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(54) Titre : NOUVEAUX VARIANTS D'EPISSAGE DU GENE DKKL1 HUMAIN
(54) Title: NOVEL SPLICE VARIANTS OF HUMAN DKKL1

(57) **Abrégé/Abstract:**

The present invention relates to novel sequences for use in detection, diagnosis and treatment of diseases, including cancer. The invention provides novel splice forms of human DKKL 1 gene. The present invention provides methods of using polynucleotides having the novel splice variants of the human DKKL 1 sequences, their corresponding gene products and antibodies specific for the gene products in the detection, diagnosis, prevention and/or treatment of associated cancers.



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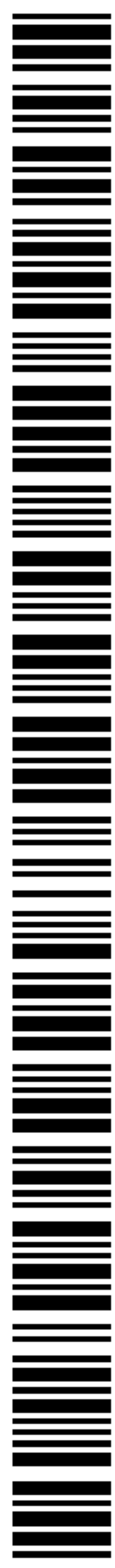
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(54) Title: NOVEL SPLICE VARIANTS OF HUMAN DKKL1

(57) Abstract: The present invention relates to novel sequences for use in detection, diagnosis and treatment of diseases, including cancer. The invention provides novel splice forms of human DKKL 1 gene. The present invention provides methods of using polynucleotides having the novel splice variants of the human DKKL 1 sequences, their corresponding gene products and antibodies specific for the gene products in the detection, diagnosis, prevention and/or treatment of associated cancers.



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NOVEL SPLICE VARIANTS OF HUMAN DKKL1

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of provisional application U.S. Ser. No. 60/507,682, filed on September 30, 2003, which is herein incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD OF THE INVENTION

[0002] This invention relates generally to the field of cancer-associated genes. Specifically, it relates to nucleotide sequences representing novel splice variants of the human DKKL1 gene in human tissue for use in diagnosis and treatment of cancer, as well as the use of the novel sequences in screening methods.

BACKGROUND OF THE INVENTION

[0003] Oncogenes are genes that can cause cancer. Carcinogenesis can occur by a wide variety of mechanisms, including infection of cells by viruses containing oncogenes, activation of protooncogenes in the host genome, and mutations of protooncogenes and tumor suppressor genes. Carcinogenesis is fundamentally driven by somatic cell evolution (i.e. mutation and natural selection of variants with progressive loss of growth control). The genes that serve as targets for these somatic mutations are classified as either protooncogenes or tumor suppressor genes, depending on whether their mutant phenotypes are dominant or recessive, respectively.

[0004] The pattern of gene expression in a particular living cell is characteristic of its current state. Nearly all differences in the state or type of a cell are reflected in qualitative and quantitative differences in RNA levels of one or more genes. For example, oncogenes are positive regulators of tumorigenesis, while tumor suppressor genes are

negative regulators of tumorigenesis. (Marshall, Cell, 64: 313-326 (1991); Weinberg, Science, 254: 1138-1146 (1991)).

[0005] Secreted proteins are involved in signaling between cells that are not in direct contact and play a role in differentiation of cells in mammals. The wnt gene family encodes a class of secreted proteins related to the Int1/Wnt1 protooncogene (Cadigan and Nusse, Genes & Development 11:3286-3305 (1997). Dickkopf (Dkk) is a negative regulator of Wnt signaling (Glinka A, et al. Nature. 1998 Jan 22;391(6665):357-362; Niehrs C Trends Genet. 1999 Aug;15(8):314-319). The Dkk protein is secreted and rich in cysteines. A family of human Dickkopf proteins (also referred to as "Cysteine-Rich Secreted Proteins" or CRSPs) have been reported. (see WO 00/52047 (McCarthy)). There are 4 Dkk members in the human genome with different activities. Some do NOT inhibit Wnt signaling (Wu W, et al. Cur Biol. 2000 Dec 14-28;10(24):1611-1614). There are no non-vertebrate homologs of Dkk.

[0006] Additional members of the human Dickkopf gene family were identified by sequence homology. A Dkk-like protein referred to as DKKL-1 (or Soggy-1) has been reported. (Krupnick VE, et al. Gene 238(2): 301-313(1999); see WO 00/52047 (McCarthy)). The mouse ortholog of DKKL-1 has been reported. (Kaneko KJ et al., Nuc. Acids Res. 28(20): 3982-3990 (2000)).

[0007] Immunotherapy, or the use of antibodies for therapeutic purposes has been used in recent years to treat cancer. Passive immunotherapy involves the use of monoclonal antibodies in cancer treatments. See for example, *Cancer: Principles and Practice of Oncology*, 6th Edition (2001) Ch. 20 pp. 495-508. Inherent therapeutic biological activity of these antibodies include direct inhibition of tumor cell growth or survival, and the ability to recruit the natural cell killing activity of the body's immune system. These agents are administered alone or in conjunction with radiation or chemotherapeutic agents. Rituxan® and Herceptin®, approved for treatment of lymphoma and breast cancer, respectively, are two examples of such therapeutics. Alternatively, antibodies are used to make antibody conjugates where the antibody is linked to a toxic agent and directs that agent to the tumor by specifically binding to the tumor. Mylotarg® is an example of an approved antibody conjugate used for the treatment of leukemia.

[0008] Accordingly, it is another object of this invention to provide antigens (cancer-associated polypeptides) associated with a variety of cancers as targets for diagnostic and/or therapeutic antibodies. These antigens are also useful for drug discovery (*e.g.*, small molecules) and for further characterization of cellular regulation, growth, and differentiation.

SUMMARY OF THE INVENTION

[0009] In accordance with the objects outlined above, the present invention provides two novel splice variants of the human DKKL-1 gene.

[0010] In one aspect, the invention provides polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 3A-3E. A novel isoform 2 comprises the novel splice junction comprising at least 4, 6, 10, 15, 20, 25, or 30 consecutive nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E and hybridizes to a DKKL-1 polynucleotide sequence or complement thereof. A novel isoform 3 comprises the novel splice junction comprising at least 4, 6, 10, 15, 20, 25, or 30 consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E and hybridizes to a DKKL-1 polynucleotide sequence, or complement thereof.

[0011] In another aspect, the invention provides polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B. A novel isoform 2 comprises the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B and comprises a DKKL-1 polypeptide sequence or fragment thereof. A novel isoform 3 comprises the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B and comprises a DKKL-1 polypeptide sequence or fragment thereof.

[0012] The present invention provides human cancer indication by expression profiling of DKKL1 splice variants on primary tumors. In one embodiment, novel splice variants are associated with DNA amplification of the DKKL1 loci. In one embodiment, mis-regulation of the splicing events of the DKKL1 locus associated with the formation of novel isoforms 2 and 3 occur in different primary tumors.

[0013] In one aspect, a method of screening drug candidates comprises providing a cell that expresses a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. Preferred embodiments are differentially expressed in cancer cells, preferably lymphatic, breast, prostate, testicular or epithelial cells, compared to other cells. The methods further include adding a drug candidate to the cell and determining the effect of the drug candidate on the expression of the polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products.

[0014] In one embodiment, the method of screening drug candidates includes comparing the level of expression in the absence of the drug candidate to the level of expression in the presence of the drug candidate.

