *Science*, 251:90-93, the contents of which are incorporated herein by reference. Several regions of FGF have been implicated in receptor binding, notably beta-strands 1-3, and the loop
30 between strands 8 and 9. The loop between strands 10 and 11 is believed to be involved in binding heparin.

The JAFFA proteins of the present invention contain a significant number of structural characteristics in common with members of the FGF family. An alignment of

human JAFFA with FGF family members (FGF-1, -2, -3 (int-2), -8, -15, -16, -17, -18, and – 19) is depicted in Figure 4. A comparison of the FGF sequences with a consensus FGF amino acid sequence (shown as "majority") revealed a number of highly conserved amino acid residues present on all of the FGF sequence analyzed (including JAFFA). For example, 5 the following amino acids were present on all FGF sequences analyzed: amino acids leucine and tyrosine at positions 73-74; leucine at position 107; cysteine at position 148; phenylalanine at position 150; glutamate at position 152; tyrosine at position 159; and phenylalanine at position 211 of SEQ ID NO: 6 (see Figure 4). The amino acid numbers are based on the consensus sequence.

10 Thus, a JAFFA molecule of the present invention can be identified based on the presence of a "fibroblast growth factor domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "fibroblast growth factor domain" includes a protein domain having an amino acid sequence of about 20-100 amino acid residues and having a bit score for the alignment of the sequence to the fibroblast growth factor domain (HMM) of 15 at least 20. Preferably, a fibroblast growth factor domain includes at least about 20-100, more preferably about 40-90 amino acid residues, or about 60-80 amino acids and has a bit score for the alignment of the sequence to the fibroblast growth factor domain (HMM) of at least 25, 30, 35, 50 or greater. The fibroblast growth factor domain (HMM) has been assigned the PFAM Accession PF00167 (http;//genome.wustl.edu/Pfam/.html). An 20 alignment of the fibroblast growth factor domain (amino acids 60 to 140 of SEQ ID NO:2) of human JAFFA with a consensus amino acid sequence derived from a hidden Markov model is depicted in Figure 3.

To identify the presence of a "fibroblast growth factor domain" domain in a JAFFA protein, and make the determination that a protein of interest has a particular profile, the 25 amino acid sequence of the protein is searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for 30 determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.*(1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.*(1987)

Proc. Natl. Acad. Sci. USA 84:4355-4358; Krogh *et al.*(1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.*(1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of a "fibroblast growth factor domain" domain in the amino acid sequence of
5 human JAFFA at about residues 60-140 of SEQ ID NO:2 (see Figure 1).

A member of this novel subfamily of JAFFA proteins has a "fibroblast growth factor domain" which includes at least about 20-100 amino acid residues and has at least about 50-60% identity with a "fibroblast growth factor domain" of human JAFFA (e.g., residues 60-140 of SEQ ID NO:2). Preferably, a "fibroblast growth factor domain" includes at least
10 about 40-90 amino acid residues, or about 60-80, and has at least 60-70% identity, preferably about 70-80%, more preferably about 80-90% identity with a "fibroblast growth factor domain" of human JAFFA (e.g., residues 60-140 of SEQ ID NO:2).

Accordingly, JAFFA proteins having at least 50-60% identity, preferably about 60-70%, more preferably about 70-80%, or about 80-90% identity with a fibroblast growth
15 factor domain of human JAFFA are within the scope of the invention.

A JAFFA molecule can further include a signal sequence. As used herein, a "signal sequence" refers to a peptide of about 20-30 amino acid residues in length which occurs at the N-terminus of secretory and integral membrane proteins and which contains a majority of hydrophobic amino acid residues. For example, a signal sequence contains at least about
20 15-45 amino acid residues, preferably about 20-40 amino acid residues, more preferably about 21-33 amino acid residues, and more preferably about 23-28 amino acid residues, and has at least about 40-70%, preferably about 50-65%, and more preferably about 55-60% hydrophobic amino acid residues (e.g., alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, or proline). Such a "signal sequence", also referred to in the art as a
25 "signal peptide", serves to direct a protein containing such a sequence to a lipid bilayer. For example, in one embodiment, a JAFFA protein contains a signal sequence of about amino acids 1-28 of SEQ ID NO:2. The "signal sequence" is cleaved during processing of the mature protein. The mature JAFFA protein corresponds to amino acids 29 to 209.

Isolated proteins of the present invention, preferably JAFFA proteins, have an amino
30 acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, or are encoded by a nucleotide sequence sufficiently homologous to SEQ ID NO:1, 3 or 5. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an

amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently homologous.

As the JAFFA proteins of the present invention may modulate JAFFA-mediated activities, they may be useful for developing novel diagnostic and therapeutic agents for JAFFA-associated disorders, as described below.

The genomic sequence encoding JAFFA localizes to a complementary strand of H-type GDP-L-Fucose:□-D-Galactosidase 2-□-L-Fucosyltransferase gene (FUT1 gene), also referred to herein as "□(1,2)fucosyltransferase". See Figure 6. □(1,2)fucosyltransferase forms the H blood group antigen and catalyzes the transfer of fucose in the □(1,2) linkage to the terminal galactose of a precursor molecule. This enzyme is essential for the expression of ABO blood group antigens (Koda, Y. et al. (1997) *J. Biol. Chem.* 272(11):7501-7505). Based on the overlapping location of the JAFFA and the □(1,2)fucosyltransferase genes, it is predicted that a number of pathological conditions, e.g., genetic disorders, cancers, associated with the □(1,2)fucosyltransferase gene will also affect the JAFFA gene. Accordingly, the JAFFA nucleic acids and proteins of the invention may be useful for developing novel diagnostic and therapeutic agents for cancers and other disorders that also may demonstrate ABO(H) blood group disorders.

As used herein, an "ABO(H) blood group disorder" or a "disorder characterized by alterations in cell group antigens" includes any pathological condition having an abnormal level of an ABO(H) histoblood group antigens, e.g., A, B, H, Le^a, Le^b, Le^x and Le^y. Exemplary ABO(H) blood group disorder include malignant transformation and genetic disorders. For example, alterations in the level of ABO(H) surface blood group antigens have been associated with a number of neoplasms, including carcinomas (e.g., colon, ovarian, prostate, lung, or gastric carcinomas); thyroid neoplasms (e.g., follicular adenomas,

follicular carcinomas, papillary carcinomas and medullary carcinomas); and leukemias (e.g., myeloid leukemia) (Gonzalez-Campora, R. et al. (1998) *Arch. Pathol. Lab. Med.* 122:957). Blood group antigens are more abundantly expressed in malignant neoplasms compared to benign neoplasms. Moreover, the presence of metastasis was correlated with the loss of A
5 or B antigens.

Similarly, aberrant expression of the A, B and H antigens has been associated with rare genetic defects, e.g., Bombay and para-Bombay phenotypes, or Rambam-Hasharon Syndrome. (Frydman, M. et al. (1992) *Am. J. Med. Genetics* 44:297-302; Frydman, M. et al. (1996) *Prenatal Diagnosis* 16:266-269; Wang, B. et al. (1997) Vox Sang 72:31-35). For
10 example, the Bombay phenotype is characterized by the absence of ABH antigens on erythrocyte membranes and in secretions (e.g., saliva) because of mutations in the H gene encoding □(1,2)fucosyltransferase. Therefore, the JAFFA nucleic acids and proteins of the invention may be useful for developing novel diagnostic and therapeutic agents for cancers and genetic disorders such as Bombay and para-Bombay phenotypes, or Rambam-Hasharon
15 Syndrome, that have ABO(H) blood group disorder as their phenotype.

Based on sequence similarities, the JAFFA molecules of the present invention are predicted to have similar biological activities as FGF family members. FGF family members modulate the proliferation, motility, differentiation, and survival of a variety of cells of mesodermal, neuroectodermal, ectodermal and endodermal origin, including
20 fibroblast, chondrocytes, myoblasts, endothelial cells, astrocytes, neuroblast, keratinocytes, osteoblasts, and smooth muscle cells. (Burgess, W.H. et al. (1989) *Ann. Rev. Biochem.*, 58:575-606; Galzie, Z. et al. (1997) *Biochem. Cell. Biol.*, 75:669-685). FGF family members display a broad range of biological activities as mitogens, motogens, angiogenic factors, neurotropic factors, differentiation factors, and oncogenes (Galzie, Z. et al. (1997)
25 Biochem. Cell., 75:669-685). These proteins are important in developmental processes including limb formation, mesoderm induction, and induction and patterning of neural tissues, as well as in the maintenance of tissues and in wound healing and repair.

FGF family members and their receptors have also been implicated in a variety of pathological conditions, including cancers and genetic disorders, e.g., Pfeiffer syndrome,
30 Crouzon syndrome and achondroplasia (Wilkie, A.O. et al. (1995) *Current Biology* Vol 5 (5): 500-507). With respect to a role in cancers, expression of FGF family members has been shown in a number of neoplastic cell types (both in cultured cells and in tissues). For example, expression of bFGF has been detected in adrenal pheochromocytoma,

chemodetectoma, carcinomas (e.g., renal cell, bladder, breast, hepatocellular carcinomas, and carcinomas of the digestive tract), brain tumors and astrocytomas, melanomas, among others (Stauto et al. (1993) *Int. J. Cancer* 53:5-10; Singh et al. (1994) *Am. J. Pathol.* 145:365-374; Allen and Maher (1993) *J. Cell. Physiol.* 155:368-375; Li et al. (1994) *J.*
5 *Pathol.* 172:171-175; Brem et al. (1992) *Cancer* 70:2673-2680; Wilkinson et al. (1993) *Int. J. Oncol.* 3:933-936; and Ueki et al. (1995) *J. Pathol.* 177:353-361). In addition, FGF family members have been shown to act as angiogenic and autocrine factors in stimulating tumor cell growth (Galzie, Z. et al. (1997) *supra*).

Accordingly, the JAFFA molecules of the present invention may play a role in
10 regulating cellular growth signaling mechanisms. As used herein, the term "cellular growth signaling mechanism" includes the ability to interact with, e.g., bind to, a cell surface receptor, e.g., a tyrosine kinase; a proteoglycan, e.g., a heparan sulphate proteoglycan; and/or a polysaccharide, e.g. heparan sulphate, and modulate, e.g., activate, one or more of: (1) induction of receptor dimerization, (2) tyrosine kinase activation, (3) phosphorylation of
15 signaling molecules, e.g., phospholipase C-gamma and GTPase activating protein, and/or (4) induction gene expression; thereby regulating one or more of: (5) cell proliferation, (6) cell differentiation, (7) cell survival, (8) chemotaxis, (9) migration, and/or (10) apoptosis, of a cell (e.g., a mesodermal, ectodermal, neural (e.g., neuroectodermal), endodermal or hematopoietic cell). Jaffa molecules may regulate a variety of processes including
20 embryonic development and differentiation, tissue maintenance, wound healing and repair, neuronal function, angiogenesis, and pathological conditions, e.g. tumorigenesis and metastasis, and neuronal degeneration. Thus, the JAFFA molecules, by participating in cellular growth signaling mechanisms, may modulate cell behavior and act as therapeutic agents for controlling proliferation, differentiation, survival, apoptosis, chemotaxis, and/or
25 migration, of a cell.

Northern blot analysis revealed moderate to low expression of JAFFA mRNA in the fetal liver and cancer cell lines, e.g., the human cervical carcinoma HeLa cells. In addition, expression of JAFFA transcript in normal and diseased hematopoietic cells is predicted based on an analysis of the 5'untranslated region of the JAFFA gene (Figure 6; SEQ ID
30 NO:5), which revealed multiple consensus sequence for DNA binding sites sites for various hematopoietic transcription factors. For example, five Ik-2 sites, four AML-1a sites, thirteen GATA-1 sites, ten GATA-2 sites, and three GATA-3 sites were identified. Ikaros transcription factors, e.g., Ik-2, which belong to a family of zinc finger transcription factors,

have been shown to bind to a number of lymphocyte-specific regulatory elements. (Molnar, A. et al. (1994) *Mol. Cell Biol.* 14(12):8292-303). GATA factors are members of a zinc finger family of transcription factors, and are believed to be essential for the development of erythroid lineages (Zhang, P. et al. (1999) *Proc. Natl. Acad. Sci.* USA 96(15):8705-10;
5 Rekhtman, N. et al. (1999) *Genes Dev.* 13(11):1398-411). In addition to developmental expression, a number of these trancription factor have been shown to be expressed in hematopoietic disorders. For example, AML hematopoietic transcription factors are believed to be involved in the pathogenesis of myeloid and lymphoid leukemias (Coco, L. et al. (1997) *Haematologica* 82(3): 364-70). GATA1 has been shown to be expressed in
10 myelodysplastic syndrome, acute myeloid leukemia and acute lymphoblastic leukemia (Patmasisiwat, P. et al. (1999) *Leukemia* 13(6):891-900). Mutant forms of the Ikaros transcription factors have been identified in T-cell acute lymphoblastic leukemia (Sun, L. et al. (1999) *Clin. Cancer Res.* 5(8):2112-20; Sun, L. et al. (1999) *Proc. Natl. Acad. Sci.* USA 96(2):680-5). The identification of numerous consensus binding sequences for
15 hematopoietic transcription factors in the 5' untranslated region of the JAFFA gene suggests that expression of human JAFFA may be upregulated in hematopoietic cells during normal development and in diseased conditions.

In addition, endogenous human JAFFA gene expression was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System which employs TaqMan technology.
20 Briefly, TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific oligonucleotide (referred to as a probe) which has a fluorescent dye coupled to its 5' end (typically 6-FAM) and a quenching dye at the 3' end (typically TAMRA). When the fluorescently tagged oligonucleotide is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of taq polymerase digest the
25 labeled primer, producing a free nucleotide labeled with 6-FAM, which is now detected as a fluorescent signal. The PCR cycle where fluorescence is first released and detected is directly proportional to the starting amount of the gene of interest in the test sample, thus providing a way of quantitating the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe specific for a housekeeping gene
30 such as GAPDH which has been labeled with a different fluor on the 5' end (typically VIC).

To determine the level of JAFFA in various human tissues a primer/probe set was designed (Figure 7) using Primer Express (Perkin-Elmer) software and primary cDNA sequence information. Total RNA was prepared from a series of human tissues using an

RNeasy kit from Qiagen. First strand cDNA was prepared from one ug total RNA using an oligo dT primer and Superscript II reverse transcriptase (Gibco/BRL). cDNA obtained from approximately 50 ng total RNA was used per TaqMan reaction. Normal tissues tested include human colon, breast, prostate, liver and fetal liver. In addition, a variety of human tumor samples were assayed for JAFFA expression including tumors of the colon, breast, prostate as well as highly metastatic colon and prostate tumors. Expression of JAFFA was restricted to metastatic colon and prostate samples as well as a moderate amount in normal liver. JAFFA mRNA was completely absent from normal colon, breast and prostate samples as well as from non-metastatic tumors of colon, breast and prostate.

As the JAFFA mRNA is expressed in hematopoietic cells and cancer cell lines, as well as colon and prostate metastatic tumors, it is likely that JAFFA molecules of the present invention may be involved in disorders characterized by aberrant activity of these cells. For example, altered expression and/or activity of a JAFFA molecule can lead to perturbed cellular proliferation of these cells, which in turn can lead to cellular proliferative and/or differentiative disorders. As used herein, a "cellular proliferative disorder" includes a disorder, disease, or condition characterized by a deregulated, e.g., upregulated or downregulated, growth response. As used herein, a "cellular differentiative disorder" includes a disorder, disease, or condition characterized by aberrant cellular differentiation. As used herein, metastatic refers to the ability of a tumor cell to form implants at a site distant from the original tumor. Thus, the JAFFA molecules can act as novel diagnostic targets and therapeutic agents for controlling cellular proliferative and/or differentiative disorders.

Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, or hematopoietic neoplastic disorders, e.g., leukemias; and disorders involving aberrant angiogenesis and/or vascularity, e.g., tumor angiogenesis and metastasis; immune disorders, e.g., inflammatory diseases; neural disorders (e.g., CNS disorders), among others. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, breast and liver origin. Expression of other members of the FGF family have been found to correlate with a metastatic phenotype in angiosarcomas (Yamamoto *et al.* (1999) *J. Am. Acad. Dermatol.* 41127-129), MCF-7 breast carcinoma cells (Zhang *et al.* (1997) *Oncogene* 15:2093-2108), advanced ovarian cancers (Fujimoto *et al.* (1997) *Eur. J. Gynaecol. Oncol.* 18:349-352), and immortalized human salivary gland clones (Azuma *et al.* (1997) *Int. J. Cancer* 71:891-896).

As used herein, the terms “cancer”, "hyperproliferative" and "neoplastic" are used interchangeably, and include those cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

The terms “cancer” or “neoplasms” include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

As used herein, the term “hematopoietic neoplastic disorders” includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Exemplary disorders include myeloid disorders including, but not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hemotol.* 11:267-97); lymphoid malignancies

include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin
5 lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF) and Hodgkin's disease.

As the JAFFA mRNA is expressed in the fetal liver, the JAFFA nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of immune disorders.
10 Exemplary immune disorders include autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease,
15 aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions,leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia,
20 idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

Examples of CNS disorders include neurodegenerative disorders, e.g., Alzheimer's
25 disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, and Jakob-Creutzfieldt disease; psychiatric disorders, e.g., depression, schizophrenic disorders, Korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, e.g., amnesia or age-related
30 memory loss; and neurological disorders, e.g., migraine.

The JAFFA protein is predicted to be a secreted protein which may modulate the activity of a cell surface receptor, e.g., a tyrosine kinase receptor (e.g., an FGF receptor), and thus participate in signaling pathways within cells. As used herein, a signaling pathway

refers to the modulation (e.g., the stimulation or inhibition) of a cellular function/activity upon the binding of a JAFFA protein to a cell surface receptor. Examples of such functions include dimerization of the tyrosine kinase receptor, induction of receptor autophosphorylation, induction of receptor clustering and/or receptor internalization. Based on the activity of FGF family members, it is predicted that activation of JAFFA will result in phosphorylation of signaling molecules, such as phospholipase C-gamma and GTPase activating protein (GAP) (Galzie, Z. et al. (1997) *supra*). These signaling molecules have been shown to interact with phosphorylated regions in growth factor receptors through their src-homology domain. Mobilization of these signaling molecules is expected to modulate the activity of signal transduction pathways, e.g., phosphatidylinositol 4,5-bisphosphate (PIP_2), inositol 1,4,5-triphosphate (IP_3), or MAP kinase pathways, ultimately resulting in changes in gene expression.

Based on the structural similarities between JAFFA and FGF family members, it is expected that the JAFFA proteins may interact with, e.g., bind to, glycosaminoglycans, e.g., a heparan sulphate proteoglycans (HSPG). HSPGs have been shown to be low-affinity receptors for FGF-like molecules such as bFGF (dissociation constant 10-100 nM). HSPGs are complex and highly variable mammalian proteoglycans that consist of a core protein (e.g, a syndecan, a glypican, a perlecan, or a betaglycan) to which a variable number of glycosaminoglycan chains are linked (Gallagher et al. (1990) *Biochem. Soc. Trans.* 18:207-209). The JAFFA protein may also interact with a saccharide, e.g., heparin, heparan sulphate (HS). HS consists of a disaccharide repeat of □□-linked glucosamine and hexuronic acid (Galzie, Z. et al. (1997) *supra*). HS have been associated with storage and sequestration of soluble proteins, e.g., FGF family members, as well as mediating ligand presentation to a cell surface receptor (Galzie, Z. et al. (1997) *supra*). Interference with the binding of an FGF family member with HSPG or HS has been shown to inhibit tumor growth (Galzie, Z. et al. (1997) *supra*). Thus, modulation of an interaction of HSPG- or HS-like molecules with JAFFA proteins of the invention may be used therapeutically, e.g., to inhibit tumor cell growth.

As used interchangeably herein, an “JAFFA activity”, “biological activity of JAFFA” or "functional activity of JAFFA", refers to an activity exerted by a JAFFA protein, polypeptide or nucleic acid molecule on a JAFFA responsive cell or on a JAFFA protein substrate, as determined *in vivo* or *in vitro*, according to standard techniques (e.g., an activity as described herein). In one embodiment, a JAFFA activity is a direct activity, such

as an association with a JAFFA target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a JAFFA protein binds or interacts in nature, such that JAFFA-mediated function is achieved. A JAFFA target molecule can be a non-JAFFA molecule or a JAFFA protein or polypeptide of the present invention. In an
5 exemplary embodiment, a JAFFA target molecule is a JAFFA receptor, e.g., a cell surface receptor (e.g., a tyrosine kinase receptor), a glycosaminoglycan, or a saccharide. A JAFFA activity can also be an indirect activity, e.g., a cellular signaling activity mediated by interaction of the JAFFA protein with a JAFFA receptor The biological activities of JAFFA are described herein. For example, the JAFFA proteins of the present invention can have
10 one or more of the following activities: (1) induction of receptor dimerization, (2) tyrosine kinase activation, (3) phosphorylation of signaling molecules, e.g., phospholipase C-gamma and GTPase activating protein, (4) induction of gene expression, (5) modulation of cell proliferation, (6) modulation of cell differentiation, (7) modulation of cell survival, (8) modulation of chemotaxis, (9) modulation of migration, and/or (10) modulation of
15 apoptosis, of a cell (e.g., a mesodermal, ectodermal, neural (e.g., neuroectodermal), endodermal or hematopoietic cell).

Accordingly, another embodiment of the invention features isolated JAFFA proteins and polypeptides having a JAFFA activity. Preferred proteins are JAFFA proteins including at least one fibroblast growth factor domain, and, preferably, having a JAFFA activity, e.g.,
20 a JAFFA activity as described herein. Further preferred proteins include at least one fibroblast growth factor domain, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3 or 5.

25 Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode
30 JAFFA proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify JAFFA -encoding nucleic acid molecules (e.g., JAFFA mRNA) and fragments for use as PCR primers for the amplification or mutation of JAFFA nucleic acid molecules. As used herein, the term

"nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

5 The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences 10 which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated JAFFA nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the 15 nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule 20 having the nucleotide sequence of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with 25 ATCC as Accession Number _____ as hybridization probes, JAFFA nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

30 Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, 3 or

5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to JAFFA nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human JAFFA cDNA. This cDNA comprises sequences encoding the human JAFFA protein (i.e., "the coding region", from nucleotides 158-784 of SEQ I NO:1), as well as 5' untranslated sequences (nucleotides 1-157 of SEQ ID NO:1). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (e.g., nucleotides 158-784, corresponding to SEQ ID NO:3).

In yet another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:5. The sequence of SEQ ID NO:5 corresponds to the human JAFFA genomic sequence. This genomic sequence comprises four introns located at about nucleotides 1-846, 1084-1537, 1640-2504, and 2793-4033 of SEQ ID NO:5, and three exons located at about 847-1083, 1538-1639, and 2505-2792 of SEQ ID NO:5.

In one embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO: 1, 3, or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ such that it can hybridize to the nucleotide sequence shown in SEQ ID NO: 1, 3, or 5 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1, 3, or 5, or the entire
5 length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences.or a portion of any of these nucleotide sequences.

A. JAFFA Nucleic Acid Fragments

10 The nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a JAFFA protein, e.g., an immunogenic or biologically active portion of a JAFFA protein.
15 For example, the fragment can comprise nucleotides 337 to 577 of SEQ ID NO:1, which encodes a fibroblast growth factor domain of human JAFFA. The nucleotide sequence determined from the cloning of the JAFFA gene allows for the generation of probes and primers designed for use in identifying and/or cloning other JAFFA family members, as well as JAFFA homologues from other species.

20 The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1, 3, or 5, or the nucleotide sequence of the DNA insert of the
25 plasmid deposited with ATCC as Accession Number _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In certain embodiments, the nucleic acid molecules are at least 7, more preferably 15 (e.g., contiguous) nucleotides in length and hybridize under stringent conditions to
30 nucleotides 1-22, 265-308, and 425-559 of SEQ ID NO:1. In another embodiment, the nucleic acid molecules comprise 1-22, 265-308, and 425-559 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecules consist of nucleotides 1-22, 265-308, and 425-559 of SEQ ID NO:1.

In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than 195, 200, 300, 400, 500, 550, 550-600, 600-650, 650-700, 700-750 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, or 3, or the
5 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In yet another embodiment, the nucleic acid molecule includes a fragment which is less than 196 nucleotides and has a nucleotide sequence at least 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence of SEQ ID NO:1, or 3, or a complement thereof.

10 A nucleic acid fragment encoding a "biologically active portion of a JAFFA protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, which encodes a polypeptide having a JAFFA biological activity (e.g., the biological activities of the JAFFA proteins are described herein), expressing the
15 encoded portion of the JAFFA protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the JAFFA protein. For example, a nucleic acid fragment encoding a biologically active portion of JAFFA includes a fibroblast growth factor domain, e.g., amino acid residues 60 to 140 of SEQ ID NO:2. A nucleic acid fragment encoding a biologically active portion of a JAFFA protein, may comprise a
20 nucleotide sequence which is greater than 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 549, 549-600, 600-627 or more nucleotides in length.

B. JAFFA Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the
25 nucleotide sequence shown in SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ due to degeneracy of the genetic code and thus encode the same JAFFA proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In another
30 embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.

In addition to the JAFFA nucleotide sequences shown in SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

Accession Number _____, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the JAFFA proteins may exist within a population (e.g., the human population). Such genetic polymorphism in the JAFFA genes may exist among individuals within a population due to
5 natural allelic variation.

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a JAFFA protein, preferably a mammalia JAFFA protein, and can further include non-coding regulatory sequences, and introns.

10 Allelic variants of JAFFA, e.g., human JAFFA, include both functional and non-functional JAFFA proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the JAFFA protein within a population that maintain the ability to bind a JAFFA receptor or substrate, and/or modulate cell growth and migration mechanisms. Functional allelic variants will typically contain only conservative substitution of one or
15 more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally-occurring amino acid sequence variants of the JAFFA, e.g., human JAFFA, protein within a population that do not have the ability to either bind a JAFFA receptor, or modulate cell growth or migration mechanisms. Non-
20 functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

The present invention further provides orthologues of the human JAFFA protein. Orthologues of the human JAFFA protein are proteins that are isolated from non-human
25 organisms and possess the same JAFFA receptor or substrate binding mechanisms, and/or modulation of cell growth or migration mechanisms of the human JAFFA protein. Orthologues of the human JAFFA protein can readily be identified as comprising an amino acid sequence that is substantially homologous to SEQ ID NO:2.

Moreover, nucleic acid molecules encoding other JAFFA family members and, thus,
30 which have a nucleotide sequence which differs from the JAFFA sequences of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention. For example, another JAFFA cDNA can be identified based on the nucleotide sequence of

human JAFFA. Moreover, nucleic acid molecules encoding JAFFA proteins from different species, and which, thus, have a nucleotide sequence which differs from the JAFFA sequences of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention. For example, a mouse JAFFA cDNA can be identified based on the nucleotide sequence of a human JAFFA.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the JAFFA cDNAs of the invention can be isolated based on their homology to the JAFFA nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic variants and homologues of the JAFFA cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the JAFFA gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 7, 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 253, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 784, 800, 900, 1000, 1500, 2000, 2500, 3,000, 3,500, 4000 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45□C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45□C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at

about 45□C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45□C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent 5 conditions to the sequence of SEQ ID NO:1, 3 or 5, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the JAFFA sequences that may 10 exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby leading to changes in the amino acid sequence of the encoded JAFFA proteins, without altering the functional ability of the JAFFA proteins. For 15 example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of JAFFA (e.g., the sequence of SEQ ID NO:2) without altering the 20 biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the JAFFA proteins of the present invention, e.g., those present in the JAFFA fibroblast growth factor domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the JAFFA proteins of the present invention and other 25 members of the JAFFA family are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding JAFFA proteins that contain changes in amino acid residues that are not essential for activity. Such JAFFA proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises 30 a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to SEQ ID NO:2.

An isolated nucleic acid molecule encoding a JAFFA protein homologous to the protein of SEQ ID NO:2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession
5 Number _____ such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, 3 or 5, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at
10 one or more predicted non-essential amino acid residues.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g.,
15 aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in
20 a JAFFA protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a JAFFA coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for JAFFA biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3,or 5, or the nucleotide sequence
25 of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant JAFFA protein can be assayed for the ability to (1) interact with a target receptor, e.g., a cell surface receptor; (2) induce receptor
30 dimerization, (3) activate tyrosine kinase activity, (4) phosphorylate signaling molecules, e.g., phospholipase C-gamma and GTPase activating protein, (5) induce gene expression, (6) modulate cell proliferation, (7) modulate cell differentiation, (8) modulate cell survival, (9) modulate chemotaxis, (10) modulate migration, and/or (11) modulate apoptosis, of a cell

(e.g., a mesodermal, ectodermal, neural (e.g., neuroectodermal), endodermal or hematopoietic cell); or (12) modulate angiogenic processes.

C. Antisense JAFFA Nucleic Acid Molecules

5 In addition to the nucleic acid molecules encoding JAFFA proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA 10 sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire JAFFA coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding JAFFA. The term "coding region" refers to the region of the nucleotide sequence 15 comprising codons which are translated into amino acid residues (e.g., the coding region of human JAFFA corresponds to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding JAFFA. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also 20 referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding JAFFA disclosed herein (e.g., SEQ ID NO:3), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of JAFFA mRNA, but more preferably is an oligonucleotide 25 which is antisense to only a portion of the coding or noncoding region of JAFFA mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of JAFFA mRNA. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length. An antisense nucleic acid of the invention can be constructed using 30 chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical

stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a JAFFA protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs

in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

D. JAFFA-Specific Ribozymes

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave JAFFA mRNA transcripts to thereby inhibit translation of JAFFA mRNA. A ribozyme having specificity for a JAFFA-encoding nucleic acid can be designed based upon the nucleotide sequence of a JAFFA cDNA disclosed herein (i.e., SEQ ID NO:1 or 3). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a JAFFA-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, JAFFA mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, JAFFA gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the JAFFA (e.g., the JAFFA promoter and/or enhancers) to form triple helical structures that prevent transcription of the JAFFA gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

E. Modified JAFFA Nucleic Acid Molecules

In yet another embodiment, the JAFFA nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate
5 peptide nucleic acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA
10 under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) *supra*; Perry-O'Keefe et al. Proc. Natl. Acad. Sci. 93: 14670-675.

PNAs of JAFFA nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-
15 specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of JAFFA nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or
20 hybridization (Hyrup B. et al. (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of JAFFA can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of JAFFA nucleic acid
25 molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the
30 nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. et al. (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside

analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et al. (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. et al. (1996) *supra*).
5 Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. et al. (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA*
10 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide
15 may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric to permit ease of detection. Such labels and the criteria by which one label
20 would be selected over another are well known to those skilled in the art.

One variety of detectable label which is particularly well-suited to the methods of the invention is a molecular beacon, since this technology permits detection of the label only in the instance where the oligonucleotide molecule bearing the molecular beacon is hybridized to a target sequence. The invention therefore includes molecular beacon
25 oligonucleotide primer and probe molecules having at least one region which is complementary to a JAFFA nucleic acid of the invention, such that the molecular beacon is useful for quantitating the presence of the JAFFA nucleic acid of the invention in a sample. A "molecular beacon" oligonucleotide is a nucleic acid comprising a pair of complementary regions and having a fluorophore and fluorescent quencher associated therewith. The
30 fluorophore and quencher are associated with different portions of the nucleic acid in such an orientation that when the complementary regions are annealed with one another, fluorescence of the fluorophore is quenched by the quencher. When the complementary regions of the nucleic acid are not annealed with one another, such as is the case when the

primer or probe is hybridized to its target sequence, the fluorophore and quencher are distanced, and the fluorescence of the fluorophore is quenched to a lesser degree. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

5

II. Isolated JAFFA Proteins

One aspect of the invention pertains to isolated JAFFA proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to
10 raise anti-JAFFA antibodies. In one embodiment, native JAFFA proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, JAFFA proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a JAFFA protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

15 An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the JAFFA protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of JAFFA protein in which the protein is
20 separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of JAFFA protein having less than about 30% (by dry weight) of non-JAFFA protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-JAFFA protein, still more preferably less than about 10% of non-
25 JAFFA protein, and most preferably less than about 5% non-JAFFA protein. When the JAFFA protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

30 The language "substantially free of chemical precursors or other chemicals" includes preparations of JAFFA protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes

preparations of JAFFA protein having less than about 30% (by dry weight) of chemical precursors or non-JAFFA chemicals, more preferably less than about 20% chemical precursors or non-JAFFA chemicals, still more preferably less than about 10% chemical precursors or non-JAFFA chemicals, and most preferably less than about 5% chemical
5 precursors or non-JAFFA chemicals.

As used herein, a "biologically active portion" of a JAFFA protein includes a fragment of a JAFFA protein which participates in an interaction between a JAFFA molecule and a non-JAFFA molecule. Biologically active portions of a JAFFA protein include peptides comprising amino acid sequences sufficiently homologous to or derived
10 from the amino acid sequence of the JAFFA protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length JAFFA proteins, and exhibit at least one activity of a JAFFA protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the JAFFA protein, e.g., modulating cell growth and/or migration mechanisms. A biologically active portion of a JAFFA protein
15 can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a JAFFA protein can be used as targets for developing agents which modulate a JAFFA mediated activity, e.g., a cell proliferation, differentiation, migration, apoptosis, or angiogenic signaling mechanism.

In one embodiment, a biologically active portion of a JAFFA protein comprises at
20 least one fibroblast growth factor domain. It is to be understood that a preferred biologically active portion of a JAFFA protein of the present invention may contain at least one fibroblast growth factor domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native JAFFA protein.

25 In a preferred embodiment, the JAFFA protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the JAFFA protein is substantially homologous to SEQ ID NO:2, and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the JAFFA protein is a protein
30 which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to SEQ ID NO:2.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can

be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%,
5 even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the JAFFA amino acid sequence of SEQ ID NO:2 having 209 amino acid residues, at least 80, preferably at least 120, more preferably at least 150, even more preferably at least 180, and even more preferably at least 200, or 209 amino acid residues are aligned). The amino acid
10 residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the
15 two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred
20 embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another
25 preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller
30 (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to JAFFA nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to JAFFA protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

A. JAFFA Chimeric or Fusion Proteins

The invention also provides JAFFA chimeric or fusion proteins. As used herein, a JAFFA "chimeric protein" or "fusion protein" comprises a JAFFA polypeptide operatively linked to a non-JAFFA polypeptide. An "JAFFA polypeptide" refers to a polypeptide having an amino acid sequence corresponding to JAFFA, whereas a "non-JAFFA polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the JAFFA protein, e.g., a protein which is different from the JAFFA protein and which is derived from the same or a different organism. Within a JAFFA fusion protein the JAFFA polypeptide can correspond to all or a portion of a JAFFA protein. In a preferred embodiment, a JAFFA fusion protein comprises at least one biologically active portion of a JAFFA protein. In another preferred embodiment, a JAFFA fusion protein comprises at least two biologically active portions of a JAFFA protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the JAFFA polypeptide and the non-JAFFA polypeptide are fused in-frame to each other. The non-JAFFA polypeptide can be fused to the N-terminus or C-terminus of the JAFFA polypeptide.