[0015] Also provided herein is a method of screening for a bioactive agent capable of binding to a polypeptide encoded by a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products, the method comprising combining the polypeptide and a candidate bioactive agent, and determining the binding of the candidate agent to the polypeptide.

[0016] Further provided herein is a method for screening for a bioactive agent capable of modulating the activity of the polypeptide. In one embodiment, the method comprises combining the polypeptide and a candidate bioactive agent, and determining the effect of the candidate agent on the bioactivity of the polypeptide.

[0017] Also provided is a method of evaluating the effect of a candidate cancer drug comprising administering the drug to a patient and removing a cell sample from the patient. The expression profile of the cell is then determined for the expression of a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. This method may further comprise comparing the expression profile of the patient to an expression profile of a healthy individual.

[0018] In a further aspect, a method for inhibiting the activity of a protein encoded by a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products is provided. In one embodiment, the method comprises administering to a patient an inhibitor of a protein encoded by a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. A method of neutralizing the effect of the protein is also provided. Preferably, the method comprises contacting an agent specific for said protein with said protein in an amount sufficient to effect neutralization.

[0019] Moreover, provided herein is a biochip comprising a nucleic acid segment polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. Also provided herein is a method for diagnosing or determining the propensity to cancers, especially lymphoma or leukemia or carcinoma by sequencing at least one carcinoma or lymphoma gene of an individual. In yet another aspect of the invention, a method is provided for determining cancer including lymphoma and leukemia gene copy numbers in an individual.

[0020] The invention provides an isolated nucleic acid comprising at least 10, 12, 15, 20 or 30 contiguous nucleotides of a sequence selected from the group consisting of the sequences of a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products or its complement, or an expression vector comprising the isolated nucleic acids and host cells comprising them.

[0021] In some embodiments, the polynucleotide, or its complement or a fragment thereof, further comprises a detectable label, is attached to a solid support, is prepared at least in part by chemical synthesis, is an antisense fragment, is single stranded, is double stranded or comprises a microarray.

[0022] The invention provides an isolated polypeptide, encoded within an open reading frame of a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. The invention provides an isolated polypeptide, wherein said polypeptide comprises the amino acid sequence of a polypeptide encoded by a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. The invention further provides an isolated polypeptide, comprising the amino

acid sequence of an epitope of the amino acid sequence of a polypeptide encoded by a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products. In one embodiment the invention provides an isolated antibody (monoclonal or polyclonal) or antigen binding fragment thereof, that binds to such a polypeptide. The isolated antibody or antigen binding fragment thereof may be attached to a solid support, or further comprises a detectable label.

[0023] In one embodiment, the invention provides a kit for diagnosing the presence of cancer in a test sample, said kit comprising at least one polynucleotide that selectively hybridizes to a polynucleotide sequence comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products or its complement. In another embodiment, the invention provides an electronic library comprising a polynucleotide, a polypeptide, or fragment thereof comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products.

[0024] In one embodiment, the invention provides a method of screening for anticancer activity comprising: (a) providing a cell that expresses a cancer associated (CA) gene encoded by a nucleic acid sequence selected from the group consisting of a polynucleotide comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products, or fragment thereof; (b) contacting a tissue sample derived from a cancer cell with an anticancer drug candidate; (c) monitoring an effect of the anticancer drug candidate on an expression of the polynucleotide in the tissue sample, and optionally (d) comparing the level of expression in the absence of said drug candidate to the level of expression in the presence of the drug candidate.

[0025] In one embodiment, the invention provides a method for detecting cancer associated with expression of a polypeptide in a test cell sample, comprising the steps of: (i) detecting a level of expression of at least one polypeptide selected from the group consisting of the polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A and 4B or a fragment thereof; and (ii) comparing the level of expression of the polypeptide in the test sample with a level of expression of polypeptide in a normal cell sample, wherein an altered level of expression

of the polypeptide in the test cell sample relative to the level of polypeptide expression in the normal cell sample is indicative of the presence of cancer in the test cell sample.

[0026] In another embodiment, the invention provides a method for detecting cancer associated with expression of a polypeptide in a test cell sample, comprising the steps of: (i) detecting a level of activity of at least one polypeptide selected from the group consisting of the group consisting of the polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A and 4B or a fragment thereof; and (ii) comparing the level of activity of the polypeptide in the test sample with a level of activity of polypeptide in a normal cell sample, wherein an altered level of activity of the polypeptide in the test cell sample relative to the level of polypeptide activity in the normal cell sample is indicative of the presence of cancer in the test cell sample.

[0027] In another embodiment, the invention provides a method for detecting cancer associated with the presence of an antibody in a test serum sample, comprising the steps of: (i) detecting a level of an antibody against an antigenic polypeptide selected from the group consisting of the group consisting of the polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A and 4B or a fragment thereof; and (ii) comparing said level of said antibody in the test sample with a level of said antibody in the control sample, wherein an altered level of antibody in said test sample relative to the level of antibody in the control sample is indicative of the presence of cancer in the test serum sample.

[0028] The invention provides a method for screening for a bioactive agent capable of modulating the activity of a protein, wherein said protein is encoded by a nucleic acid comprising a nucleic acid sequence selected from the group consisting of the polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 3A-3E or a fragment thereof, said method comprising: a) combining said protein and a candidate bioactive agent; and b) determining the effect of the candidate agent on the bioactivity of said protein.

[0029] In one embodiment, the invention provides a method for diagnosing cancer comprising: a) determining the expression of one or more genes comprising a nucleic

acid sequence selected from the group consisting of the polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 3A-3E, in a first tissue type of a first individual; and b) comparing said expression of said gene(s) from a second normal tissue type from said first individual or a second unaffected individual; wherein a difference in said expression indicates that the first individual has cancer.

[0030] In another embodiment the invention provides a method for treating cancers comprising administering to a patient a bioactive agent modulating the activity of a protein, wherein said protein is encoded by a nucleic acid comprising a nucleic acid sequence selected from the group consisting of the polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 3A-3E and further wherein the bioactive agent binds to the protein which has an activity selected from the group consisting of: interaction with a Dkk receptor, interaction with an intracellular protein via a membrane bound Dkk receptor, interaction with extracellular proteins, modulation of cellular signal transduction, modulation of *wnt*-mediated signal transduction, regulation of gene expression in a cell involved in development or differentiation, .

[0031] The invention also provides a method for detecting a presence or an absence of cancer cells in an individual, the method comprising: contacting cells from the individual with the antibody according to the invention; and detecting a complex of a protein encoded by the novel isoforms of DKKL1 from the cancer cells and the antibody, wherein detection of the complex correlates with the presence of cancer cells in the individual. In one embodiment the invention provides a method for inhibiting growth of cancer cells in an individual, the method comprising: administering to the individual an effective amount of a pharmaceutical composition according to the invention. In another embodiment the invention provides a method for delivering a therapeutic agent to cancer cells in an individual, the method comprising: administering to the individual an effective amount of a pharmaceutical composition according to the invention.

[0032] Novel sequences associated with cancer are also provided herein. Other aspects of the invention will become apparent to the skilled artisan by the following description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0033] **Figure 1** shows alignment of Celera DKKL-1 transcript with the novel splice variants.

[0034] **Figure 2** shows alignment of transcripts of splice variant isoforms of DKKL-1 in terms of complexity.

[0035] **Figures 3A-3E** shows alignment of transcripts of splice variant isoforms of DKKL-1 by nucleotide sequence.