For example, in one embodiment, the fusion protein is a GST-JAFFA fusion protein in which the JAFFA sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant JAFFA.

In another embodiment, the fusion protein is a JAFFA protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of JAFFA can be increased through use of a heterologous signal sequence.

5 The JAFFA fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The JAFFA fusion proteins can be used to affect the bioavailability of a JAFFA substrate. Use of JAFFA fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a JAFFAprotein; (ii) mis-regulation of the

10 JAFFA gene; and (iii) aberrant post-translational modification of a JAFFA protein.

Moreover, the JAFFA-fusion proteins of the invention can be used as immunogens to produce anti-JAFFA antibodies in a subject, to purify JAFFA ligands and in screening assays to identify molecules which inhibit the interaction of JAFFA with a JAFFA substrate.

Preferably, a JAFFA chimeric or fusion protein of the invention is produced by

15 standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining,

20 and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example,

25 *Current Protocols in Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A JAFFA-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the JAFFA protein.

30 B. Variants of JAFFA Proteins

The present invention also pertains to variants of the JAFFA proteins which function as either JAFFA agonists (mimetics) or as JAFFA antagonists. Variants of the JAFFA proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation

of a JAFFA protein. An agonist of the JAFFA proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a JAFFA protein. An antagonist of a JAFFA protein can inhibit one or more of the activities of the naturally occurring form of the JAFFA protein by, for example, competitively modulating a JAFFA-
5 mediated activity of a JAFFA protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the JAFFA protein.

10 In one embodiment, variants of a JAFFA protein which function as either JAFFA agonists (mimetics) or as JAFFA antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a JAFFA protein for JAFFA protein agonist or antagonist activity. In one embodiment, a variegated library of JAFFA variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a
15 variegated gene library. A variegated library of JAFFA variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential JAFFA sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of JAFFA sequences therein. There are a variety of methods which can
20 be used to produce libraries of potential JAFFA variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential *JAFFA* sequences. Methods for
25 synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a JAFFA protein coding sequence can be used to generate a variegated population of JAFFA fragments for screening and subsequent
30 selection of variants of a JAFFA protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a JAFFA coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double

stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the JAFFA protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of JAFFA proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify JAFFA variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA 89*:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated JAFFA library. For example, a library of expression vectors can be transfected into a cell line, e.g., an endothelial cell line, which ordinarily responds to JAFFA in a particular JAFFA substrate-dependent manner. The transfected cells are then contacted with JAFFA and the effect of the expression of the mutant on signaling by the JAFFA substrate can be detected, e.g., by measuring intracellular calcium and inositol 1,4,5-trisphosphate (IP3) levels, cell growth, and cell migration. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the JAFFA substrate, and the individual clones further characterized.

III. Anti-JAFFA Antibodies

An isolated JAFFA protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind JAFFA using standard techniques for polyclonal and monoclonal antibody preparation. A full-length JAFFA protein can be used or, alternatively, the invention provides antigenic peptide fragments of JAFFA for use as

immunogens. The antigenic peptide of JAFFA comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of JAFFA such that an antibody raised against the peptide forms a specific immune complex with JAFFA. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more
5 preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of JAFFA that are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human JAFFA
10 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the JAFFA protein and are thus likely to constitute surface residues useful for targeting antibody production.

A JAFFA immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An
15 appropriate immunogenic preparation can contain, for example, recombinantly expressed JAFFA protein or a chemically synthesized JAFFA polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic JAFFA preparation induces a polyclonal anti-JAFFA antibody response.

20 Accordingly, another aspect of the invention pertains to anti-JAFFA antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as JAFFA. Examples of immunologically active portions of immunoglobulin molecules include F(ab)
25 and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind JAFFA. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of JAFFA. A monoclonal
30 antibody composition thus typically displays a single binding affinity for a particular JAFFA protein with which it immunoreacts.

Polyclonal anti-JAFFA antibodies can be prepared as described above by immunizing a suitable subject with a JAFFA immunogen. The anti-JAFFA antibody titer in

the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized JAFFA. If desired, the antibody molecules directed against JAFFA can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A
5 chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-JAFFA antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown et al. (1981) *J. Immunol.* 127:539-46; Brown et al. (1980) *J.*
10 *Biol. Chem* 255:4980-83; Yeh et al. (1976) *Proc. Natl. Açad. Sci. USA* 76:2927-31; and Yeh et al. (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well
15 known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.,* 54:387-402; M. L. Gefter et al. (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a JAFFA immunogen as described
20 above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds JAFFA.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-JAFFA monoclonal antibody (see, e.g., G. Galfre et al. (1977) *Nature* 266:55052; Gefter et al.
25 *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by
30 fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can

be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then
5 selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind JAFFA, e.g., using a standard ELISA assay.

10 Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-JAFFA antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with JAFFA to thereby isolate immunoglobulin library members that bind JAFFA. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia
15 *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; Dower et al. PCT International
20 Publication No. WO 91/17271; Winter et al. PCT International Publication WO 92/20791; Markland et al. PCT International Publication No. WO 92/15679; Breitling et al. PCT International Publication WO 93/01288; McCafferty et al. PCT International Publication No. WO 92/01047; Garrard et al. PCT International Publication No. WO 92/09690; Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology*
25 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J. Mol. Biol.* 226:889-896; Clarkson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas et al. (1991) *Proc.*
30 *Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty et al. *Nature* (1990) 348:552-554.

Additionally, recombinant anti-JAFFA antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention.

Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al. European Patent Application 125,023; Better et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi et al. (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide corresponding to a marker of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.*, 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see *e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016 and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers et al. (1994) *Bio/technology* 12:899-
5 903).

Alternatively, an appropriate single-chain antibody (scFV) may be engineered (see, for example, Colcher, D., et al. *Ann N Y Acad Sci* 1999 Jun 30;880:263-80; and Reiter, Y. *Clin Cancer Res* 1996 Feb;2(2):245-52). Such molecules contain only the Fv portion of the antibody (the portion of the antibody which specifically recognizes the antigen epitope) and
10 none of the typical bioactive portions of the antibody. As such, they are significantly smaller in size than a regular antibody, and may conveniently be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target JAFFA protein.

An anti-JAFFA antibody (e.g., monoclonal antibody) can be used to isolate JAFFA
15 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-JAFFA antibody can facilitate the purification of natural JAFFA from cells and of recombinantly produced JAFFA expressed in host cells. Moreover, an anti-JAFFA antibody can be used to detect JAFFA protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the JAFFA protein. Anti-JAFFA
20 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive
25 materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, □-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a
30 luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

IV. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a JAFFA protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide

sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into
5 host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., JAFFA proteins, mutant forms of JAFFA proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of JAFFA proteins in prokaryotic or eukaryotic cells. For example, JAFFA proteins can be
10 expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

15 Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to
20 increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their
25 cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

30 Purified fusion proteins can be utilized in JAFFA activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for JAFFA proteins, for example. In a preferred embodiment, a JAFFA fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect

bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the JAFFA expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, JAFFA proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the

expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and
5 Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific
10 regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and
15 Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also
20 encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the □-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That
25 is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to JAFFA mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or
30 enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the

activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

5 Another aspect of the invention pertains to host cells into which a JAFFA nucleic acid molecule of the invention is introduced, e.g., a JAFFA nucleic acid molecule within a recombinant expression vector or a JAFFA nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is 10 understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

15 A host cell can be any prokaryotic or eukaryotic cell. For example, a JAFFA protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional 20 transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in 25 Sambrook, et al. (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may 30 integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and

methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a JAFFA protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while
5 the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a JAFFA protein. Accordingly, the invention further provides methods for producing a JAFFA protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a
10 recombinant expression vector encoding a JAFFA protein has been introduced) in a suitable medium such that a JAFFA protein is produced. In another embodiment, the method further comprises isolating a JAFFA protein from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte
15 or an embryonic stem cell into which JAFFA-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous JAFFA sequences have been introduced into their genome or homologous recombinant animals in which endogenous JAFFA sequences have been altered. Such animals are useful for studying the function and/or activity of a JAFFA protein and for identifying and/or
20 evaluating modulators of JAFFA activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the
25 genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous JAFFA gene has been altered by homologous
30 recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a JAFFA-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The JAFFA cDNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of a human JAFFA gene, such as a rat or mouse JAFFA gene, can be used as a transgene. Alternatively, a JAFFA gene homologue, such as another JAFFA family member, can be isolated based on hybridization to the JAFFA cDNA sequences of SEQ ID NO:1 or 3 (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a JAFFA transgene to direct expression of a JAFFA protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Patent No. 4,873,191 by Wagner et al. and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a JAFFA transgene in its genome and/or expression of JAFFA mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a JAFFA protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a JAFFA gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the JAFFA gene. The JAFFA gene can be a human gene (e.g., the cDNA of SEQ ID NO:3), but more preferably, is a non-human homolog of a human JAFFA gene (e.g., a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1), For example, a mouse JAFFA gene can be used to construct a homologous recombination nucleic acid molecule, e.g., a vector, suitable for altering an endogenous JAFFA gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous JAFFA gene is functionally disrupted (i.e., no

longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous JAFFA gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to
5 thereby alter the expression of the endogenous JAFFA protein). In the homologous recombination nucleic acid molecule, the altered portion of the JAFFA gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the JAFFA gene to allow for homologous recombination to occur between the exogenous JAFFA gene carried by the homologous recombination nucleic acid molecule and an endogenous JAFFA gene in a cell,
10 e.g., an embryonic stem cell. The additional flanking JAFFA nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, e.g., Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The
15 homologous recombination nucleic acid molecule is introduced into a cell, e.g., an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced JAFFA gene has homologously recombined with the endogenous JAFFA gene are selected (see e.g., Li, E. et al. (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g., Bradley, A. in
20 *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline
25 transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, e.g., vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec et al.; WO 91/01140 by Smithies et al.; WO 92/0968 by Zijlstra et al.; and WO 93/04169 by Berns et al.

30 In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Lakso et al. (1992) *Proc. Natl.*

Acad. Sci. USA 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. et al. (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

V. Pharmaceutical Compositions

The JAFFA nucleic acid molecules, fragments of JAFFA proteins, and anti-JAFFA antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include

parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,
5 glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The
10 parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration,
15 suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or
20 dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various
25 antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum
30 monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a fragment of a JAFFA protein or an anti-JAFFA antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as

required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of
5 preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral
10 therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The
15 tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring
20 agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For
25 transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are
30 formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.,* for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form

employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, 5 vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., 10 mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents 15 (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, 20 pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor 25 ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled 30 Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of

Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second
5 antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic
10 injection (see e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical
15 preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

20 VI. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic). As
25 described herein, a JAFFA protein of the invention has one or more of the following activities: (1) it interacts with a non-JAFFA protein molecule, e.g., a JAFFA substrate, such as a JAFFA receptor; (2) it activates a JAFFA-dependent signal transduction pathway; (3) it modulates cell proliferation, differentiation, and/or migration mechanisms; (4) it modulates angiogenesis, and, thus, can be used to, for example, (1) modulate the interaction with a
30 non-JAFFA protein molecule; (2) to activate a JAFFA-dependent signal transduction pathway; (3) to modulate cell proliferation, differentiation, and/or migration mechanisms; (4) to modulate angiogenesis.

The isolated nucleic acid molecules of the invention can be used, for example, to express JAFFA protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect JAFFA mRNA (e.g., in a biological sample) or a genetic alteration in a JAFFA gene, and to modulate JAFFA activity, as described further below.
5 The JAFFA proteins can be used to treat disorders characterized by insufficient or excessive production of a JAFFA substrate or production of JAFFA inhibitors. In addition, the JAFFA proteins can be used to screen for naturally occurring JAFFA substrates, to screen for drugs or compounds which modulate JAFFA activity, as well as to treat disorders characterized by insufficient or excessive production of JAFFA protein or production of
10 JAFFA protein forms which have decreased, aberrant or unwanted activity compared to JAFFA wild type protein (e.g., cell proliferation and/or differentiation disorders, such as disorders characterized by aberrant angiogenesis). Moreover, the anti-JAFFA antibodies of the invention can be used to detect and isolate JAFFA proteins, regulate the bioavailability of JAFFA proteins, and modulate JAFFA activity.

15

A. Screening Assays:

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to JAFFA proteins,
20 have a stimulatory or inhibitory effect on, for example, JAFFA expression or JAFFA activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a JAFFA substrate. Compounds thus identified can be used to modulate the activity of target gene products in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.
25 The preferred target genes/products used in this embodiment are the JAFFA genes of the present invention.

Assays for the Detection of Binding Between a Test Compound and the JAFFA Protein Product

30 In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a JAFFA protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening

candidate or test compounds which bind to or modulate the activity of a JAFFA protein or polypeptide or biologically active portion thereof.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including:
5 biological libraries; peptoid libraries [libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive] (see, e.g., Zuckermann, R.N. et al. *J. Med. Chem.* 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound'
10 library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art,
15 for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al. (1994) *J. Med. Chem.* 37:1233.

20 Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull et al. (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla et al.
25 (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a JAFFA protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate JAFFA activity is determined. Determining the
30 ability of the test compound to modulate JAFFA activity can be accomplished by monitoring, for example, intracellular calcium and inositol 1,4,5-trisphosphate (IP3) levels, cell growth, and cell chemotaxis. The cell, for example, can be of mammalian origin, e.g., an endothelial cell.

The ability of the test compound to modulate JAFFA binding to a substrate or to bind to JAFFA can also be determined. Determining the ability of the test compound to modulate JAFFA binding to a substrate can be accomplished, for example, by coupling the JAFFA substrate with a radioisotope or enzymatic label such that binding of the JAFFA substrate to JAFFA can be determined by detecting the labeled JAFFA substrate in a complex. Alternatively, JAFFA could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate JAFFA binding to a JAFFA substrate in a complex. Determining the ability of the test compound to bind JAFFA can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to JAFFA can be determined by detecting the labeled JAFFA compound in a complex. For example, compounds (e.g., JAFFA substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (e.g., a JAFFA substrate) to interact with JAFFA without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with JAFFA without the labeling of either the compound or the JAFFA. McConnell, H. M. et al. (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and JAFFA.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a JAFFA target molecule (e.g., a JAFFA substrate) with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the JAFFA target molecule. Determining the ability of the test compound to modulate the activity of a JAFFA target molecule can be accomplished, for example, by determining the ability of the JAFFA protein to bind to or interact with the JAFFA target molecule.

Determining the ability of the JAFFA protein or a biologically active fragment thereof, to bind to or interact with a JAFFA target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the JAFFA protein to bind to or interact with a JAFFA target
5 molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (i.e., intracellular calcium or IP3), detecting catalytic/enzymatic activity of the target molecule upon an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element
10 operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a target-regulated cellular response (i.e., cell growth or migration).

In yet another embodiment, an assay of the present invention is a cell-free assay in which a JAFFA protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the JAFFA protein or biologically
15 active portion thereof is determined. Preferred biologically active portions of the JAFFA proteins to be used in assays of the present invention include fragments which participate in interactions with non-JAFFA molecules, e.g., fragments with high surface probability scores.

The cell-free assays of the present invention are amenable to use of both soluble
20 and/or membrane-bound forms of isolated proteins (e.g., JAFFA proteins or biologically active portions thereof). In the case of cell-free assays in which a membrane-bound form of an isolated protein is used it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-
25 dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

30 The principle of the assays used to identify compounds that bind to the target gene product involves preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected in the

reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring target gene product or the test substance onto a solid phase and detecting target gene product/test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein and which are well-known to one skilled in the art.

It is also possible to directly detect the interaction of two molecules without further sample manipulation, for example utilizing the technique of fluorescence energy transfer (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment of this assay method, determining the ability of the JAFFA protein to bind to a JAFFA target molecule can be accomplished without labeling either interactant using a technology such as real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo et al. (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "surface plasmon resonance" or "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either JAFFA or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a JAFFA protein, or interaction of a JAFFA protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/JAFFA fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or JAFFA protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of JAFFA binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a JAFFA protein or a JAFFA target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated JAFFA protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). In certain embodiments, the protein-immobilized surfaces can be prepared in advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously nonimmobilized component is pre-labeled, the detection of label immobilized on the surface

indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

5 In one embodiment, this assay is performed utilizing antibodies reactive with JAFFA protein or target molecules but which do not interfere with binding of the JAFFA protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or JAFFA protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-

10 immobilized complexes, include immunodetection of complexes using antibodies reactive with the JAFFA protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the JAFFA protein or target molecule.

Alternatively, in another embodiment, an assay can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of

15 a number of standard techniques, including but not limited to: differential centrifugation, chromatography, electrophoresis and immunoprecipitation. In differential centrifugation, complexes of molecules may be separated from uncomplexed molecules through a series of centrifugal steps, due to the different sedimentation equilibria of complexes based on their different sizes and densities (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem*

20 *Sci* 1993 Aug;18(8):284-7). Standard chromatographic techniques may also be utilized to separate complexed molecules from uncomplexed ones. For example, gel filtration chromatography separates molecules based on size, and through the utilization of an appropriate gel filtration resin in a column format, for example, the relatively larger complex may be separated from the relatively smaller uncomplexed components. Similarly,

25 the relatively different charge properties of the complex as compared to the uncomplexed molecules may be exploited to differentially separate the complex from the remaining individual reactants, for example through the use of ion-exchange chromatography resins. Such resins and chromatographic techniques are well known to one skilled in the art (see, e.g., Heegaard, N.H., *J Mol Recognit* 1998 Winter;11(1-6):141-8; Hage, D.S., and Tweed,

30 S.A. *J Chromatogr B Biomed Sci Appl* 1997 Oct 10;699(1-2):499-525). Gel electrophoresis may also be employed to separate complexed molecules from unbound species (see, e.g., Ausubel, F. et al., eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York.). In this technique, protein or nucleic acid complexes are separated based on size or charge,

for example. In order to maintain the binding interaction during the electrophoretic process, nondenaturing gels in the absence of reducing agent are typically preferred, but conditions appropriate to the particular interactants will be well known to one skilled in the art. Immunoprecipitation is another common technique utilized for the isolation of a protein-protein complex from solution (see, for example, Ausubel, F. et al., eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York). In this technique, all proteins binding to an antibody specific to one of the binding molecules are precipitated from solution by conjugating the antibody to a polymer bead that may be readily collected by centrifugation. The bound proteins are released from the beads (through a specific proteolysis event or other technique well known in the art which will not disturb the protein-protein interaction in the complex), and a second immunoprecipitation step is performed, this time utilizing antibodies specific for a different interacting protein. In this manner, only the complex should remain attached to the beads. The captured complex may be visualized using gel electrophoresis. The presence of a molecular complex (which may be identified by any of these techniques) indicates that a specific binding event has occurred, and that the introduced compound specifically binds to the target protein. Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the JAFFA protein or biologically active portion thereof with a known compound which binds JAFFA to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a JAFFA protein, wherein determining the ability of the test compound to interact with a JAFFA protein comprises determining the ability of the test compound to preferentially bind to JAFFA or biologically active portion thereof as compared to the known compound.

In yet another embodiment, the cell-free assay involves contacting a JAFFA protein or biologically active portion thereof with a known compound which binds the JAFFA protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the JAFFA protein, wherein determining the ability of the test compound to interact with the JAFFA protein comprises determining the ability of the JAFFA protein to preferentially bind to or modulate the activity of a JAFFA target molecule.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity
5 of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the JAFFA genes herein identified. Towards this purpose, in an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a JAFFA protein through modulation of the
10 activity of a downstream effector of a JAFFA target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined as previously described.

The basic principle of the assay systems used to identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular
15 binding partner or partners involves preparing a reaction mixture containing the target gene product, and the binding partner under conditions and for a time sufficient to allow the two products to interact and bind, thus forming a complex. In order to test an agent for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be
20 added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the
25 compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds
30 that disrupt interactions of mutant but not normal target gene products.

The assay for compounds that interfere with the interaction of the target gene products and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding

partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere
5 with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance; i.e., by adding the test substance to the reaction mixture prior to or simultaneously with the target gene product and interactive cellular or extracellular binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds
10 with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface, while the non-
15 anchored species is labeled, either directly or indirectly. In practice, microtitre plates are conveniently utilized. The anchored species can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished simply by coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored can be used
20 to anchor the species to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will
25 remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the
30 initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for
5 the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the target gene product and the interactive cellular
10 or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above
15 background. In this way, test substances that disrupt target gene product-cellular or extracellular binding partner interaction can be identified.

Assays for the Detection of the Ability of a Test Compound to Modulate Expression of JAFFA

20 In another embodiment, modulators of JAFFA expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of JAFFA mRNA or protein in the cell is determined. The level of expression of JAFFA mRNA or protein in the presence of the candidate compound is compared to the level of expression of JAFFA mRNA or protein in the absence of the candidate compound. The candidate compound can
25 then be identified as a modulator of JAFFA expression based on this comparison. For example, when expression of JAFFA mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of JAFFA mRNA or protein expression. Alternatively, when expression of JAFFA mRNA or protein is less (statistically
30 significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of JAFFA mRNA or protein expression. The level of JAFFA mRNA or protein expression in the cells can be determined by methods described herein for detecting JAFFA mRNA or protein.

In yet another aspect of the invention, the JAFFA proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Biotechniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with JAFFA ("JAFFA-binding proteins" or "JAFFA-bp") and are involved in JAFFA activity. Such JAFFA-binding proteins are also likely to be involved in the propagation of signals by the JAFFA proteins or JAFFA targets as, for example, downstream elements of a JAFFA-mediated signaling pathway. Alternatively, such JAFFA-binding proteins are likely to be JAFFA inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a JAFFA protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a JAFFA-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the JAFFA protein.

Combination Assays

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a JAFFA protein can be confirmed *in vivo*, e.g., in an animal such as an animal model for angiogenesis, or for cellular transformation and/or tumorigenesis.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an

agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a JAFFA modulating agent, an antisense JAFFA nucleic acid molecule, a JAFFA-specific antibody, or a JAFFA-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

The choice of assay format will be based primarily on the nature and type of sensitivity/resistance protein being assayed. A skilled artisan can readily adapt protein activity assays for use in the present invention with the genes identified herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the JAFFA nucleotide sequences, described herein, can be used to map the location of the JAFFA genes on a chromosome. The mapping of the JAFFA sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, JAFFA genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the JAFFA nucleotide sequences. Computer analysis of the JAFFA sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then

be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the JAFFA sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al. (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the JAFFA nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a JAFFA sequence to its chromosome include *in situ* hybridization (described in Fan, Y. et al. (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good

results at a reasonable amount of time. For a review of this technique, see Verma et al., Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al. (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the JAFFA gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The JAFFA sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The

sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the JAFFA nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The JAFFA nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from JAFFA nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial JAFFA Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make

such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

5 The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for 10 identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or 5 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the JAFFA nucleotide sequences or portions thereof, e.g., 15 fragments derived from the noncoding regions of SEQ ID NO:1 or 5 having a length of at least 20 bases, preferably at least 30 bases.

The JAFFA nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., a tissue containing 20 endothelial cells. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such JAFFA probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., JAFFA primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different 25 types of cells in a culture).

4. Use of JAFFA Molecules as Surrogate Markers

The JAFFA molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of 30 disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the JAFFA molecules of the invention may be detected, and may be correlated with one or more biological states in vivo. For example, the JAFFA molecules of the invention may serve as

surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) J. Mass. Spectrom. 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The JAFFA molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a JAFFA marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described

herein, anti-[GENENAME] antibodies may be employed in an immune-based detection system for a JAFFA protein marker, or JAFFA-specific radiolabeled probes may be used to detect a JAFFA mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) Env. Health Perspect. 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am, J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The JAFFA molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35:1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., JAFFA protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in JAFFA DNA may correlate JAFFA drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining JAFFA protein and/or nucleic acid expression as well as JAFFA activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted JAFFA expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of

developing a disorder associated with JAFFA protein, nucleic acid expression or activity. For example, mutations in a JAFFA gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with JAFFA
5 protein, nucleic acid expression or activity.

As an alternative to making determinations based on the absolute expression level of selected genes, determinations may be based on the normalized expression levels of these genes. Expression levels are normalized by correcting the absolute expression level of a JAFFA gene by comparing its expression to the expression of a gene that is not a JAFFA
10 gene, e.g., a housekeeping gene that is constitutively expressed. Suitable genes for normalization include housekeeping genes such as the actin gene. This normalization allows the comparison of the expression level in one sample, e.g., a patient sample, to another sample, e.g., a non-disease sample, or between samples from different sources.

Alternatively, the expression level can be provided as a relative expression level.
15 To determine a relative expression level of a gene, the level of expression of the gene is determined for 10 or more samples of different endothelial or hematopoietic cell isolates, preferably 50 or more samples, prior to the determination of the expression level for the sample in question. The mean expression level of each of the genes assayed in the larger number of samples is determined and this is used as a baseline expression level for the
20 gene(s) in question. The expression level of the gene determined for the test sample (absolute level of expression) is then divided by the mean expression value obtained for that gene. This provides a relative expression level and aids in identifying extreme cases of disease, e.g., cancer.

Preferably, the samples used in the baseline determination will be from diseased,
25 e.g., cancerous, or from non-diseased cells of endothelial or hematopoietic tissue. The choice of the cell source is dependent on the use of the relative expression level. Using expression found in normal tissues as a mean expression score aids in validating whether the JAFFA gene assayed is hematopoietic cell-type specific (versus normal cells). Such a use is particularly important in identifying whether a JAFFA gene can serve as a target gene. In
30 addition, as more data is accumulated, the mean expression value can be revised, providing improved relative expression values based on accumulated data. Expression data from hematopoietic cells provides a means for grading the severity of the disease state, e.g., cancer.

Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of JAFFA in clinical trials.

These and other agents are described in further detail in the following sections.

5

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of JAFFA protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of
10 detecting JAFFA protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes JAFFA protein such that the presence of JAFFA protein or nucleic acid is detected in the biological sample. The level of expression of the JAFFA gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the JAFFA genes; measuring the amount of protein encoded by the JAFFA genes; or measuring the activity of
15 the protein encoded by the JAFFA genes.

The level of mRNA corresponding to the JAFFA gene in a cell can be determined both by *in situ* and by *in vitro* formats in a biological sample using methods known in the art. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A
20 preferred biological sample is a serum sample isolated by conventional means from a subject. Many JAFFA expression detection methods use isolated RNA. For *in vitro* methods, any RNA isolation technique that does not select against the isolation of mRNA can be utilized for the purification of RNA from the hematopoieitic or endothelial cells (*see*, e.g., Ausubel et al., eds., 1987-1997, Current Protocols in Molecular Biology, John Wiley &
25 Sons, Inc. New York). Additionally, large numbers of tissue samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski (1989, U.S. Patent No. 4,843,155).

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction
30 analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length JAFFA nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3,

or 5, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to JAFFA mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of
5 the invention are described herein. Hybridization of an mRNA with the probe indicates that the gene in question is being expressed.

In one format, the mRNA is immobilized on a solid surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the
10 probes are immobilized on a solid surface and the mRNA is contacted with the probes, for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the JAFFA genes of the present invention.

An alternative method for determining the level of mRNA in a sample that is
15 encoded by one of the JAFFA genes of the present invention involves the process of nucleic acid amplification, e.g., by rtPCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany, 1991, Proc. Natl. Acad. Sci. USA *88*:189-193), self sustained sequence replication (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA *87*:1874-1878), transcriptional amplification system (Kwoh et al., 1989, Proc. Natl.
20 Acad. Sci. USA *86*:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, Bio/Technology *6*:1197), rolling circle replication (Lizardi et al., U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in
25 very low numbers. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such
30 primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers. Suitable primers for the amplification of the JAFFA gene are described herein.

For *in situ* methods, mRNA does not need to be isolated from the hemaotopoietic or endothelial cells prior to detection. In such methods, a cell or tissue sample is prepared/processed using known histological methods. The sample is then immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to
5 mRNA that encodes the JAFFA gene being analyzed.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting JAFFA mRNA, or genomic DNA, such that the presence of JAFFA mRNA or genomic DNA is detected in the biological sample, and comparing the presence
10 of JAFFA mRNA or genomic DNA in the control sample with the presence of JAFFA mRNA or genomic DNA in the test sample.

A variety of methods can be used to determine the level of protein encoded by one or more of the JAFFA genes of the present invention. In general, these methods involve the use of an agent that selectively binds to the protein, such as an antibody. In a preferred
15 embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by
20 reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The detection methods of the invention can be used to detect JAFFA protein in a
25 biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of JAFFA protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vivo* techniques for detection of JAFFA protein include introducing into a subject a labeled anti-JAFFA antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject
30 can be detected by standard imaging techniques.

Proteins from hematopoietic or endothelial cells can be isolated using techniques that are well known to those of skill in the art. The protein isolation methods employed can, for example, be such as those described in Harlow and Lane (Harlow and Lane, 1988,

Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

A variety of formats can be employed to determine whether a sample contains a protein that binds to a given antibody. Examples of such formats include, but are not
5 limited to, enzyme immunoassay (EIA), radioimmunoassay (RIA), Western blot analysis and enzyme linked immunoabsorbant assay (ELISA). A skilled artisan can readily adapt known protein/antibody detection methods for use in determining whether hematopoietic or endothelial cells express a protein encoded by one or more of the JAFFA genes of the present invention.

10 In one format, antibodies, or antibody fragments, can be used in methods such as Western blots or immunofluorescence techniques to detect the expressed proteins. In such uses, it is generally preferable to immobilize either the antibody or protein on a solid support. Suitable solid phase supports or carriers include any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene,
15 polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.

One skilled in the art will know many other suitable carriers for binding antibody or antigen, and will be able to adapt such support for use with the present invention. For example, protein isolated from hematopoietic or endothelial cells can be run on a
20 polyacrylamide gel electrophoresis and immobilized onto a solid phase support such as nitrocellulose. The support can then be washed with suitable buffers followed by treatment with the detectably labeled JAFFA gene specific antibody. The solid phase support can then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on the solid support can then be detected by conventional means.

25 In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting JAFFA protein, such that the presence of JAFFA protein is detected in the biological sample, and comparing the presence of JAFFA protein in the control sample with the presence of JAFFA protein in the test sample.

30 The invention also encompasses kits for detecting the presence of JAFFA in a biological sample. For example, the kit can comprise a compound or agent capable of detecting JAFFA protein or mRNA in a biological sample; means for determining the amount of JAFFA in the sample; and means for comparing the amount of JAFFA in the

sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect JAFFA protein or nucleic acid.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (e.g.,
5 attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic
10 acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample
15 or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

20 2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted JAFFA expression or activity. As used herein, the term "aberrant" includes a JAFFA expression or activity which deviates from the wild type JAFFA expression or
25 activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant JAFFA expression or activity is intended to include the cases in which a mutation in the JAFFA gene causes the JAFFA gene to be under-expressed or over-expressed and situations in
30 which such mutations result in a non-functional JAFFA protein or a protein which does not function in a wild-type fashion, e.g., a protein which does not interact with a JAFFA substrate, e.g., a JAFFA receptor, or one which interacts with a non-JAFFA substrate. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a

biological response such as pain or deregulated cell proliferation. For example, the term unwanted includes a JAFFA expression or activity which is undesirable in a subject.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in JAFFA protein activity or nucleic acid expression, such as a cell proliferation and/or differentiation disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in JAFFA protein activity or nucleic acid expression, such as a cell proliferation and/or differentiation disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant or unwanted JAFFA expression or activity in which a test sample is obtained from a subject and JAFFA protein or nucleic acid (e.g., mRNA or genomic DNA) is detected, wherein the presence of JAFFA protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted JAFFA expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted JAFFA expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a cell proliferation and/or differentiation disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted JAFFA expression or activity in which a test sample is obtained and JAFFA protein or nucleic acid expression or activity is detected (e.g., wherein the abundance of JAFFA protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted JAFFA expression or activity).

The methods of the invention can also be used to detect genetic alterations in a JAFFA gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in JAFFA protein activity or nucleic acid expression, such as a cell proliferation and/or differentiation disorder. In preferred embodiments, the methods

include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a JAFFA-protein, or the mis-expression of the JAFFA gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a
5 deletion of one or more nucleotides from a JAFFA gene; 2) an addition of one or more nucleotides to a JAFFA gene; 3) a substitution of one or more nucleotides of a JAFFA gene, 4) a chromosomal rearrangement of a JAFFA gene; 5) an alteration in the level of a messenger RNA transcript of a JAFFA gene, 6) aberrant modification of a JAFFA gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type
10 splicing pattern of a messenger RNA transcript of a JAFFA gene, 8) a non-wild type level of a JAFFA-protein, 9) allelic loss of a JAFFA gene, and 10) inappropriate post-translational modification of a JAFFA-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a JAFFA gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a
15 subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa et
20 al. (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the JAFFA-gene (see Abravaya et al. (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically
25 hybridize to a JAFFA gene under conditions such that hybridization and amplification of the JAFFA-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting
30 mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177),

Q-Beta Replicase (Lizardi, P.M. et al. (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low 5 numbers.

In an alternative embodiment, mutations in a JAFFA gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and 10 compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in JAFFA can be identified by hybridizing 15 a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. et al. (1996) *Human Mutation* 7: 244-255; Kozal, M.J. et al. (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in JAFFA can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first 20 hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or 25 mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the JAFFA gene and detect mutations by comparing the sequence of the sample JAFFA with the corresponding wild-type (control) sequence. 30 Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques*

19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the JAFFA gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type JAFFA sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in JAFFA cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a JAFFA sequence, e.g., a wild-type JAFFA sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in JAFFA genes. For example, single strand conformation polymorphism (SSCP)

may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control JAFFA nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.*

(1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a JAFFA gene.