[0036] **Figures 4A-4B** shows amino acid sequence alignment of transcripts of splice variant isoforms of DKKL-1.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

[0037] A “polynucleotide comprising novel isoform 2” comprises the novel splice junction comprising at least 4, 6, 10, 15, 20, 25, or 30 consecutive nucleotides spanning positions 329 and 330 of the nucleotide s

[0038] Sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E and hybridizes to a DKKL-1 polynucleotide sequence or complement thereof. A “polynucleotide comprising novel isoform 3” comprises the novel splice junction comprising at least 4, 6, 10, 15, 20, 25, or 30 consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E and hybridizes to a DKKL-1 polynucleotide sequence, or complement thereof.

[0039] A “polypeptide comprising novel isoform 2” comprises the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning

positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B and comprises a DKKL-1 polypeptide sequence or fragment thereof. A “polypeptide comprising novel isoform 3” comprises the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B and comprises a DKKL-1 polypeptide sequence or fragment thereof.

[0040] Suitable cancers that can be diagnosed or screened for using the methods of the present invention include cancers classified by site or by histological type. Cancers classified by site include cancer of the oral cavity and pharynx (lip, tongue, salivary gland, floor of mouth, gum and other mouth, nasopharynx, tonsil, oropharynx, hypopharynx, other oral/pharynx); cancers of the digestive system (esophagus; stomach; small intestine; colon and rectum; anus, anal canal, and anorectum; liver; intrahepatic bile duct; gallbladder; other biliary; pancreas; retroperitoneum; peritoneum, omentum, and mesentery; other digestive); cancers of the respiratory system (nasal cavity, middle ear, and sinuses; larynx; lung and bronchus; pleura; trachea, mediastinum, and other respiratory); cancers of the mesothelioma; bones and joints; and soft tissue, including heart; skin cancers, including melanomas and other non-epithelial skin cancers; Kaposi's sarcoma and breast cancer; cancer of the female genital system (cervix uteri; corpus uteri; uterus, nos; ovary; vagina; vulva; and other female genital); cancers of the male genital system (prostate gland; testis; penis; and other male genital); cancers of the urinary system (urinary bladder; kidney and renal pelvis; ureter; and other urinary); cancers of the eye and orbit; cancers of the brain and nervous system (brain; and other nervous system); cancers of the endocrine system (thyroid gland and other endocrine, including thymus); lymphomas (Hodgkin's disease and non-Hodgkin's lymphoma), multiple myeloma, and leukemias (lymphocytic leukemia; myeloid leukemia; monocytic leukemia; and other leukemias).

[0041] Other cancers, classified by histological type, that may be associated with the sequences of the invention include, but are not limited to, Neoplasm, malignant; Carcinoma, NOS; Carcinoma, undifferentiated, NOS; Giant and spindle cell carcinoma; Small cell carcinoma, NOS; Papillary carcinoma, NOS; Squamous cell carcinoma, NOS;

Lymphoepithelial carcinoma; Basal cell carcinoma, NOS; Pilomatrix carcinoma;
 Transitional cell carcinoma, NOS; Papillary transitional cell carcinoma;
 Adenocarcinoma, NOS; Gastrinoma, malignant; Cholangiocarcinoma; Hepatocellular
 carcinoma, NOS; Combined hepatocellular carcinoma and cholangiocarcinoma;
 Trabecular adenocarcinoma; Adenoid cystic carcinoma; Adenocarcinoma in
 adenomatous polyp; Adenocarcinoma, familial polyposis coli; Solid carcinoma, NOS;
 Carcinoid tumor, malignant; Bronchiolo-alveolar adenocarcinoma; Papillary
 adenocarcinoma, NOS; Chromophobe carcinoma; Acidophil carcinoma; Oxyphilic
 adenocarcinoma; Basophil carcinoma; Clear cell adenocarcinoma, NOS; Granular cell
 carcinoma; Follicular adenocarcinoma, NOS; Papillary and follicular adenocarcinoma;
 Nonencapsulating sclerosing carcinoma; Adrenal cortical carcinoma; Endometroid
 carcinoma; Skin appendage carcinoma; Apocrine adenocarcinoma; Sebaceous
 adenocarcinoma; Ceruminous adenocarcinoma; Mucoepidermoid carcinoma;
 Cystadenocarcinoma, NOS; Papillary cystadenocarcinoma, NOS; Papillary serous
 cystadenocarcinoma; Mucinous cystadenocarcinoma, NOS; Mucinous adenocarcinoma;
 Signet ring cell carcinoma; Infiltrating duct carcinoma; Medullary carcinoma, NOS;
 Lobular carcinoma; Inflammatory carcinoma; Paget's disease, mammary; Acinar cell
 carcinoma; Adenosquamous carcinoma; Adenocarcinoma w/ squamous metaplasia;
 Thymoma, malignant; Ovarian stromal tumor, malignant; Thecoma, malignant;
 Granulosa cell tumor, malignant; Androblastoma, malignant; Sertoli cell carcinoma;
 Leydig cell tumor, malignant; Lipid cell tumor, malignant; Paraganglioma, malignant;
 Extra-mammary paraganglioma, malignant; Pheochromocytoma; Glomangiosarcoma;
 Malignant melanoma, NOS; Amelanotic melanoma; Superficial spreading melanoma;
 Malig melanoma in giant pigmented nevus; Epithelioid cell melanoma; Blue nevus,
 malignant; Sarcoma, NOS; Fibrosarcoma, NOS; Fibrous histiocytoma, malignant;
 Myxosarcoma; Liposarcoma, NOS; Leiomyosarcoma, NOS; Rhabdomyosarcoma, NOS;
 Embryonal rhabdomyosarcoma; Alveolar rhabdomyosarcoma; Stromal sarcoma, NOS;
 Mixed tumor, malignant, NOS; Mullerian mixed tumor; Nephroblastoma;
 Hepatoblastoma; Carcinosarcoma, NOS; Mesenchymoma, malignant; Brenner tumor,
 malignant; Phyllodes tumor, malignant; Synovial sarcoma, NOS; Mesothelioma,
 malignant; Dysgerminoma; Embryonal carcinoma, NOS; Teratoma, malignant, NOS;

Struma ovarii, malignant; Choriocarcinoma; Mesonephroma, malignant; Hemangiosarcoma; Hemangioendothelioma, malignant; Kaposi's sarcoma; Hemangiopericytoma, malignant; Lymphangiosarcoma; Osteosarcoma, NOS; Juxtacortical osteosarcoma; Chondrosarcoma, NOS; Chondroblastoma, malignant; Mesenchymal chondrosarcoma; Giant cell tumor of bone; Ewing's sarcoma; Odontogenic tumor, malignant; Ameloblastic odontosarcoma; Ameloblastoma, malignant; Ameloblastic fibrosarcoma; Pinealoma, malignant; Chordoma; Glioma, malignant; Ependymoma, NOS; Astrocytoma, NOS; Protoplasmic astrocytoma; Fibrillary astrocytoma; Astroblastoma; Glioblastoma, NOS; Oligodendroglioma, NOS; Oligodendroblastoma; Primitive neuroectodermal; Cerebellar sarcoma, NOS; Ganglioneuroblastoma; Neuroblastoma, NOS; Retinoblastoma, NOS; Olfactory neurogenic tumor; Meningioma, malignant; Neurofibrosarcoma; Neurilemmoma, malignant; Granular cell tumor, malignant; Malignant lymphoma, NOS; Hodgkin's disease, NOS; Hodgkin's; paraganuloma, NOS; Malignant lymphoma, small lymphocytic; Malignant lymphoma, large cell, diffuse; Malignant lymphoma, follicular, NOS; Mycosis fungoides; Other specified non-Hodgkin's lymphomas; Malignant histiocytosis; Multiple myeloma; Mast cell sarcoma; Immunoproliferative small intestinal disease; Leukemia, NOS; Lymphoid leukemia, NOS; Plasma cell leukemia; Erythroleukemia; Lymphosarcoma cell leukemia; Myeloid leukemia, NOS; Basophilic leukemia; Eosinophilic leukemia; Monocytic leukemia, NOS; Mast cell leukemia; Megakaryoblastic leukemia; Myeloid sarcoma; and Hairy cell leukemia.

[0042] Similarly, a "recombinant protein" is a protein made using recombinant techniques, i.e. through the expression of a recombinant nucleic acid as depicted above. A recombinant protein is distinguished from naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated or purified away from some or all of the proteins and compounds with which it is normally associated in its wild type host, and thus may be substantially pure. For example, an isolated protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of the total protein in a given sample. A substantially pure protein comprises about 50-75% by weight of the total protein, with about 80% being preferred,

and about 90% being particularly preferred. The definition includes the production of a cancer-associated protein from one organism in a different organism or host cell.

Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Alternatively, the protein may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions, as discussed below.

[0043] A nucleic acid of the present invention generally contains phosphodiester bonds, although in some cases, as outlined below (for example, in antisense applications or when a nucleic acid is a candidate drug agent), nucleic acid analogs may have alternate backbones, comprising, for example, phosphoramidate (Beaucage et al., *Tetrahedron* 49(10):1925 (1993) and references therein; Letsinger, *J. Org. Chem.* 35:3800 (1970); Sprinzl et al., *Eur. J. Biochem.* 81:579 (1977); Letsinger et al., *Nucl. Acids Res.* 14:3487 (1986); Sawai et al., *Chem. Lett.* 805 (1984), Letsinger et al., *J. Am. Chem. Soc.* 110:4470 (1988); and Pauwels et al., *Chemica Scripta* 26:141 (1986)), phosphorothioate (Mag et al., *Nucleic Acids Res.* 19:1437 (1991); and U.S. Patent No. 5,644,048), phosphorodithioate (Briu et al., *J. Am. Chem. Soc.* 111:2321 (1989), O-methylphosphoroamidite linkages (see Eckstein, *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, *J. Am. Chem. Soc.* 114:1895 (1992); Meier et al., *Chem. Int. Ed. Engl.* 31:1008 (1992); Nielsen, *Nature*, 365:566 (1993); Carlsson et al., *Nature* 380:207 (1996), all of which are incorporated by reference). Other analog nucleic acids include those with positive backbones (Denpcy et al., *Proc. Natl. Acad. Sci. USA* 92:6097 (1995); non-ionic backbones (U.S. Patent Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Kiedrowshi et al., *Angew. Chem. Intl. Ed. English* 30:423 (1991); Letsinger et al., *J. Am. Chem. Soc.* 110:4470 (1988); Letsinger et al., *Nucleoside & Nucleotide* 13:1597 (1994); Chapters 2 and 3, *ASC Symposium Series* 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker et al., *Bioorganic & Medicinal Chem. Lett.* 4:395 (1994); Jeffs et al., *J. Biomolecular NMR* 34:17 (1994); *Tetrahedron Lett.* 37:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and

Chapters 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within one definition of nucleic acids (see Jenkins et al., Chem. Soc. Rev. (1995) pp169-176). Several nucleic acid analogs are described in Rawls, C & E News June 2, 1997 page 35. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone may be done for a variety of reasons, for example to increase the stability and half-life of such molecules in physiological environments for use in anti-sense applications or as probes on a biochip.

[0044] As will be appreciated by those in the art, all of these nucleic acid analogs may find use in the present invention. In addition, mixtures of naturally occurring nucleic acids and analogs can be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

[0045] The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand "Watson" also defines the sequence of the other strand "Crick"; thus the sequences described herein also includes the complement of the sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine, hypoxanthine, isocytosine, isoguanine, etc. As used herein, the term "nucleoside" includes nucleotides and nucleoside and nucleotide analogs, and modified nucleosides such as amino modified nucleosides. In addition, "nucleoside" includes non-naturally occurring analog structures. Thus for example the individual units of a peptide nucleic acid, each containing a base, are referred to herein as a nucleoside.

[0046] As used herein, the term "tag," "sequence tag" or "primer tag sequence" refers to an oligonucleotide with specific nucleic acid sequence that serves to identify a batch of polynucleotides bearing such tags therein. Polynucleotides from the same biological source are covalently tagged with a specific sequence tag so that in subsequent analysis

the polynucleotide can be identified according to its source of origin. The sequence tags also serve as primers for nucleic acid amplification reactions.

[0047] A “microarray” is a linear or two-dimensional array of preferably discrete regions, each having a defined area, formed on the surface of a solid support. The density of the discrete regions on a microarray is determined by the total numbers of target polynucleotides to be detected on the surface of a single solid phase support, preferably at least about 50/cm², more preferably at least about 100/cm², even more preferably at least about 500/cm², and still more preferably at least about 1,000/cm². As used herein, a DNA microarray is an array of oligonucleotide primers placed on a chip or other surfaces used to amplify or clone target polynucleotides. Since the position of each particular group of primers in the array is known, the identities of the target polynucleotides can be determined based on their binding to a particular position in the microarray.

[0048] A “linker” is a synthetic oligodeoxyribonucleotide that contains a restriction site. A linker may be blunt end-ligated onto the ends of DNA fragments to create restriction sites that can be used in the subsequent cloning of the fragment into a vector molecule.

[0049] The term “label” refers to a composition capable of producing a detectable signal indicative of the presence of the target polynucleotide in an assay sample. Suitable labels include radioisotopes, nucleotide chromophores, enzymes, substrates, fluorescent molecules, chemiluminescent moieties, magnetic particles, bioluminescent moieties, and the like. As such, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, chemical, or any other appropriate means. The term “label” is used to refer to any chemical group or moiety having a detectable physical property or any compound capable of causing a chemical group or moiety to exhibit a detectable physical property, such as an enzyme that catalyzes conversion of a substrate into a detectable product. The term “label” also encompasses compounds that inhibit the expression of a particular physical property. The label may also be a compound that is a member of a binding pair, the other member of which bears a detectable physical property.

[0050] The term “support” refers to conventional supports such as beads, particles, dipsticks, fibers, filters, membranes, and silane or silicate supports such as glass slides.

[0051] The term “amplify” is used in the broad sense to mean creating an amplification product which may include, for example, additional target molecules, or target-like molecules or molecules complementary to the target molecule, which molecules are created by virtue of the presence of the target molecule in the sample. In the situation where the target is a nucleic acid, an amplification product can be made enzymatically with DNA or RNA polymerases or reverse transcriptases.

[0052] As used herein, a “biological sample” refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, blood, plasma, serum, spinal fluid, lymph fluid, skin, respiratory, intestinal and genitourinary tracts, tears, saliva, milk, cells (including but not limited to blood cells), tumors, organs, and also samples of *in vitro* cell culture constituents.

[0053] The term “biological sources” as used herein refers to the sources from which the target polynucleotides are derived. The source can be of any form of “sample” as described above, including but not limited to, cell, tissue or fluid. “Different biological sources” can refer to different cells/tissues/organs of the same individual, or cells/tissues/organs from different individuals of the same species, or cells/tissues/organs from different species.

[0054] DKKL-1 proteins of the present invention are generally secreted proteins, the secretion of which can be either constitutive or regulated. These proteins have a signal peptide or signal sequence that targets the molecule to the secretory pathway. Secreted proteins are involved in numerous physiological events; by virtue of their circulating nature, they serve to transmit signals to various other cell types. The secreted protein may function in an autocrine manner (acting on the cell that secreted the factor), a paracrine manner (acting on cells in close proximity to the cell that secreted the factor) or an endocrine manner (acting on cells at a distance). Thus secreted molecules find use in modulating or altering numerous aspects of physiology. Secreted proteins are particularly preferred in the present invention as they serve as good targets for diagnostic markers, for example in blood tests.