Furthermore, any cell type or tissue in which JAFFA is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a JAFFA protein (e.g., the modulation of cell growth, differentiation, migration, and/or apoptosis mechanisms) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase JAFFA gene expression, protein levels, or upregulate JAFFA activity, can be monitored in clinical trials of subjects exhibiting decreased JAFFA gene expression, protein levels, or downregulated JAFFA activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease JAFFA gene expression, protein levels, or downregulate JAFFA activity, can be monitored in clinical trials of subjects exhibiting increased JAFFA gene expression, protein levels, or upregulated JAFFA activity. In such clinical trials, the expression or activity of a JAFFA gene, and preferably, other genes that have been implicated in, for example, a JAFFA-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including JAFFA, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule)

which modulates JAFFA activity (e.g., identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on JAFFA-associated disorders (e.g., disorders characterized by deregulated cell growth, differentiation and/or migration mechanisms), for example, in a clinical trial, cells can be isolated and RNA prepared and
5 analyzed for the levels of expression of JAFFA and other genes implicated in the JAFFA-associated disorder, respectively. The levels of gene expression (e.g., a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of JAFFA or other genes. In this
10 way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist,
15 peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a JAFFA protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the
20 subject; (iv) detecting the level of expression or activity of the JAFFA protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the JAFFA protein, mRNA, or genomic DNA in the pre-administration sample with the JAFFA protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For
25 example, increased administration of the agent may be desirable to increase the expression or activity of JAFFA to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of JAFFA to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, JAFFA expression or activity
30 may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted JAFFA expression or activity. As used herein, the term "treating" or "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the JAFFA molecules of the present invention or JAFFA modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted JAFFA expression or activity, by administering to the subject a JAFFA or an agent which modulates JAFFA expression or at least one JAFFA activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted JAFFA expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the JAFFA aberrance, such that a disease or disorder is prevented or, alternatively, delayed in

its progression. Depending on the type of JAFFA aberrance, for example, a JAFFA, JAFFA agonist or JAFFA antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

5 2. Therapeutic Methods

Treatment of a Proliferative and/or Differentiative Disorder by Modulation of JAFFA Genes or Gene Products

Proliferative and/or differentiative disorders can be treated by negatively modulating the expression of a target gene or the activity of a target gene product.
10 "Negative modulation," refers to a reduction in the level and/or activity of target gene product relative to the level and/or activity of the target gene product in the absence of the modulatory treatment.

It is possible that some proliferative and/or differentiative disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene
15 product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of proliferative and/or differentiative disorder symptoms.

Negative Modulatory Techniques

20 As discussed, successful treatment of proliferative and/or differentiative disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products.

For example, compounds, e.g., an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with
25 the invention to prevent and/or ameliorate symptoms of proliferative and/or differentiative disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, $F(ab')_2$ and FAb expression library fragments, scFV molecules, and epitope-binding
30 fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene

expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity.

Among the compounds that can exhibit the ability to prevent and/or ameliorate symptoms of proliferative and/or differentiative disorders are antisense, ribozyme, and triple
5 helix molecules. Such molecules can be designed to reduce or inhibit either wild type, or if appropriate, mutant target gene activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. With respect
10 to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see, for example, Rossi, 1994, Current Biology 4:469-
15 471.) The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA and must include the well-known catalytic sequence responsible for mRNA cleavage. For this sequence, see U.S. Pat. No. 5,093,246,
20 that is incorporated by reference herein in its entirety. As such within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of RNA sequences encoding target gene proteins.

Specific ribozyme cleavage sites within any potential RNA target are initially
25 identified by scanning the molecule of interest for ribozyme cleavage sites that include the following sequences, GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of
30 candidate sequences can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Nucleic acid molecules to be used in triplex helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base

composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, that generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences can be pyrimidine-based, that will result in TAT and CGC^+ triplets across the three associated
5 strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarily to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules can be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in that the majority of the purine residues are
10 located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation can be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they
15 base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to reduce or inhibit mutant gene expression, it is possible that the technique utilized can also efficiently reduce or inhibit the transcription (triple helix)
20 and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles such that the possibility can arise wherein the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can
25 be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Anti-sense RNA and DNA, ribozyme and triple helix molecules of the invention
30 can be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules can be generated by *in*

vitro and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or
5 inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various well-known modifications to the DNA molecules can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than
10 phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Another method by which nucleic acid molecules may be utilized in treatment or prevention of a disease state characterized by JAFFA expression is through the use of aptamer molecules specific for JAFFA protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g.,
15 Osborne, et al. *Curr. Opin. Chem Biol.* 1997, 1(1): 5-9; and Patel, D.J. *Curr Opin Chem Biol* 1997 Jun;1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which JAFFA protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

20 Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, by administered in instances whereby negative modulatory techniques are appropriate for the treatment of proliferative and/or differentiative disorders. Antibodies can be generated using standard techniques against the proteins themselves or against peptides corresponding to portions of
25 the proteins. The antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single chain antibodies, scFV molecules, chimeric antibodies, and the like, as described herein.

In circumstances wherein injection of an animal or a human subject with a JAFFA protein or epitope for the purpose of stimulating antibody production is harmful to
30 the subject, due to the nature of the JAFFA protein or portion thereof, it is possible to generate an immune response against JAFFA through the use of anti-idiotypic antibodies (see, for example, Herlyn, D. *Ann Med* 1999;31(1):66-78; and

Bhattacharya-Chatterjee, M., and Foon, K.A. *Cancer Treat Res* 1998;94:51-68). Anti-idiotypic antibodies are antibodies which specifically recognize the antigen-binding portion of another antibody, and as such, their antigen-binding domain should be nearly identical in structure to an epitope of the antigen to which the first antibody was specific. For example, 5 an anti-idiotypic antibody specific for the antigen-binding domain of an anti-JAFFA antibody should have an antigen-binding domain structure similar to that of some portion of the JAFFA protein. If such an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the JAFFA protein. Vaccines directed to a disease state characterized 10 by JAFFA expression may also be generated in this fashion.

In instances where the target gene protein to that the antibody is directed to is intracellular and whole antibodies are used, internalizing antibodies may be preferred. However, lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target gene epitope into cells. Where fragments of the antibody 15 are used, the smallest inhibitory fragment that binds to the target protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the antibody that binds to the target gene protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (e.g., see Creighton, 1983, *supra*; and 20 Sambrook et al., 1989, *supra*). Alternatively, single chain neutralizing antibodies that bind to intracellular target gene product epitopes can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (1993, Proc. Natl. Acad. Sci. USA 25 90:7889-7893).

Therapeutic Treatment

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or 30 ameliorate proliferative and/or differentiative disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of proliferative and/or differentiative disorders.

Effective Dose

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

Another example of determination of effective dose for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject. Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. The compound which is able to modulate JAFFA activity is used as a template, or "imprinting molecule", to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated "negative image" of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al* (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J. (1994) *Trends in Polymer Science* **2**:166-173.

Such "imprinted" affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al* (1993) *Nature* **361**:645-647. Through the use of isotope-labeling, the "free" concentration of
5 compound which modulates the expression or activity of JAFFAcan be readily monitored and used in calculations of IC_{50}.

Such "imprinted" affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using
10 appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC_{50}. An rudimentary example of such a "biosensor" is discussed in Kriz, D. *et al* (1995) *Analytical Chemistry* **67**:2142-2144.

Another aspect of the invention pertains to methods of modulating JAFFA expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment,
15 the modulatory method of the invention involves contacting a cell with a JAFFA or agent that modulates one or more of the activities of JAFFA protein activity associated with the cell. An agent that modulates JAFFA protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a JAFFA protein (e.g., a JAFFA substrate or receptor), a JAFFA antibody, a JAFFA agonist or antagonist, a
20 peptidomimetic of a JAFFA agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more JAFFA activities. Examples of such stimulatory agents include active JAFFA protein and a nucleic acid molecule encoding JAFFA that has been introduced into the cell. In another embodiment, the agent inhibits one or more JAFFA activities. Examples of such inhibitory agents include antisense JAFFA
25 nucleic acid molecules, anti-JAFFA antibodies, and JAFFA inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a JAFFA protein or nucleic
30 acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) JAFFA expression or activity. In another

embodiment, the method involves administering a JAFFA protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted JAFFA expression or activity.

Stimulation of JAFFA activity is desirable in situations in which JAFFA is abnormally downregulated and/or in which increased JAFFA activity is likely to have a
5 beneficial effect. For example, stimulation of JAFFA activity is desirable in situations in which a JAFFA is downregulated and/or in which increased JAFFA activity is likely to have a beneficial effect. Likewise, inhibition of JAFFA activity is desirable in situations in which JAFFA is abnormally upregulated and/or in which decreased JAFFA activity is likely to have a beneficial effect.

10

3. Pharmacogenomics

The JAFFA molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on JAFFA activity (e.g., JAFFA gene expression) as identified by a screening assay described herein can be administered to
15 individuals to treat (prophylactically or therapeutically) JAFFA-associated disorders (e.g., cell proliferation and/or differentiation disorders, or disorders characterized by aberrant angiogenesis) associated with aberrant or unwanted JAFFA activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be
20 considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a JAFFA molecule or JAFFA modulator as well as tailoring the dosage and/or
25 therapeutic regimen of treatment with a JAFFA molecule or JAFFA modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11) :983-985 and Linder, M.W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two types
30 of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or

as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

5 One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be 10 compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs 15 in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be 20 tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a JAFFA protein of the present invention), all common variants 25 of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and 30 cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor

metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug 5 response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene 10 amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a JAFFA molecule or JAFFA modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

15 Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a JAFFA molecule or 20 JAFFA modulator, such as a modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new anti-proliferative agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the JAFFA genes of the 25 present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the JAFFA genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., hematopoietic cells, will become sensitive to treatment with an agent that the 30 unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a JAFFA protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase JAFFA gene expression,

protein levels, or upregulate JAFFA activity, can be monitored in clinical trials of subjects exhibiting decreased JAFFA gene expression, protein levels, or downregulated JAFFA activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease JAFFA gene expression, protein levels, or downregulate JAFFA activity, can be
5 monitored in clinical trials of subjects exhibiting increased JAFFA gene expression, protein levels, or upregulated JAFFA activity. In such clinical trials, the expression or activity of a JAFFA gene, and preferably, other genes that have been implicated in, for example, a JAFFA-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

10 Other Embodiments

In another aspect, the invention features, a method of analyzing a plurality of capture probes. The method can be used, e.g., to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and
15 each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence; contacting the array with a JAFFA, preferably purified, nucleic acid, preferably purified, polypeptide, preferably purified, or antibody, and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label
20 attached to the JAFFA nucleic acid, polypeptide, or antibody.

The capture probes can be a set of nucleic acids from a selected sample, e.g., a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

The method can include contacting the JAFFA nucleic acid, polypeptide, or antibody with a first array having a plurality of capture probes and a second array having a different
25 plurality of capture probes. The results of each hybridization can be compared, e.g., to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, e.g., a wild type, normal, or non-diseased, non-stimulated, sample, e.g., a biological fluid, tissue, or cell sample. The second plurality of capture probes can be from an experimental sample, e.g., a mutant type, at risk, disease-state
30 or disorder-state, or stimulated, sample, e.g., a biological fluid, tissue, or cell sample.

The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of JAFFA. Such methods can be used to diagnose a subject, e.g., to evaluate risk for a disease or disorder, to evaluate suitability of a

selected treatment for a subject, to evaluate whether a subject has a disease or disorder. JAFFA is associated with a proliferative disorders, thus it is useful for evaluating the same.

The method can be used to detect SNPs, as described above.

In another aspect, the invention features, a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express JAFFA or from a cell or subject in which a JAFFA mediated response has been elicited, e.g., by contact of the cell with JAFFA nucleic acid or protein, or administration to the cell or subject JAFFA nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express JAFFA (or does not express as highly as in the case of the JAFFA positive plurality of capture probes) or from a cell or subject which in which a JAFFA mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a JAFFA nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features, a method of analyzing a plurality of probes or a sample. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, contacting the array with a first sample from a cell or subject which express or mis express JAFFA or from a cell or subject in which a JAFFA mediated response has been elicited, e.g., by contact of the cell with JAFFA nucleic acid or protein, or administration to the cell or subject JAFFA nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the

plurality having a unique capture probe, and contacting the array with a second sample from a cell or subject which does not express JAFFA (or does not express as highly as in the case of the JAFFA positive plurality of capture probes) or from a cell or subject which in which a JAFFA mediated response has not been elicited (or has been elicited to a lesser extent than
5 in the first sample); and comparing the binding of the first sample with the binding of the second sample. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody. The same array can be used for both samples or different arrays can be used. If different arrays are used the plurality of
10 addresses with capture probes should be present on both arrays.

In another aspect, the invention features, a method of analyzing JAFFA, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a JAFFA nucleic acid or amino acid sequence; comparing the JAFFA sequence with one or more preferably a plurality of sequences from a collection
15 of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze JAFFA.

The method can include evaluating the sequence identity between a JAFFA sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the internet.

In another aspect, the invention features, a set of oligonucleotides, useful, e.g., for
20 identifying SNP's, or identifying specific alleles of JAFFA. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation position, e.g., an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotides
25 which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

The sequence of a JAFFA molecules is provided in a variety of mediums to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or amino acid molecule, which contains a JAFFA. Such a manufacture can provide a
30 nucleotide or amino acid sequence, e.g., an open reading frame, in a form which allows examination of the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form.

A JAFFA nucleotide or amino acid sequence can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the
5 computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBI).

Thus, the invention features a method of making a computer readable record of a sequence of a JAFFA sequence which includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the
10 following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention features, a method of analyzing a sequence. The
15 method includes: providing a JAFFA sequence, or record, in computer readable form; comparing a second sequence to the JAFFA sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, e.g., determining if the JAFFA sequence includes a sequence being compared. In a preferred embodiment the JAFFA or second sequence is
20 stored on a first computer, e.g., at a first site and the comparison is performed, read, or recorded on a second computer, e.g., at a second site. E.g., the JAFFA or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of
25 a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent
30 applications cited throughout this application are incorporated herein by reference.

EXAMPLES

Example 1: Identification and Characterization of Human JAFFA cDNA and Genomic Sequence

In this example, the identification and characterization of the gene encoding human
5 JAFFA is described.

Isolation of the human JAFFA cDNA

The invention is based, at least in part, on the discovery of a human nucleic acid molecule encoding a novel JAFFA polypeptide.

10 Analysis of rare sequences from the Millennium database led to the identification of an est from a human prostate tumor library which coded for a novel hypothetical signal peptide. Further analysis led to the identification of JAFFA.

The nucleotide sequence encoding the human JAFFA protein is shown in Figure 1 and is set forth as SEQ ID NO:1. The full length protein encoded by this nucleic acid
15 comprises about 209 amino acids and has the amino acid sequence shown in Figure 1 and set forth as SEQ ID NO:2. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. Signal peptide algorithms predict that human JAFFA contains a signal peptide (about amino acids 1-28 of SEQ ID NO:2). The mature protein is approximately 181 amino acid residues in length (from about amino acid 28 to amino acid
20 209 of SEQ ID NO:2). The clone comprising the entire coding region of human JAFFA was deposited with the American Type Culture Collection (ATCC®), Rockville, Maryland, on _________, 1999, and assigned Accession No. _______, presently in Manassas, Virginia.

The genomic sequence containing the three exons encoding JAFFA protein is shown in Figure 6 and is set forth as SEQ ID NO:5. Four introns located at about nucleotides 1-
25 846, 1084-1537, 1640-2504, and 2793-4033 of SEQ ID NO:5, and three exons located at about 847-1083, 1538-1639, and 2505-2792 of SEQ ID NO:5 were identified. Nucleotides 1-168 correspond to exons 1 and 2 of the □(1,2)-fucosyltransferase (FUT1) gene (Figure 6).

Analysis of the JAFFA Molecules

30 A BLASTN 1.4.9MP-WashU search (Altschul et al. (1990) *J. Mol. Biol.* 215:403-10) of the nucleotide sequence of human JAFFA cDNA revealed a sequence identity a 100% between human JAFFA nucleotides 10-391 of SEQ ID NO:1 and nucleotides 2951-3332 of the H-type □(1,2)fucosyltransferase gene (AB006136); 99% identity between

human JAFFA nucleotides 491-784 of SEQ ID NO:1 and nucleotides 1240-1533 of the □(1,2)fucosyltransferase gene (AB006136); and 96% identity between human JAFFA nucleotides 382-495 of SEQ ID NO:1 and nucleotides 2393-2506 of the □(1,2)fucosyltransferase gene (AB006136). The human JAFFA gene is in reverse
5 orientation and in the complementary strand with respect to the □(1,2)fucosyltransferase gene. These results indicate that the genomic sequence encoding human JAFFA (SEQ ID NO:5) co-localizes with the H-type □(1,2)fucosyltransferase. (See Figure 6). The locus of the H-type □(1,2)fucosyltransferase gene has been mapped to 19q13.3 (Kelly, R.J. et al. (1994) *Proc. Natl. Acad. Sci.* USA 91:5843-5847).

10 A BLASTN 1.4.9MP-WashU search (Gish, W. et al. (1993) Nat. Genet. 3:266-272; Altschul et al. (1990) *J. Mol. Biol.* 215:403-10) of the amino acid sequence encoded by the human JAFFA cDNA revealed weak homology to FGF family members, human FGF-19, mouse FGF-15, and human FGF-6 (hst-2). For example, a 45% identity was identified between amino acids 61 to 151 of SEQ ID NO:2 (corresponding to translated nucleotides
15 335-604 of SEQ ID NO:1) of human JAFFA, and amino acids 60-149 of human FGF-19 (AB018122). A 34% identity was identified between amino acids 13 to 143 of SEQ ID NO:2 (corresponding to translated nucleotides 191-580 of SEQ ID NO:1) of human JAFFA, and amino acids 19-148 of human FGF-19 (AB018122). A 31% identity was identified between amino acids 60 to 120 of SEQ ID NO:2 (corresponding to translated nucleotides
20 332-511 of SEQ ID NO:1) of human JAFFA, and amino acids 87-146 of human FGF-6 (X63454).