Nucleic acids of novel isoforms of DKKL-1

[0055] In one aspect, the invention provides polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 3A-3E. Once isolated from its natural source, e.g., contained within a plasmid or other vector or excised therefrom as a linear nucleic acid segment, the recombinant nucleic acid can be further used as a probe to identify the expression of DKKL-1 splice variants.

[0056] In a first embodiment, nucleic acid probes hybridizable to polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products are made and attached to biochips to be used in screening and diagnostic methods, or for gene therapy and/or antisense applications. Alternatively, the polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products that include coding regions of DKKL-1 can be put into expression vectors for the expression of proteins, again either for screening purposes or for administration to a patient.

[0057] DNA microarray technology make it possible to conduct a large scale assay of a plurality of target nucleic acid molecules on a single solid phase support. U.S. Pat. No. 5,837,832 (Chee *et al.*) and related patent applications describe immobilizing an array of oligonucleotide probes for hybridization and detection of specific nucleic acid sequences in a sample. Target polynucleotides of interest isolated from a tissue of interest are hybridized to the DNA chip and the specific sequences detected based on the target polynucleotides' preference and degree of hybridization at discrete probe locations. One important use of arrays is in the analysis of differential gene expression, where the profile of expression of genes in different cells, often a cell of interest and a control cell, is compared and any differences in gene expression among the respective cells are identified. Such information is useful for the identification of the types of genes expressed in a particular cell or tissue type and diagnosis of cancer conditions based on the expression profile.

[0058] Typically, RNA from the sample of interest is subjected to reverse transcription to obtain labeled cDNA. *See* U.S. Pat. No. 6,410,229 (Lockhart *et al.*) The cDNA is then hybridized to oligonucleotides or cDNAs of known sequence arrayed on a chip or other surface in a known order. The location of the oligonucleotide to which the labeled cDNA

hybridizes provides sequence information on the cDNA, while the amount of labeled hybridized RNA or cDNA provides an estimate of the relative representation of the RNA or cDNA of interest. *See* Schena, *et al.* Science 270:467-470 (1995). For example, use of a cDNA microarray to analyze gene expression patterns in human cancer is described by DeRisi, *et al.* (Nature Genetics 14:457-460 (1996)).

[0059] In a preferred embodiment, nucleic acid probes corresponding to polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products (both the nucleic acid sequences outlined in the figures and/or the complements thereof) are made. Typically, these probes are synthesized based on the disclosed sequences of this invention. The nucleic acid probes attached to the biochip are designed to be substantially complementary to the polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products, i.e. the target sequence (either the target sequence of the sample or to other probe sequences, for example in sandwich assays), such that specific hybridization of the target sequence and the probes of the present invention occurs. As outlined below, this complementarity need not be perfect, in that there may be any number of base pair mismatches that will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. It is expected that the overall homology of the genes at the nucleotide level probably will be about 40% or greater, probably about 60% or greater, and even more probably about 80% or greater; and in addition that there will be corresponding contiguous sequences of about 8-12 nucleotides or longer. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by “substantially complementary” herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under normal reaction conditions, particularly high stringency conditions, as outlined herein. Whether or not a sequence is unique to polynucleotides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products according to this invention can be determined by techniques known to those of skill in the art. For example, the sequence can be compared to sequences in databanks, e.g., GeneBank, to determine whether it is present in the

uninfected host or other organisms. The sequence can also be compared to the known sequences of other viral agents, including those that are known to induce cancer.

[0060] A nucleic acid probe is generally single stranded but can be partly single and partly double stranded. The strandedness of the probe is dictated by the structure, composition, and properties of the target sequence. In general, the oligonucleotide probes range from about 6, 8, 10, 12, 15, 20, 30 to about 100 bases long, with from about 10 to about 80 bases being preferred, and from about 30 to about 50 bases being particularly preferred. That is, generally entire genes are rarely used as probes. In some embodiments, much longer nucleic acids can be used, up to hundreds of bases. The probes are sufficiently specific to hybridize to complementary template sequence under conditions known by those of skill in the art. The number of mismatches between the probes sequences and their complementary template (target) sequences to which they hybridize during hybridization generally do not exceed 15%, usually do not exceed 10% and preferably do not exceed 5%, as determined by FASTA (default settings).

[0061] Oligonucleotide probes can include the naturally-occurring heterocyclic bases normally found in nucleic acids (uracil, cytosine, thymine, adenine and guanine), as well as modified bases and base analogues. Any modified base or base analogue compatible with hybridization of the probe to a target sequence is useful in the practice of the invention. The sugar or glycoside portion of the probe can comprise deoxyribose, ribose, and/or modified forms of these sugars, such as, for example, 2'-O-alkyl ribose. In a preferred embodiment, the sugar moiety is 2'-deoxyribose; however, any sugar moiety that is compatible with the ability of the probe to hybridize to a target sequence can be used.

[0062] In one embodiment, the nucleoside units of the probe are linked by a phosphodiester backbone, as is well known in the art. In additional embodiments, internucleotide linkages can include any linkage known to one of skill in the art that is compatible with specific hybridization of the probe including, but not limited to phosphorothioate, methylphosphonate, sulfamate (*e.g.*, U.S. Patent No. 5,470,967) and polyamide (*i.e.*, peptide nucleic acids). Peptide nucleic acids are described in Nielsen *et*

al. (1991) *Science* 254: 1497-1500, U.S. Patent No. 5,714,331, and Nielsen (1999) *Curr. Opin. Biotechnol.* 10:71-75.

[0063] In certain embodiments, the probe can be a chimeric molecule; *i.e.*, can comprise more than one type of base or sugar subunit, and/or the linkages can be of more than one type within the same primer. The probe can comprise a moiety to facilitate hybridization to its target sequence, as are known in the art, for example, intercalators and/or minor groove binders. Variations of the bases, sugars, and internucleoside backbone, as well as the presence of any pendant group on the probe, will be compatible with the ability of the probe to bind, in a sequence-specific fashion, with its target sequence. A large number of structural modifications, both known and to be developed, are possible within these bounds. Advantageously, the probes according to the present invention may have structural characteristics such that they allow the signal amplification, such structural characteristics being, for example, branched DNA probes as those described by Urdea et al. (Nucleic Acids Symp. Ser., 24:197-200 (1991)) or in the European Patent No. EP-0225,807. Moreover, synthetic methods for preparing the various heterocyclic bases, sugars, nucleosides and nucleotides that form the probe, and preparation of oligonucleotides of specific predetermined sequence, are well-developed and known in the art. A preferred method for oligonucleotide synthesis incorporates the teaching of U.S. Patent No. 5,419,966.

[0064] Probes may be in solution, such as in wells or on the surface of a micro-array, or attached to a solid support. Examples of solid support materials that can be used include a plastic, a ceramic, a metal, a resin, a gel and a membrane. Useful types of solid supports include plates, beads, magnetic material, microbeads, hybridization chips, membranes, crystals, ceramics and self-assembling monolayers. A preferred embodiment comprises a two-dimensional or three-dimensional matrix, such as a gel or hybridization chip with multiple probe binding sites (Pevzner et al., J. Biomol. Struc. & Dyn. 9:399-410, 1991; Maskos and Southern, Nuc. Acids Res. 20:1679-84, 1992). Hybridization chips can be used to construct very large probe arrays that are subsequently hybridized with a target nucleic acid. Analysis of the hybridization pattern of the chip can assist in the identification of the target nucleotide sequence. Patterns can be manually or computer analyzed, but it is clear that positional sequencing by hybridization lends itself to

computer analysis and automation. Algorithms and software, which have been developed for sequence reconstruction, are applicable to the methods described herein (R. Drmanac et al., *J. Biomol. Struc. & Dyn.* 5:1085-1102, 1991; P. A. Pevzner, *J. Biomol. Struc. & Dyn.* 7:63-73, 1989).

[0065] As will be appreciated by those in the art, nucleic acids can be attached or immobilized to a solid support in a wide variety of ways. By “immobilized” herein is meant the association or binding between the nucleic acid probe and the solid support is sufficient to be stable under the conditions of binding, washing, analysis, and removal as outlined below. The binding can be covalent or non-covalent. By “non-covalent binding” and grammatical equivalents herein is meant one or more of either electrostatic, hydrophilic, and hydrophobic interactions. Included in non-covalent binding is the covalent attachment of a molecule, such as streptavidin, to the support and the non-covalent binding of the biotinylated probe to the streptavidin. By “covalent binding” and grammatical equivalents herein is meant that the two moieties, the solid support and the probe, are attached by at least one bond, including sigma bonds, pi bonds and coordination bonds. Covalent bonds can be formed directly between the probe and the solid support or can be formed by a cross linker or by inclusion of a specific reactive group on either the solid support or the probe or both molecules. Immobilization may also involve a combination of covalent and non-covalent interactions.

[0066] Nucleic acid probes may be attached to the solid support by covalent binding such as by conjugation with a coupling agent or by, covalent or non-covalent binding such as electrostatic interactions, hydrogen bonds or antibody-antigen coupling, or by combinations thereof. Typical coupling agents include biotin/avidin, biotin/streptavidin, *Staphylococcus aureus* protein A/IgG antibody F_c fragment, and streptavidin/protein A chimeras (T. Sano and C. R. Cantor, *Bio/Technology* 9:1378-81 (1991)), or derivatives or combinations of these agents. Nucleic acids may be attached to the solid support by a photocleavable bond, an electrostatic bond, a disulfide bond, a peptide bond, a diester bond or a combination of these sorts of bonds. The array may also be attached to the solid support by a selectively releasable bond such as 4,4'-dimethoxytrityl or its derivative. Derivatives which have been found to be useful include 3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic

acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-hydroxymethyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-chloromethyl-benzoic acid, and salts of these acids.

[0067] In general, the probes are attached to the biochip in a wide variety of ways, as will be appreciated by those in the art. As described herein, the nucleic acids can either be synthesized first, with subsequent attachment to the biochip, or can be directly synthesized on the biochip.

[0068] The biochip comprises a suitable solid substrate. By “substrate” or “solid support” or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of the nucleic acid probes and is amenable to at least one detection method. The solid phase support of the present invention can be of any solid materials and structures suitable for supporting nucleotide hybridization and synthesis. Preferably, the solid phase support comprises at least one substantially rigid surface on which the primers can be immobilized and the reverse transcriptase reaction performed. The substrates with which the polynucleotide microarray elements are stably associated may be fabricated from a variety of materials, including plastics, ceramics, metals, acrylamide, cellulose, nitrocellulose, glass, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, polysilicates, polycarbonates, Teflon®, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, polypropylfumerate, collagen, glycosaminoglycans, and polyamino acids. Substrates may be two-dimensional or three-dimensional in form, such as gels, membranes, thin films, glasses, plates, cylinders, beads, magnetic beads, optical fibers, woven fibers, etc. A preferred form of array is a three-dimensional array. A preferred three-dimensional array is a collection of tagged beads. Each tagged bead has different primers attached to it. Tags are detectable by signaling means such as color (Luminex, Illumina) and electromagnetic field (Pharmaseq) and signals on tagged beads can even be remotely detected (e.g., using optical fibers). The size of the solid support can be any of the standard microarray sizes, useful for DNA microarray technology, and the size may be tailored to fit the particular machine being used to conduct a reaction of

the invention. In general, the substrates allow optical detection and do not appreciably fluoresce.

[0069] In a preferred embodiment, the surface of the biochip and the probe may be derivatized with chemical functional groups for subsequent attachment of the two. Thus, for example, the biochip is derivatized with a chemical functional group including, but not limited to, amino groups, carboxy groups, oxo groups and thiol groups, with amino groups being particularly preferred. Using these functional groups, the probes can be attached using functional groups on the probes. For example, nucleic acids containing amino groups can be attached to surfaces comprising amino groups, for example using linkers as are known in the art; for example, homo-or hetero-bifunctional linkers as are well known (see 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated herein by reference). In addition, in some cases, additional linkers, such as alkyl groups (including substituted and heteroalkyl groups) may be used.

[0070] In this embodiment, the oligonucleotides are synthesized as is known in the art, and then attached to the surface of the solid support. As will be appreciated by those skilled in the art, either the 5' or 3' terminus may be attached to the solid support, or attachment may be via an internal nucleoside. In an additional embodiment, the immobilization to the solid support may be very strong, yet non-covalent. For example, biotinylated oligonucleotides can be made, which bind to surfaces covalently coated with streptavidin, resulting in attachment.

[0071] The arrays may be produced according to any convenient methodology, such as preforming the polynucleotide microarray elements and then stably associating them with the surface. Alternatively, the oligonucleotides may be synthesized on the surface, as is known in the art. A number of different array configurations and methods for their production are known to those of skill in the art and disclosed in WO 95/25116 and WO 95/35505 (photolithographic techniques), U.S. Pat. No. 5,445,934 (in situ synthesis by photolithography), U.S. Pat. No. 5,384,261 (in situ synthesis by mechanically directed flow paths); and U.S. Pat. No. 5,700,637 (synthesis by spotting, printing or coupling); the disclosure of which are herein incorporated in their entirety by reference. Another

method for coupling DNA to beads uses specific ligands attached to the end of the DNA to link to ligand-binding molecules attached to a bead. Possible ligand-binding partner pairs include biotin-avidin/streptavidin, or various antibody/antigen pairs such as digoxigenin-antidigoxigenin antibody (Smith et al., "Direct Mechanical Measurements of the Elasticity of Single DNA Molecules by Using Magnetic Beads," *Science* 258:1122-1126 (1992)). Covalent chemical attachment of DNA to the support can be accomplished by using standard coupling agents to link the 5'-phosphate on the DNA to coated microspheres through a phosphoamidate bond. Methods for immobilization of oligonucleotides to solid-state substrates are well established. *See* Pease *et al.*, *Proc. Natl. Acad. Sci. USA* 91(11):5022-5026 (1994). A preferred method of attaching oligonucleotides to solid-state substrates is described by Guo *et al.*, *Nucleic Acids Res.* 22:5456-5465 (1994). Immobilization can be accomplished either by in situ DNA synthesis (Maskos and Southern, *Nucleic Acids Research*, 20:1679-1684 (1992) or by covalent attachment of chemically synthesized oligonucleotides (Guo et al., *supra*) in combination with robotic arraying technologies.