A Hidden Markov Model ("HMM") search (HMMER 2.1) of the amino acid sequence of human JAFFA (SEQ ID NO:2) identified a fibroblast growth factor domain ((Accession No. PF00167) with a score of 52.6 (E-value 2.4e-14)), located at about amino
25 acids 60 to 140 of SEQ ID NO:2 (Figure 2). The results of the HMM search are set forth in Figure 3.

The JAFFA protein is also predicted to have two protein kinase C phosphorylation sites (PS00005), at about amino acid residues 98 to 100 and 122-124 of SEQ ID NO:2. The JAFFA protein is predicted to have two casein kinase II phosphorylation sites (PS00006), at
30 about amino acid residues 6 to 9 and 122 to 125 of SEQ ID NO:2. In addition, the JAFFA protein is predicted to have five N-myristoylation sites (PS00008), at about amino acid residues 12-17, 20-25, 67-72, 95-100, and 108-113 of SEQ ID NO:2.

Example 2: Tissue Distribution of JAFFA mRNA by Large-Scale Tissue-Specific Library Sequencing and by Northern Blot Hybridization

This Example describes the tissue distribution of JAFFA mRNA.

5 Northern blot hybridizations with various RNA samples was performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the JAFFA cDNA (SEQ ID NO:1) was used. The DNA was radioactively labeled with ^{32}P-dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse
10 hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) were probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

Low expression of JAFFA mRNA was detected in the fetal liver. Moderate expression of this message was also detected in the human cell line HeLa 53.

15

Example 3: Recombinant Expression of JAFFA in Bacterial Cells

In this example, JAFFA is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, JAFFA is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g.,
20 strain PEB199. Expression of the GST-JAFFA fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

25

Example 4: Expression of Recombinant JAFFA Protein in COS Cells

To express the JAFFA gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding
5 the entire JAFFA protein and an HA tag (Wilson et al. (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the JAFFA DNA sequence is amplified by PCR using two
10 primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the JAFFA coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the JAFFA coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested
15 with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the JAFFA_gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5□, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on
20 ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the JAFFA-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for
25 transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the JAFFA polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D.
30 *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS,

0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the JAFFA coding sequence is cloned directly into
5 the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the JAFFA polypeptide is detected by radiolabelling and immunoprecipitation using a JAFFA specific monoclonal antibody.

10 **Equivalents**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1, or a complement thereof;

(b) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:3, or a complement thereof.

2. An isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2, or a complement thereof.

3. An isolated nucleic acid molecule comprising the nucleotide sequence contained in the plasmid deposited with ATCC as Accession Number _______ .

4. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2, or a complement thereof.

5. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 196 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

6. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 under stringent conditions.

7. An isolated nucleic acid molecule comprising the nucleic acid molecule of
5 any one of claims 1, 2, 3, 4, or 5, and a nucleotide sequence encoding a heterologous polypeptide.

8. A vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.

10

9. The vector of claim 8, which is an expression vector.

10. A host cell transfected with the expression vector of claim 9.

15 11. A method of producing a polypeptide comprising culturing the host cell of claim 10 in an appropriate culture medium to, thereby, produce the polypeptide.

12. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of
20 SEQ ID NO:2, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule consisting of SEQ ID NO:1 or 3
25 under stringent conditions;

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1or 3; and

d) a polypeptide comprising an amino acid sequence which is at least
30 60% identical to the amino acid sequence of SEQ ID NO:2.

13. The isolated polypeptide of claim 12, comprising the amino acid sequence of SEQ ID NO:2.

14. The polypeptide of claim 12, further comprising heterologous amino acid sequences.

5 15. An antibody which selectively binds to a polypeptide of claim 12.

16. A method for detecting the presence of a polypeptide of claim 12 in a sample comprising:

a) contacting the sample with a compound which selectively binds to the
10 polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 12 in the sample.

17. The method of claim 16, wherein the compound which binds to the
15 polypeptide is an antibody.

18. A kit comprising a compound which selectively binds to a polypeptide of claim 12 and instructions for use.

20 19. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a
25 nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in the sample.

20. The method of claim 19, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

30

21. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 and instructions for use.

22. A method for identifying a compound which binds to a polypeptide of claim 12 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

5 b) determining whether the polypeptide binds to the test compound.

23. The method of claim 22, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide

10 binding;

b) detection of binding using a competition binding assay; and

c) detection of binding using an assay for JAFFA activity.

24. A method for modulating the activity of a polypeptide of claim 12

15 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

25. A method for identifying a compound which modulates the activity of a

20 polypeptide of claim 12 comprising:

a) contacting a polypeptide of claim 12 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

25

JAFFA

ACGCGTCCGAGCTGAGGATCCAGCCGAAAGAGGAGCCAGGCACTCAGGCCA
CCTGAGTCTACTCACCTGGACAACTGGAATCTGGCACCAATTCTAAACCACTC
AGCTTCTCCGAGCTCACACCCCGGAGATCACCTGAGGACCCGAGCCATTGAT
GGACTCGGACGAGACCGGGTTCGAGCACTCAGGGCTGTGGGTTTCTGTGCTG
GCTGGTCTTCTGCTGGGAGCCTGCCAGGCACACCCCATCCCTGACTCCAGTCC
TCTCCTGCAATTCGGGGGCCAAGTCCGGCAGCGGTACCTCTACACAGATGAT
GCCCAGCAGACAGAAGCCCACCTGGAGATCAGGGAGGATGGGACGGTGGGG
GGCGCTGCTGACCAGAGCCCCGAAAGTCTCCTGCAGCTGAAAGCCTTGAAGC
CGGGAGTTATTCAAATCTTGGGAGTCAAGACATCCAGGTTCCTGTGCCAGCG
GCCAGATGGGGCCCTGTATGGATCGCTCCACTTTGACCCTGAGGCCTGCAGC
TTCCGGGAGCTGCTTCTTGAGGACGGATACAATGTTTACCAGTCCGAAGCCC
ACGGCCTCCCGCTGCACCTGCCAGGGAACAAGTCCCCACACCGGGACCCTGC
ACCCCGAGGACCAGCTCGCTTCCTGCCACTACCAGGCCTGCCCCCGGCACCC
CCGGAGCCACCCGGAATCCTGGCCCCCCAGCCCCCCGATGTGGGCTCCTCGG
ACCCTCTGAGCATGGTGGGACCTTCCCAGGGCCGAAGCCCCAGCTACGCTTC
CTGA

Coding sequence
ATGGACTCGGACGAGACCGGGTTCGAGCACTCAGGGCTGTGGGTTTCTGTGC
TGGCTGGTCTTCTGCTGGGAGCCTGCCAGGCACACCCCATCCCTGACTCCAGT
CCTCTCCTGCAATTCGGGGGCCAAGTCCGGCAGCGGTACCTCTACACAGATG
ATGCCCAGCAGACAGAAGCCCACCTGGAGATCAGGGAGGATGGGACGGTGG
GGGGCGCTGCTGACCAGAGCCCCGAAAGTCTCCTGCAGCTGAAAGCCTTGAA
GCCGGGAGTTATTCAAATCTTGGGAGTCAAGACATCCAGGTTCCTGTGCCAG
CGGCCAGATGGGGCCCTGTATGGATCGCTCCACTTTGACCCTGAGGCCTGCA
GCTTCCGGGAGCTGCTTCTTGAGGACGGATACAATGTTTACCAGTCCGAAGC
CCACGGCCTCCCGCTGCACCTGCCAGGGAACAAGTCCCCACACCGGGACCCT
GCACCCCGAGGACCAGCTCGCTTCCTGCCACTACCAGGCCTGCCCCCGGCAC
CCCCGGAGCCACCCGGAATCCTGGCCCCCCAGCCCCCCGATGTGGGCTCCTC
GGACCCTCTGAGCATGGTGGGACCTTCCCAGGGCCGAAGCCCCAGCTACGCT
TCCTGA

A.A sequence
MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLYTDDA
QQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGA
LYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRG
PARFLPLPGLPPAPPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS.

FIGURE 1

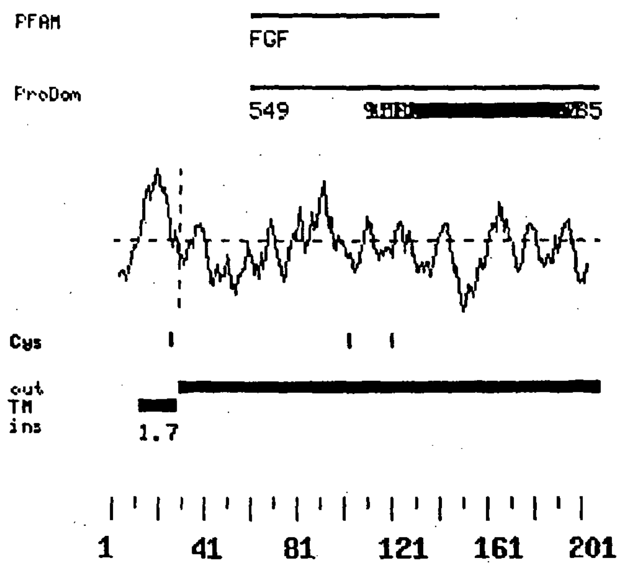

FIGURE 2

```
FGF: domain 1 of 1, from 60 to 140: score 52.6, E = 2.4e-14
                   *->HLqilPdGrVnGthEddnpysiLeisaverGiVsIrGveSglYLAMn
                      HL+i  dG+V G+++  +p s+L++ a   G++ I Gv + ++L+
      mfgf-2    60    HLEIREDGTVGGAAD-QSPESLLQLKALKPGVIQILGVKTSRFLCQR 105

                   kkGkLYASkkGltee.CvFrErleeNnYNTYaSaky<-*
                    +G LY+S + +  e C FrE+l+e +YN Y+S  +
      mfgf-2   106 PDGALYGSLH-FDPEaCSFRELLLEDGYNVYQSEAH    140

//
```