[0072] In addition to the solid-phase technology represented by biochip arrays, gene expression can also be quantified using liquid-phase arrays. One such system is kinetic polymerase chain reaction (PCR). Kinetic PCR allows for the simultaneous amplification and quantification of specific nucleic acid sequences. The specificity is derived from synthetic oligonucleotide primers designed to preferentially adhere to single-stranded nucleic acid sequences bracketing the target site. This pair of oligonucleotide primers form specific, non-covalently bound complexes on each strand of the target sequence. These complexes facilitate *in vitro* transcription of double-stranded DNA in opposite orientations. Temperature cycling of the reaction mixture creates a continuous cycle of primer binding, transcription, and re-melting of the nucleic acid to individual strands. The result is an exponential increase of the target dsDNA product. This product can be quantified in real time either through the use of an intercalating dye or a sequence specific probe. SYBR® Greene I, is an example of an intercalating dye, that preferentially binds to dsDNA resulting in a concomitant increase in the fluorescent signal. Sequence specific probes, such as used with TaqMan® technology, consist of a fluorochrome and a quenching molecule covalently bound to opposite ends of an

oligonucleotide. The probe is designed to selectively bind the target DNA sequence between the two primers. When the DNA strands are synthesized during the PCR reaction, the fluorochrome is cleaved from the probe by the exonuclease activity of the polymerase resulting in signal dequenching. The probe signaling method can be more specific than the intercalating dye method, but in each case, signal strength is proportional to the dsDNA product produced. Each type of quantification method can be used in multi-well liquid phase arrays with each well representing primers and/or probes specific to nucleic acid sequences of interest. When used with messenger RNA preparations of tissues or cell lines, an array of probe/primer reactions can simultaneously quantify the expression of multiple gene products of interest. See Germer, S., et al., *Genome Res.* 10:258-266 (2000); Heid, C. A., et al., *Genome Res.* 6, 986-994 (1996).

Expression of novel isoforms of DKKL-1 protein

[0073] In a preferred embodiment, nucleic acids encoding polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B are used to make a variety of expression vectors to express the proteins which can then be used in screening assays, as described below. In a preferred embodiment the polypeptides comprise the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B, or the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B.

[0074] The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the protein. The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0075] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the protein. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

[0076] In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences.

[0077] Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

[0078] In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences that flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by

selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

[0079] In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

[0080] The proteins of the present invention are produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B, under the appropriate conditions to induce or cause expression of the polypeptide. The conditions appropriate for protein expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

[0081] Appropriate host cells include yeast, bacteria, archaeobacteria, fungi, and insect, plant and animal cells, including mammalian cells. Of particular interest are *Drosophila melanogaster* cells, *Saccharomyces cerevisiae* and other yeasts, *E. coli*, *Bacillus subtilis*, Sf9 cells, C129 cells, 293 cells, *Neurospora*, BHK, CHO, COS, HeLa cells, THP1 cell line (a macrophage cell line) and human cells and cell lines.

[0082] In a preferred embodiment, the polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B are expressed in mammalian cells. Mammalian expression systems are also known in the art, and include retroviral systems. A preferred expression vector system is a retroviral vector system such as is generally described in PCT/US97/01019 and PCT/US97/01048, both of which are hereby expressly incorporated by reference. Of particular use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early

promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter. Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. Examples of transcription terminator and polyadenylation signals include those derived from SV40.

[0083] The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, are well known in the art, and will vary with the host cell used. Techniques include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, viral infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0084] In a preferred embodiment, the proteins are expressed in bacterial systems. Bacterial expression systems are well known in the art. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters are also useful; for example, the tac promoter is a hybrid of the trp and lac promoter sequences. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable. The expression vector may also include a signal peptide sequence that provides for secretion of the protein in bacteria. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes that render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways. These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for *Bacillus subtilis*, *E. coli*, *Streptococcus cremoris*, and *Streptococcus lividans*, among others. The

bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

[0085] In one embodiment, proteins are produced in insect cells. Expression vectors for the transformation of insect cells, and in particular, baculovirus-based expression vectors, are well known in the art.

[0086] In a preferred embodiment, protein is produced in yeast cells. Yeast expression systems are well known in the art, and include expression vectors for *Saccharomyces cerevisiae*, *Candida albicans* and *C. maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis* and *K. lactis*, *Pichia guillermondii* and *P. pastoris*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica*.

[0087] The polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B may also be made as fusion proteins, using techniques well known in the art, for example, for the creation of monoclonal antibodies. If the desired epitope is small, the protein may be fused to a carrier protein to form an immunogen. Alternatively, the protein may be made as a fusion protein to increase expression, or for other reasons.

[0088] In one embodiment, the nucleic acids, proteins and antibodies of the invention are labeled. By "labeled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the nucleic acids, proteins and antibodies at any position. For example, the label should be capable of producing, either directly or indirectly, a detectable signal. The detectable moiety may be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the label may be employed, including those methods described by Hunter et al., *Nature*, 144:945 (1962); David et al., *Biochemistry*, 13:1014

(1974); Pain et al., *J. Immunol. Meth.*, 40:219 (1981); and Nygren, J. *Histochem. and Cytochem.*, 30:407 (1982).

[0089] In general, the term "polypeptide" as used herein refers to both the full-length polypeptide encoded by the recited polynucleotide, the polypeptide encoded by the gene represented by the recited polynucleotide, as well as portions or fragments thereof.

[0090] The present invention includes variants of polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B include mutants, fragments, and fusions. Mutants can include amino acid substitutions, additions or deletions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acids, such as to alter a glycosylation site, a phosphorylation site or an acetylation site, or to minimize misfolding by substitution or deletion of one or more cysteine residues that are not necessary for function. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/ hydrophilicity, and/or steric bulk of the amino acid substituted. Variants can be designed so as to retain or have enhanced biological activity of a particular region of the protein (*e.g.*, a functional domain and/or, where the polypeptide is a member of a protein family, a region associated with a consensus sequence). Selection of amino acid alterations for production of variants can be based upon the accessibility (interior vs. exterior) of the amino acid (see, *e.g.*, Go *et al.*, *Int. J. Peptide Protein Res.* (1980) 15:211), the thermostability of the variant polypeptide (see, *e.g.*, Querol *et al.*, *Prot. Eng.* (1996) 9:265), desired glycosylation sites (see, *e.g.*, Olsen and Thomsen, *J. Gen. Microbiol.* (1991) 137:579), desired disulfide bridges (see, *e.g.*, Clarke *et al.*, *Biochemistry* (1993) 32:4322; and Wakarchuk *et al.*, *Protein Eng.* (1994) 7:1379), desired metal binding sites (see, *e.g.*, Toma *et al.*, *Biochemistry* (1991) 30:97, and Haezebrouck *et al.*, *Protein Eng.* (1993) 6:643), and desired substitutions within proline loops (see, *e.g.*, Masul *et al.*, *Appl. Env. Microbiol.* (1994) 60:3579). Cysteine-depleted muteins can be produced as disclosed in USPN 4,959,314.

[0091] Variants also include fragments of the polypeptides disclosed herein, particularly biologically active fragments and/or fragments corresponding to functional domains. Fragments of interest will typically be at least about 8 amino acids (aa) 10 aa,

15 aa, 20 aa, 25 aa, 30 aa, 35 aa, 40 aa, to at least about 45 aa in length, usually at least about 50 aa in length, at least about 75 aa, at least about 100 aa, at least about 125 aa, at least about 150 aa in length, at least about 200 aa, at least about 300 aa, at least about 400 aa and can be as long as 500 aa in length or longer, but will usually not exceed about 1000 aa in length, where the fragment will have a stretch of amino acids that is identical to a polypeptide encoded by a polynucleotide having a sequence of any one of the polynucleotide sequences provided herein, or a homolog thereof. The protein variants described herein are encoded by polynucleotides that are within the scope of the invention. The genetic code can be used to select the appropriate codons to construct the corresponding variants.