FIGURE 3

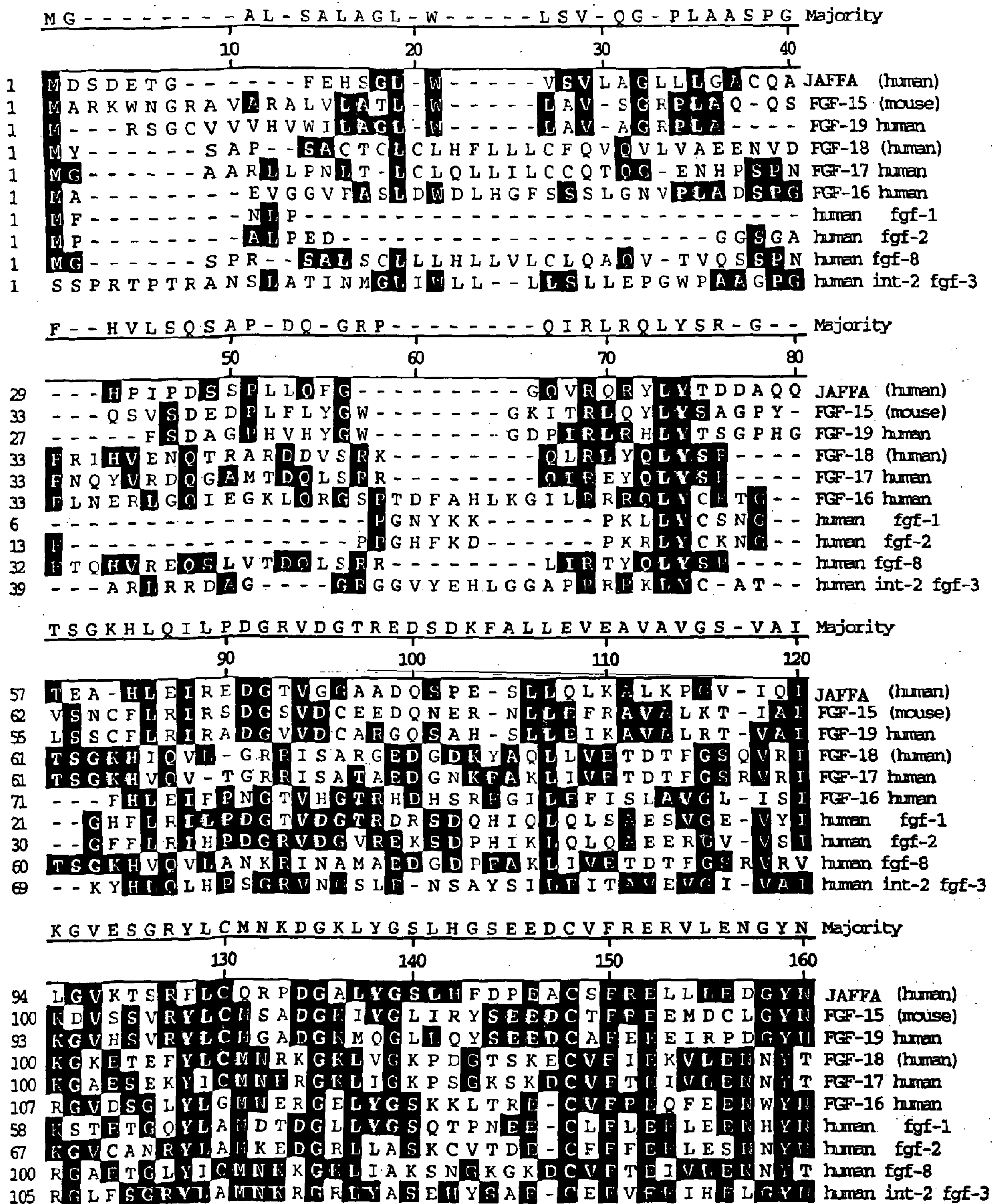

FIGURE 4

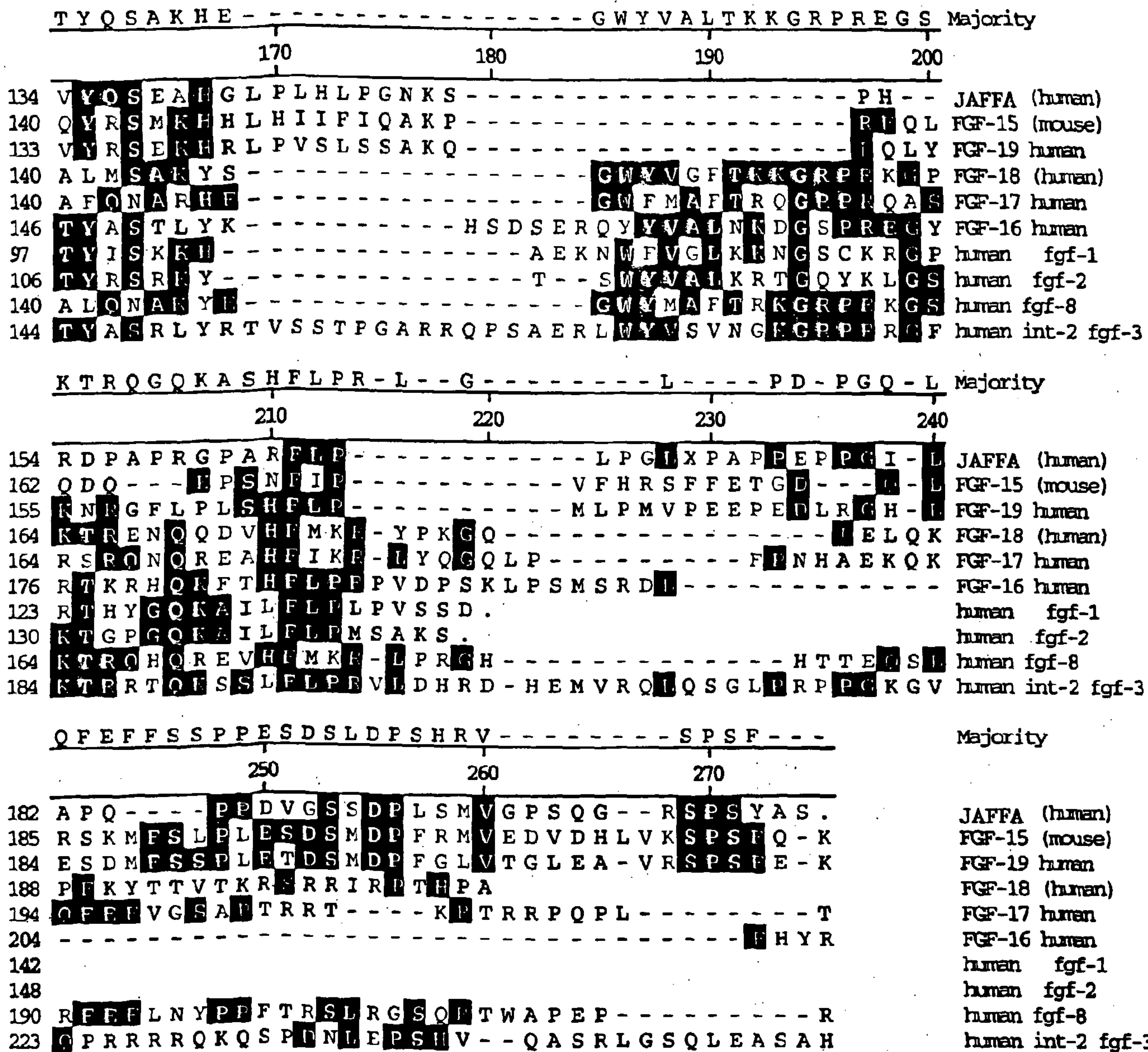

FIGURE 4 CONTINUED

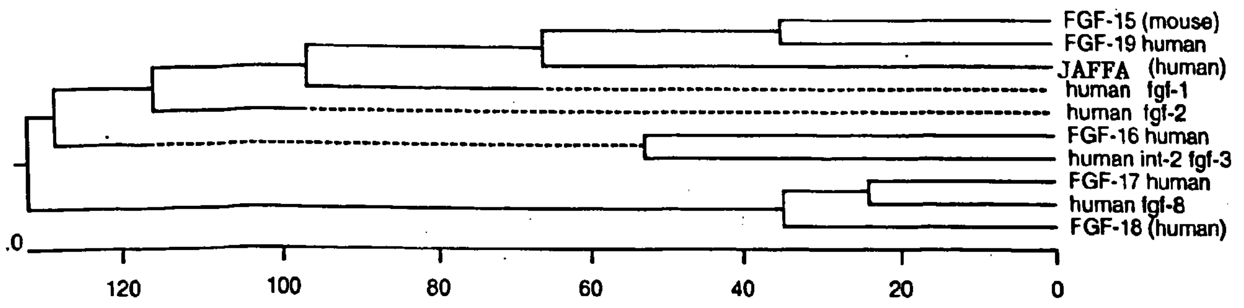

FIGURE 5

GENOMIC JAFFA

```
AGCCACCCCTCCCCCCAGGTTATTTCAGGAGCACCTGCCTGGGCCTGGGATGGC 5'
<   Exon 2 <
TTCTCTGGTGAAAGAAACACCAGGATTGCATCAGGGAGGAGGAGGCTGGGATGT
<   Exon 1 <  FUT1 (K562 Cells)
CCAGGGTCTGAGCATCTGAGCAGGGACAGATGAGGTTGAGGTTGGCCCACGGCC
AGGTGAGAGGCTTCCAAGGCAGGATACTTGTGTCTCAGATGCGGTCGCTTCTTT
CATACAGCAATTGCCGCCTTGCTGAGGATCAAGGAACCTCAGTGTCAGATCACG
CCCTCCCCCCAAACTTAGAAATTCAGATGGGGCGCAGAAATTTCTCTTGTTCTG
CGTGATCTGCATAGATGGTCCAAGAGGTGGTTTTTCCAGGAGCCCAGCACCCCT
CCTCCCTCCGACTCAGGTGCTTGAGACCCCAGATCCTTCTCTCTGAGACTCAGG
AATGTGGGCCCCCAGCCCCTTTCACCTGGGTCCCAGCTAACCCGATCCTCCCCT
CCCTCATCCCCTAGACCCAGGAGTCTGGCCCTCCATTGAAAGGACCCCAGGTTA
CATCATCCATTCAGGCTGCCCTTGCCACGATGGAATTCTGTAGCTCCTGCCAAA
TGGGTCAAATATCATGGTTCAGGCGCAGGGAGGGTGATTGGGCGGGCCTGTCTG
GGTATAAATTCTGGAGCTTCTGCATCTATCCCAAAAAACAAGGGTGTTCTGTCA
GCTGAGGATCCAGCCGAAAGAGGAGCCAGGCACTCAGGCCACCTGAGTCTACTC
ACCTGGACAACTGGAATCTGGCACCAATTCTAAACCACTCAGCTTCTCCGAGCT
CACACCCCGGAGATCACCTGAGGACCCGAGCCATTG███GACTCGGACGAGACC
                                     M  D  S  D  E  T
GGGTTCGAGCACTCAGGGCTGTGGGTTTCTGTGCTGGCTGGTCTTCTGCTGGGA
 G  F  E  H  S  G  L  W  V  S  V  L  A  G  L  L  L  G
GCCTGCCAGGCACACCCCATCCCTGACTCCAGTCCTCTCCTGCAATTCGGGGGC
 A  C  Q  A  H  P  I  P  D  S  S  P  L  L  Q  F  G  G
CAAGTCCGGCAGCGGTACCTCTACACAGATGATGCCCAGCAGACAGAAGCCCAC
 Q  V  R  Q  R  Y  L  Y  T  D  D  A  Q  Q  T  E  A  H
CTGGAGATCAGGGAGGATGGGACGGTGGGGGGCGCTGCTGACCAGAGCCCCGAA
 L  E  I  R  E  D  G  T  V  G  G  A  A  D  Q  S  P  E
AGTGAGTGTGGGCCAGAGCCTGGGTCTGAGGGAGGAGGGGCTGTGGGTCTGGAT
 S
TCCTGGGTCTGAGGGAGGAGGGGCTGGGGGCCTTGGCCCCTGGGTCTGAGGGAG
GAGGGGCTGGGGATCTGGACTCCTGGGTCTGAGGGAGGAGGGGCTGGGGATCTG
GGCCCCTGGGTCTGAGGGAGGAGGGGCTGGGTCTGGACCCCTGGGTCTGAGGGA
GGAGGGGCTGGGGGTCTGGACTCTTGGGTTTGAAGGAGGAAGGGCTGGGGTCCT
GGACTCTTGGGTCTGAGTTGGGAGGGGGCTTTGGCTTGGGCTTCTCCTGGGTCT
GAGGGAGGAGGTAGGCTGTGGGCTTGGACTCCCAGGGCTGGGACAGAGCCGGAT
GGTGGGACAGAGTCGGGTGGTGGGACAGTCCCGGGTGGGAGAGGTCCTCGAACC
ACCTTATCGCTTTCACCCCTTAGGTCTCCTGCAGCTGAAAGCCTTGAAGCCGGG
                              L  L  Q  L  K  A  L  K  P  G
AGTTATTCAAATCTTGGGAGTCAAGACATCCAGGTTCCTGTGCCAGCGGCCAGA
 V  I  Q  I  L  G  V  K  T  S  R  F  L  C  Q  R  P  D
```

EXON 1

EXON 2

FIGURE 6

TGGGGCCCTGTATGGATCGGTGAGTTTCCAGGACCCTCCTCACCACCCACCATG
G A L Y G S
CTCCTCCTATATGTCGCCCTCACAGCCTGGGGTGCCTTGTCTTGCTCATCCCCC
CCGGAGCCAGACTTGATTCTATTTGCTCTGCACGCCCCCAGCTGCAACATTTGG
AGGTTGAAGTTGTCATCAGTGTTTGCAAGATGAGGAAACTGAGGCCCAGGCCGG
GGCGCCAGTGACCTCAATCATGTGATGTGGATGCTGGAGCGGCCTGAGGCTC
AGGTTATTGGGAGTCTCGTGATTCAGTAACCCCTGCTCCTGCCCACACGGCCCC
TGTGTGCACGGCTCATGCTGGGCACAGGGACACTCGGGGAAGCCATGGCCAGTA
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CTGATGGCTCCGAGGGCTGGAATGTTCTCTGTGAAGTCTGAACTGGGAGGCAGG
TCCCTGCAGGAGAGCCCTGGGGTAAAAAACAAAACCTGCCTTGCTGTTTTGTTT
CCTAGAGGAGGGGCTGGGGGCCTGGACTCCTGGGTCTGAGGGAGGAGGGGCTGG
GGGCCTGGACCCCTGGGTCTGAGGGAGGAGGGGCTGGGGCCTGGAACCCCGGGT
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TGGGGCCTGGATCCTGGGTCTTACATCAGGAAAACAGAGGAACCCTGTCTCTGA
TCCTGTTTTTGTCCCCTAGCTCCACTTTGACCCTGAGGCCTGCAGCTTCCGGGA
L H F D P E A C S F R E
GCTGCTTCTTGAGGACGGATACAATGTTTACCAGTCCGAAGCCCACGGCCTCCC
L L L E D G Y N V Y Q S E A H G L P
GCTGCACCTGCCAGGGAACAAGTCCCCACACCGGGACCCTGCACCCCGAGGACC
L H L P G N K S P H R D P A P R G P
AGCTCGCTTCCTGCCACTACCAGGCCTGCCCCCGGCACCCCCGGAGCCACCCGG
A R F L P L P G L P P A P P E P P G
AATCCTGGCCCCCCAGCCCCCCGATGTGGGCTCCTCGGACCCTCTGAGCATGGT
I L A P Q P P D V G S S D P L S M V
GGGACCTTCCCAGGGCCGAAGCCCCAGCTACGCTTCC███AGCCAGAGGCTGTT
G P S Q G R S P S Y A S .
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CCCCCTCTACACCTCCCCTCACGTGGTCCGAGGGTCCTGGCTTCCCACTGGGCC
TCACTTTTTCTTTTCTTTTCTTTTTTTTTTTGAGACGGAGTCTCGCTCTGTGA
CCCAGGCTGGAGTGCAGTGGCGCGATCTTGGCTCACTCCAACCTCCGCCTCCCA
GGTTCAAGCAATTCTCCTGCCTCAGCCACCCGAGTAGCTGTGATTACAGGCGTG
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CAAAGTGCTGGGATTACAGGCTTGAGTCACTGTGCCCAGCCCAGCCTCACTTTT
CTACTCTGCTAAAGTGTCCCCAGGGACTGTGGACTATCCCTGCTCTCTGAAAGG
ACAAGACTGGCCGGGAGTGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGA

Exon 3

FIGURE 6 CONTINUED

CCCAGGCGGGCAAATCACCTGAGGTCAGGAGTTCGAGACCAGCCTGGCCAACAT
GGTGGAACCCCATCTCTACCAAAAATACAAAAATTAGCCAGGTGTGGTAGCACATGCCTGT
AATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCGCTTGAATCCC
GGAGGTGGAGGTTGCAGTGAGCTGAGATCGAGCCATTGCACTCCAGCCTGGGCG
ACAGAGCAAGACTCAAAAAGTCTCAAAATAAATAAATAAGTAAACAAATAAATA
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GAGGCAGGTGGATTGCCTGAGGTCAGGAGTTAGAGCAGCCTGGCCAACACAGTG
AAACCCCATCTCTACTAAAAATACAAAAACTAGCCAGACGTGGTGGCGGGCGTC
TGTTGTCCCAGCTACTCGGGGGGGGCTGAGGCAGAATCACTTGAACCTGGGAG
GTGGAGGTTGCAGGGAGCCGAGATCGTGCCACTGTCCTCCAGCCTGAGTAGTAC
AGCAAGACTCTGTCTCAAAAAAATAATAAGTTAATTT

FIGURE 6 CONTINUED

6FAM-acacagatgatgcccagcagacagaagcc-TAMRA

Top
5'CACCCCATCCCTGACTCCAGT-3'
Bottom
5'CCGTCCCATCCTCCCTGATC-3'

FIGURE 7

SEQUENCE LISTING

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<120> NOVEL LEUCINE RICH REPEAT-CONTAINING MOLECULES AND USES THEREFOR

<130> 10448-008WO1

<150> US 09/444,165
<151> 1999-11-22

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Ser Ile Thr Arg Gly Met Gly Lys Leu Leu Val Val Arg Cys Gly Thr
        10                  15                  20

gag aag gct gga cca gca gtt cca ggc ggc atg gag ggg ccc cgg agc      149
Glu Lys Ala Gly Pro Ala Val Pro Gly Gly Met Glu Gly Pro Arg Ser
    25                  30                  35

tcc acc cat gtc ccc ttg gtg ctg ccg ctt ctt gta ctt ctg ctg ctg      197
Ser Thr His Val Pro Leu Val Leu Pro Leu Leu Val Leu Leu Leu Leu
 40                  45                  50                  55

gcc ccg gct agg cag gcc gcc gcc cag cgc tgc cca cag gcc tgc atc      245
Ala Pro Ala Arg Gln Ala Ala Ala Gln Arg Cys Pro Gln Ala Cys Ile
                 60                  65                  70

tgt gac aac tcc agg cga cac gtt gcc tgc cgg tac cag aac ctc act      293
Cys Asp Asn Ser Arg Arg His Val Ala Cys Arg Tyr Gln Asn Leu Thr
             75                  80                  85

gag gtg cca gac gcc atc cct gag ctg acc cag cgg ctg gac ctg cag      341
Glu Val Pro Asp Ala Ile Pro Glu Leu Thr Gln Arg Leu Asp Leu Gln
         90                  95                 100

ggc aat ttg ctg aag gtg atc ccc gca gcc gcc ttc cag ggc gtg cct      389
Gly Asn Leu Leu Lys Val Ile Pro Ala Ala Ala Phe Gln Gly Val Pro
    105                 110                 115

cac ctc aca cac ctg gac ctg cgc cac tgc gag gtg gag ctg gtg gcc      437
His Leu Thr His Leu Asp Leu Arg His Cys Glu Val Glu Leu Val Ala
120                 125                 130                 135
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gag ggc gcc ttc cgt ggc ctg ggc cgc ctg ctc ctg ctc aac ctg gcc      485
Glu Gly Ala Phe Arg Gly Leu Gly Arg Leu Leu Leu Leu Asn Leu Ala
                140                 145                 150

tcc aac cac ctg cgt gag ctg ccc cag gag gcg ctg gac ggg ctg ggc      533
Ser Asn His Leu Arg Glu Leu Pro Gln Glu Ala Leu Asp Gly Leu Gly
            155                 160                 165

tcg ttg cgg cgg ctg gag ctg gag ggg aac gca ctg gag gag ctg cgg      581
Ser Leu Arg Arg Leu Glu Leu Glu Gly Asn Ala Leu Glu Glu Leu Arg
        170                 175                 180

ccg ggg acg ttc ggg gca ctg ggt gcg ctg gcc acg cta aac ctg gcc      629
Pro Gly Thr Phe Gly Ala Leu Gly Ala Leu Ala Thr Leu Asn Leu Ala
    185                 190                 195

cac aac gcc ctg gtt tac ctg ccc gcc atg gcc ttc cag ggg cta ctg      677
His Asn Ala Leu Val Tyr Leu Pro Ala Met Ala Phe Gln Gly Leu Leu
200                 205                 210                 215

cgc gtc cgc tgg ctg cgg ctg tcg cac aac gcg ctc agc gtg ctg gcc      725
Arg Val Arg Trp Leu Arg Leu Ser His Asn Ala Leu Ser Val Leu Ala
                220                 225                 230

ccc gag gcc ctg gct ggc ctg ccc gcc ctg aga cgg ctc agc cta cac      773
Pro Glu Ala Leu Ala Gly Leu Pro Ala Leu Arg Arg Leu Ser Leu His
            235                 240                 245

cac aac gag ctc cag gct ctg ccc ggg cct gtc ttg tcc cag gcc cgc      821
His Asn Glu Leu Gln Ala Leu Pro Gly Pro Val Leu Ser Gln Ala Arg
        250                 255                 260

ggc ctg gcc cgt ctg gag ctg ggc cac aac ccg ctc acc tac gcg ggc      869
Gly Leu Ala Arg Leu Glu Leu Gly His Asn Pro Leu Thr Tyr Ala Gly
    265                 270                 275

gag gag gac ggg ctg gcg ctg ccc ggc ctg cgg gag ctg ctg ctg gac      917
Glu Glu Asp Gly Leu Ala Leu Pro Gly Leu Arg Glu Leu Leu Leu Asp
280                 285                 290                 295

ggc ggg gcc ctg cag gcc ctg ggt ccc agg gcc ttc gca cac tgt ccg      965
Gly Gly Ala Leu Gln Ala Leu Gly Pro Arg Ala Phe Ala His Cys Pro
                300                 305                 310

cgc ctg cac acc ctc gac ctc cgc ggg aac cag cta gac acc ctg ccc     1013
Arg Leu His Thr Leu Asp Leu Arg Gly Asn Gln Leu Asp Thr Leu Pro
            315                 320                 325

ccg ctg cag ggc ccg ggc cag ctg cgc cgg ctg cgg ctg cag gga atc     1061
Pro Leu Gln Gly Pro Gly Gln Leu Arg Arg Leu Arg Leu Gln Gly Ile
        330                 335                 340

cgc tgt ggt gcg gct gcc agg cgc ggc cct act cga gtg gct ggc gcg     1109
Arg Cys Gly Ala Ala Ala Arg Arg Gly Pro Thr Arg Val Ala Gly Ala
    345                 350                 355

ggc gcg cgt gcg ctc gga cgg cgc gtg cca ggg ccg cgg cgc ctg cgg     1157
Gly Ala Arg Ala Leu Gly Arg Arg Val Pro Gly Pro Arg Arg Leu Arg
360                 365                 370                 375

ggc gag gct ctg gac gcc ctg cgg ccc tgg gac ctg cgc tgc cct ggg     1205
Gly Glu Ala Leu Asp Ala Leu Arg Pro Trp Asp Leu Arg Cys Pro Gly
                380                 385                 390

gac gcg gcg cag gaa gag gaa gag ctg gaa gag cgg gct gtg gcc ggg     1253
Asp Ala Ala Gln Glu Glu Glu Glu Leu Glu Glu Arg Ala Val Ala Gly
            395                 400                 405
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ccc cgc gcc cct ccg cgc ggc cct ccg cgc ggc ccc ggg gag gag cgg      1301
Pro Arg Ala Pro Pro Arg Gly Pro Pro Arg Gly Pro Gly Glu Glu Arg
        410                 415                 420

gca gtc gcg cct tgc cct cgc gcc tgc gtg tgc gtc ccc gag tcc cgg      1349
Ala Val Ala Pro Cys Pro Arg Ala Cys Val Cys Val Pro Glu Ser Arg
    425                 430                 435

cac agc agc tgc gag ggc tgc ggc ctg cag gcg gtg ccc cgc ggc ttc      1397
His Ser Ser Cys Glu Gly Cys Gly Leu Gln Ala Val Pro Arg Gly Phe
440                 445                 450                 455

ccc agc gac acc cag ctc ctg gac ctg agg cgg aac cac ttc ccc tcg      1445
Pro Ser Asp Thr Gln Leu Leu Asp Leu Arg Arg Asn His Phe Pro Ser
                460                 465                 470

gtg ccc cga gcg gcc ttc ccc ggc ctg ggc cac ctg gtg tcg ctg cac      1493
Val Pro Arg Ala Ala Phe Pro Gly Leu Gly His Leu Val Ser Leu His
            475                 480                 485

ctg cag cac tgc ggc atc gcg gag ctg gaa gcg ggc gcc ctg gcc ggg      1541
Leu Gln His Cys Gly Ile Ala Glu Leu Glu Ala Gly Ala Leu Ala Gly
        490                 495                 500

ctg ggc cgc ctg atc tac ctg tac ctc tcc gac aac cag ctc gca ggc      1589
Leu Gly Arg Leu Ile Tyr Leu Tyr Leu Ser Asp Asn Gln Leu Ala Gly
    505                 510                 515

ctc agc gct gct gcc ctt gca ggg gtc ccc cgc ctc ggc tac ctg tac      1637
Leu Ser Ala Ala Ala Leu Ala Gly Val Pro Arg Leu Gly Tyr Leu Tyr
520                 525                 530                 535

cta gaa cgc aac cgt ttc ctg cag gtg cca ggg gct gcc ctg cgc gcc      1685
Leu Glu Arg Asn Arg Phe Leu Gln Val Pro Gly Ala Ala Leu Arg Ala
                540                 545                 550

ctg ccc agc ctc ttc tcc ctg cac ctg cag gac aac gct gtg gac cgc      1733
Leu Pro Ser Leu Phe Ser Leu His Leu Gln Asp Asn Ala Val Asp Arg
            555                 560                 565

ctg gca cct ggg gac ctg ggg aga aca cgg gcc ttg cgc tgg gtc tac      1781
Leu Ala Pro Gly Asp Leu Gly Arg Thr Arg Ala Leu Arg Trp Val Tyr
        570                 575                 580

ctg agt gga aac cgc atc acc gaa gtg tcc ctt ggg gcg ctg ggc cca      1829
Leu Ser Gly Asn Arg Ile Thr Glu Val Ser Leu Gly Ala Leu Gly Pro
    585                 590                 595

gct cgg gag ctg gag aag ctg cac ctg gac agg aat cag ctg cga gag      1877
Ala Arg Glu Leu Glu Lys Leu His Leu Asp Arg Asn Gln Leu Arg Glu
600                 605                 610                 615

gtg ccc act ggg gcc ttg gag ggg ctg cct gcc ctc ctg gag ctg cag      1925
Val Pro Thr Gly Ala Leu Glu Gly Leu Pro Ala Leu Leu Glu Leu Gln
                620                 625                 630

ctc tcg ggc aac cca ctc agg gcc ttg cgt gac gga gcc ttc cag cct      1973
Leu Ser Gly Asn Pro Leu Arg Ala Leu Arg Asp Gly Ala Phe Gln Pro
            635                 640                 645

gtg ggc agg tcg ctg cag cac ctc ttc ctg aac agc agt ggc ctg gag      2021
Val Gly Arg Ser Leu Gln His Leu Phe Leu Asn Ser Ser Gly Leu Glu
        650                 655                 660

cag att tgt cct ggg gcc ttt tca ggc ctg ggc ccc ggg ctc cag agc      2069
Gln Ile Cys Pro Gly Ala Phe Ser Gly Leu Gly Pro Gly Leu Gln Ser
    665                 670                 675
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ctg cac ctg cag aag aac cag ctt cgg gcc ctg cct gcc ctg ccc agt      2117
Leu His Leu Gln Lys Asn Gln Leu Arg Ala Leu Pro Ala Leu Pro Ser
680                 685                 690                 695

ctc agc cag ctg gag ctc atc gac ctc agc agc aat ccc ttc cac tgt      2165
Leu Ser Gln Leu Glu Leu Ile Asp Leu Ser Ser Asn Pro Phe His Cys
                700                 705                 710

gac tgc cag ctg ctt ccg ctg cac agg tgg ctt act ggg ctg aac ctg      2213
Asp Cys Gln Leu Leu Pro Leu His Arg Trp Leu Thr Gly Leu Asn Leu
            715                 720                 725

cgg gtg ggg gcc acc tgc gcc acc cct ccc aat gcc cgt ggc cag agg      2261
Arg Val Gly Ala Thr Cys Ala Thr Pro Pro Asn Ala Arg Gly Gln Arg
        730                 735                 740

gtg aag gct gca gct gct gtc ttt gaa gac tgc ccg ggc tgg gct gcc      2309
Val Lys Ala Ala Ala Ala Val Phe Glu Asp Cys Pro Gly Trp Ala Ala
    745                 750                 755

aga aag gcc aag cgg aca cca gcc tcc agg ccc agt gcc agg aga acc      2357
Arg Lys Ala Lys Arg Thr Pro Ala Ser Arg Pro Ser Ala Arg Arg Thr
760                 765                 770                 775

ccc atc aaa gga aga cag tgt gga gca gat aag gtg ggg aag gag aag      2405
Pro Ile Lys Gly Arg Gln Cys Gly Ala Asp Lys Val Gly Lys Glu Lys
                780                 785                 790

ggt tgt ctc tgagcacgga gcaggctggt cctgccttga cccagcccgg                2454
Gly Cys Leu


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            20                  25                  30
Gly Met Glu Gly Pro Arg Ser Ser Thr His Val Pro Leu Val Leu Pro
        35                  40                  45
Leu Leu Val Leu Leu Leu Leu Ala Pro Ala Arg Gln Ala Ala Ala Gln
    50                  55                  60
Arg Cys Pro Gln Ala Cys Ile Cys Asp Asn Ser Arg Arg His Val Ala
65                  70                  75                  80
Cys Arg Tyr Gln Asn Leu Thr Glu Val Pro Asp Ala Ile Pro Glu Leu
                85                  90                  95
Thr Gln Arg Leu Asp Leu Gln Gly Asn Leu Leu Lys Val Ile Pro Ala
            100                 105                 110
Ala Ala Phe Gln Gly Val Pro His Leu Thr His Leu Asp Leu Arg His
        115                 120                 125
Cys Glu Val Glu Leu Val Ala Glu Gly Ala Phe Arg Gly Leu Gly Arg
    130                 135                 140
Leu Leu Leu Leu Asn Leu Ala Ser Asn His Leu Arg Glu Leu Pro Gln
145                 150                 155                 160
Glu Ala Leu Asp Gly Leu Gly Ser Leu Arg Arg Leu Glu Leu Glu Gly
                165                 170                 175
Asn Ala Leu Glu Glu Leu Arg Pro Gly Thr Phe Gly Ala Leu Gly Ala
            180                 185                 190
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Leu Ala Thr Leu Asn Leu Ala His Asn Ala Leu Val Tyr Leu Pro Ala
195 200 205
Met Ala Phe Gln Gly Leu Leu Arg Val Arg Trp Leu Arg Leu Ser His
210 215 220
Asn Ala Leu Ser Val Leu Ala Pro Glu Ala Leu Ala Gly Leu Pro Ala
225 230 235 240
Leu Arg Arg Leu Ser Leu His His Asn Glu Leu Gln Ala Leu Pro Gly
245 250 255
Pro Val Leu Ser Gln Ala Arg Gly Leu Ala Arg Leu Glu Leu Gly His
260 265 270
Asn Pro Leu Thr Tyr Ala Gly Glu Glu Asp Gly Leu Ala Leu Pro Gly
275 280 285
Leu Arg Glu Leu Leu Leu Asp Gly Gly Ala Leu Gln Ala Leu Gly Pro
290 295 300
Arg Ala Phe Ala His Cys Pro Arg Leu His Thr Leu Asp Leu Arg Gly
305 310 315 320
Asn Gln Leu Asp Thr Leu Pro Pro Leu Gln Gly Pro Gly Gln Leu Arg
325 330 335
Arg Leu Arg Leu Gln Gly Ile Arg Cys Gly Ala Ala Ala Arg Arg Gly
340 345 350
Pro Thr Arg Val Ala Gly Ala Gly Ala Arg Ala Leu Gly Arg Arg Val
355 360 365
Pro Gly Pro Arg Arg Leu Arg Gly Glu Ala Leu Asp Ala Leu Arg Pro
370 375 380
Trp Asp Leu Arg Cys Pro Gly Asp Ala Ala Gln Glu Glu Glu Glu Leu
385 390 395 400
Glu Glu Arg Ala Val Ala Gly Pro Arg Ala Pro Pro Arg Gly Pro Pro
405 410 415
Arg Gly Pro Gly Glu Glu Arg Ala Val Ala Pro Cys Pro Arg Ala Cys
420 425 430
Val Cys Val Pro Glu Ser Arg His Ser Ser Cys Glu Gly Cys Gly Leu
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Gln Ala Val Pro Arg Gly Phe Pro Ser Asp Thr Gln Leu Leu Asp Leu
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Arg Arg Asn His Phe Pro Ser Val Pro Arg Ala Ala Phe Pro Gly Leu
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Ser Asp Asn Gln Leu Ala Gly Leu Ser Ala Ala Ala Leu Ala Gly Val
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Pro Arg Leu Gly Tyr Leu Tyr Leu Glu Arg Asn Arg Phe Leu Gln Val
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Pro Gly Ala Ala Leu Arg Ala Leu Pro Ser Leu Phe Ser Leu His Leu
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Gln Asp Asn Ala Val Asp Arg Leu Ala Pro Gly Asp Leu Gly Arg Thr
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Arg Ala Leu Arg Trp Val Tyr Leu Ser Gly Asn Arg Ile Thr Glu Val
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Ser Leu Gly Ala Leu Gly Pro Ala Arg Glu Leu Glu Lys Leu His Leu
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Leu Asn Ser Ser Gly Leu Glu Gln Ile Cys Pro Gly Ala Phe Ser Gly
660 665 670
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675 680 685
Ala Leu Pro Ala Leu Pro Ser Leu Ser Gln Leu Glu Leu Ile Asp Leu
690 695 700
Ser Ser Asn Pro Phe His Cys Asp Cys Gln Leu Leu Pro Leu His Arg
705 710 715 720

Trp Leu Thr Gly Leu Asn Leu Arg Val Gly Ala Thr Cys Ala Thr Pro
725 730 735

Pro Asn Ala Arg Gly Gln Arg Val Lys Ala Ala Ala Ala Val Phe Glu
740 745 750

Asp Cys Pro Gly Trp Ala Ala Arg Lys Ala Lys Arg Thr Pro Ala Ser
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Asp Lys Val Gly Lys Glu Lys Gly Cys Leu
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<213> Artificial Sequence

<220>
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            20                  25                  30
Ala Ser Pro Glu Ser Leu Arg Gly Gly Pro Leu Leu Glu Leu Leu Pro
        35                  40                  45
Ser Asp Phe Ser Cys Pro
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<213> Homo sapiens

<400> 8
Leu Thr Gln Arg Leu Asp Leu Gln Gly Asn Leu Leu Lys Val Ile Pro
 1               5                  10                  15
Ala Ala Ala Phe Gln Gly Val Pro
            20


<210> 9
<211> 24
<212> PRT
<213> Homo sapiens
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<400> 9
His Leu Thr His Leu Asp Leu Arg His Cys Glu Val Glu Leu Val Ala
  1               5                  10                  15
Glu Gly Ala Phe Arg Gly Leu Gly
             20

<210> 10
<211> 24
<212> PRT
<213> Homo sapiens

<400> 10
Arg Leu Leu Leu Leu Asn Leu Ala Ser Asn His Leu Arg Glu Leu Pro
  1               5                  10                  15
Gln Glu Ala Leu Asp Gly Leu Gly
             20

<210> 11
<211> 24
<212> PRT
<213> Homo sapiens

<400> 11
Ser Leu Arg Arg Leu Glu Leu Glu Gly Asn Ala Leu Glu Glu Leu Arg
  1               5                  10                  15
Pro Gly Thr Phe Gly Ala Leu Gly
             20

<210> 12
<211> 24
<212> PRT
<213> Homo sapiens

<400> 12
Ala Leu Ala Thr Leu Asn Leu Ala His Asn Ala Leu Val Tyr Leu Pro
  1               5                  10                  15
Ala Met Ala Phe Gln Gly Leu Leu
             20

<210> 13
<211> 24
<212> PRT
<213> Homo sapiens

<400> 13
Arg Val Arg Trp Leu Arg Leu Ser His Asn Ala Leu Ser Val Leu Ala
  1               5                  10                  15
Pro Glu Ala Leu Ala Gly Leu Pro
             20

<210> 14
<211> 24
<212> PRT
<213> Homo sapiens

<400> 14
Ala Leu Arg Arg Leu Ser Leu His His Asn Glu Leu Gln Ala Leu Pro
  1               5                  10                  15
Gly Pro Val Leu Ser Gln Ala Arg
             20


<210> 15
<211> 24
<212> PRT
<213> Homo sapiens
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<400> 15
Gly Leu Ala Arg Leu Glu Leu Gly His Asn Pro Leu Thr Tyr Ala Gly
 1               5                  10                  15
Glu Glu Asp Gly Leu Ala Leu Pro
            20

<210> 16
<211> 24
<212> PRT
<213> Homo sapiens

<400> 16
Gly Leu Arg Glu Leu Leu Leu Asp Gly Gly Ala Leu Gln Ala Leu Gly
 1               5                  10                  15
Pro Arg Ala Phe Ala His Cys Pro
            20

<210> 17
<211> 22
<212> PRT
<213> Homo sapiens

<400> 17
Arg Leu His Thr Leu Asp Leu Arg Gly Asn Gln Leu Asp Thr Leu Pro
 1               5                  10                  15
Pro Leu Gln Gly Pro Gly
            20

<210> 18
<211> 30
<212> PRT
<213> Homo sapiens

<400> 18
Pro Cys Pro Arg Ala Cys Val Cys Val Pro Glu Ser Arg His Ser Ser
 1               5                  10                  15
Cys Glu Gly Cys Gly Leu Gln Ala Val Pro Arg Gly Phe Pro
            20                  25                  30

<210> 19
<211> 24
<212> PRT
<213> Homo sapiens

<400> 19
Asp Thr Gln Leu Leu Asp Leu Arg Arg Asn His Phe Pro Ser Val Pro
 1               5                  10                  15
Arg Ala Ala Phe Pro Gly Leu Gly
            20

<210> 20
<211> 24
<212> PRT
<213> Homo sapiens

<400> 20
His Leu Val Ser Leu His Leu Gln His Cys Gly Ile Ala Glu Leu Glu
 1               5                  10                  15
Ala Gly Ala Leu Ala Gly Leu Gly
            20

<210> 21
<211> 24
<212> PRT
<213> Homo sapiens
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<400> 21
Arg Leu Ile Tyr Leu Tyr Leu Ser Asp Asn Gln Leu Ala Gly Leu Ser
1 5 10 15
Ala Ala Ala Leu Ala Gly Val Pro
20

<210> 22
<211> 24
<212> PRT
<213> Homo sapiens

<400> 22
Arg Leu Gly Tyr Leu Tyr Leu Glu Arg Asn Arg Phe Leu Gln Val Pro
1 5 10 15
Gly Ala Ala Leu Arg Ala Leu Pro
20

<210> 23
<211> 24
<212> PRT
<213> Homo sapiens

<400> 23
Ser Leu Phe Ser Leu His Leu Gln Asp Asn Ala Val Asp Arg Leu Ala
1 5 10 15
Pro Gly Asp Leu Gly Arg Thr Arg
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<210> 24
<211> 24
<212> PRT
<213> Homo sapiens

<400> 24
Ala Leu Arg Trp Val Tyr Leu Ser Gly Asn Arg Ile Thr Glu Val Ser
1 5 10 15
Leu Gly Ala Leu Gly Pro Ala Arg
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<210> 25
<211> 24
<212> PRT
<213> Homo sapiens

<400> 25
Glu Leu Glu Lys Leu His Leu Asp Arg Asn Gln Leu Arg Glu Val Pro
1 5 10 15
Thr Gly Ala Leu Glu Gly Leu Pro
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<210> 26
<211> 24
<212> PRT
<213> Homo sapiens

<400> 26
Ala Leu Leu Glu Leu Gln Leu Ser Gly Asn Pro Leu Arg Ala Leu Arg
1 5 10 15
Asp Gly Ala Phe Gln Pro Val Gly
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<210> 27
<211> 24
<212> PRT
<213> Homo sapiens

<400> 27
Ser Leu Gln His Leu Phe Leu Asn Ser Ser Gly Leu Glu Gln Ile Cys
1 5 10 15
Pro Gly Ala Phe Ser Gly Leu Gly
20

<210> 28
<211> 22
<212> PRT
<213> Homo sapiens

<400> 28
Gly Leu Gln Ser Leu His Leu Gln Lys Asn Gln Leu Arg Ala Leu Pro
1 5 10 15
Ala Leu Pro Ser Leu Ser
20

<210> 29
<211> 22
<212> PRT
<213> Homo sapiens

<400> 29
Gln Leu Glu Leu Ile Asp Leu Ser Ser Asn Pro Phe His Cys Asp Cys
1 5 10 15
Gln Leu Leu Pro Leu His
20

<210> 30
<211> 49
<212> PRT
<213> Homo sapiens

<400> 30
Asn Pro Phe His Cys Asp Cys Gln Leu Leu Pro Leu His Arg Trp Leu
1 5 10 15
Thr Gly Leu Asn Leu Arg Val Gly Ala Thr Cys Ala Thr Pro Pro Asn
20 25 30
Ala Arg Gly Gln Arg Val Lys Ala Ala Ala Ala Val Phe Glu Asp Cys
35 40 45
Pro