[0092] Covalent modifications of polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N-or C-terminal residues of a polypeptide. Derivatization with bifunctional agents is useful, for instance, for crosslinking polypeptides to a water-insoluble support matrix or surface for use in the method for purifying anti-DKKL-1 antibodies or screening assays, as is more fully described below. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

[0093] Other modifications include deamidation of glutamyl and asparagyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl, threonyl or tyrosyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0094] Another type of covalent modification comprises linking the polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

[0095] Polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B of the present invention may also be modified in a way to form chimeric molecules comprising a polypeptide fused to another, heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of a polypeptide with a tag polypeptide that provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino-or carboxyl-terminus of the polypeptide, although internal fusions may also be tolerated in some instances. The presence of such epitope-tagged forms of a polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. In an alternative embodiment, the chimeric molecule may comprise a fusion of a polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule.

[0096] Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

Novel DKKL-1 antigens and antibodies thereto

[0097] In one embodiment, the invention provides specific antibodies against polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B. In a preferred embodiment, the polypeptide has at least one epitope or determinant comprising the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B or the novel splice junction comprising at least 2, 4, 6, 8, 10, 12, 15, or 20 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B. By "epitope" or "determinant" herein is meant a portion of a protein that will generate and/or bind an antibody or T-cell receptor in the context of MHC.

[0098] The antibodies of the invention specifically bind to polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B. By "specifically bind" herein is meant that the antibodies bind to the protein with a binding constant in the range of 10^{-4} - 10^{-6} M⁻¹, with a preferred range being 10^{-7} - 10^{-9} M⁻¹. In a preferred embodiment, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity.

[0099] Polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B may be analyzed to determine certain preferred regions of the polypeptide. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values that represent regions of the polypeptide that are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response. For example, the amino acid sequence of a polypeptide may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., Madison, Wis.; <http://www.dnastar.com/>).

[0100] Polypeptide features that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions (Garnier et al. *J. Mol. Biol.*, 120: 97 (1978));

Chou-Fasman alpha-regions, beta-regions, and turn-regions (*Adv. in Enzymol.*, 47:45-148 (1978)); Kyte-Doolittle hydrophilic regions and hydrophobic regions (*J. Mol. Biol.*, 157:105-132 (1982)); Eisenberg alpha- and beta-amphipathic regions; Karplus-Schulz flexible regions; Emini surface-forming regions (*J. Virol.*, 55(3):836-839 (1985)); and Jameson-Wolf regions of high antigenic index (*CABIOS*, 4(1):181-186 (1988)). Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesizing the sequence and injecting it into an appropriate animal, typically a rabbit, hamster or a mouse. Oligopeptides can be selected as candidates for the production of an antibody to the protein based upon the oligopeptides lying in hydrophilic regions, which are thus likely to be exposed in the mature protein. Additional oligopeptides can be determined using, for example, the Antigenicity Index, Welling, G.W. et al., *FEBS Lett.* 188:215-218 (1985), incorporated herein by reference.

[0101] In one embodiment, the term "antibody" includes antibody fragments, as are known in the art, including Fab, Fab₂, single chain antibodies (Fv for example), chimeric antibodies, etc., either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies.

[0102] Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include a protein encoded by a nucleic acid of the figures or fragment thereof or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and

soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[0103] The antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*. The immunizing agent will typically include a polypeptide encoded by a nucleic acid of the novel DKKL-1 isoform sequences, or fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

[0104] Monoclonal antibody technology is used in implementing research, diagnosis and therapy. Monoclonal antibodies are used in radioimmunoassays, enzyme-linked immunosorbent assays, immunocytopathology, and flow cytometry for *in vitro* diagnosis, and *in vivo* for diagnosis and immunotherapy of human disease. Waldmann, T. A. (1991) *Science* 252:1657-1662. In particular, monoclonal antibodies have been widely applied to

the diagnosis and therapy of cancer, wherein it is desirable to target malignant lesions while avoiding normal tissue. See, e.g., U.S. Pat. Nos. 4,753,894 to Frankel, et al.; 4,938,948 to Ring et al.; and 4,956,453 to Bjorn et al.

[0105] In one embodiment, the antibodies are bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent V regions and their associated CDRs fused to human constant domains (Winter et al. (1991) *Nature* 349:293-299; Lobuglio et al. (1989) *Proc. Nat. Acad. Sci. USA* 86:4220-4224; Shaw et al. (1987) *J Immunol.* 138:4534-4538; and Brown et al. (1987) *Cancer Res.* 47:3577-3583), rodent CDRs grafted into a human supporting FR prior to fusion with an appropriate human antibody constant domain (Riechmann et al. (1988) *Nature* 332:323-327; Verhoeyen et al. (1988) *Science* 239:1534-1536; and Jones et al. (1986) *Nature* 321:522-525), and rodent CDRs supported by recombinantly veneered rodent FRs (European Patent Publication No. 519,596, published Dec. 23, 1992). These "humanized" molecules are designed to minimize unwanted immunological response toward rodent antihuman antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. In the present case, one of the binding specificities is for a protein encoded by a nucleic acid of the novel DKKL-1 isoform sequences, or a fragment thereof, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit, preferably one that is tumor specific.

[0106] In a preferred embodiment, the antibodies to polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B are capable of reducing or eliminating the biological function of the polypeptide. Generally, at least a 25% decrease in activity is preferred, with at least about 50% being particularly preferred and about a 95-100% decrease being especially preferred.

[0107] In a preferred embodiment the antibodies are humanized antibodies. "Humanized" antibodies refer to a molecule having an antigen binding site that is

substantially derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and/or sequence of a human immunoglobulin. The antigen binding site may comprise either complete variable domains fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable domains. Antigen binding sites may be wild type or modified by one or more amino acid substitutions, e.g., modified to resemble human immunoglobulin more closely. Alternatively, a humanized antibody may be derived from a chimeric antibody that retains or substantially retains the antigen-binding properties of the parental, non-human, antibody but which exhibits diminished immunogenicity as compared to the parental antibody when administered to humans. The phrase "chimeric antibody," as used herein, refers to an antibody containing sequence derived from two different antibodies (*see, e.g.*, U.S. Patent No. 4,816,567) that typically originate from different species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals, while the constant portions are homologous to the sequences in antibodies derived from another. Most typically, chimeric antibodies comprise human and murine antibody fragments, generally human constant and mouse variable regions. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework residues (FR) regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., *Nature*, 321:522-525

(1986); Riechmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)). One clear advantage to such chimeric forms is that, for example, the variable regions can conveniently be derived from presently known sources using readily available hybridomas or B cells from non human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation, and the specificity is not affected by its source, the constant region being human, is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non-human source. However, the definition is not limited to this particular example.

[0108] Because humanized antibodies are far less immunogenic in humans than the parental mouse monoclonal antibodies, they can be used for the treatment of humans with far less risk of anaphylaxis. Thus, these antibodies may be preferred in therapeutic applications that involve *in vivo* administration to a human such as, *e.g.*, use as radiation sensitizers for the treatment of neoplastic disease or use in methods to reduce the side effects of, *e.g.*, cancer therapy. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeven et al., *Science* 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0109] Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381

(1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies [Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.*, 147(1):86-95 (1991)]. Humanized antibodies may be achieved by a variety of methods including, for example: (1) grafting the non-human complementarity determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as “humanizing”), or, alternatively, (2) transplanting the entire non-human variable domains, but “cloaking” them with a human-like surface by replacement of surface residues (a process referred to in the art as “veneering”). In the present invention, humanized antibodies will include both “humanized” and “veneered” antibodies. Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10, 779-783 (1992); Lonberg et al., *Nature* 368 856-859 (1994); Morrison, *Nature* 368, 812-13 (1994); Fishwild et al., *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995); Jones et al., *Nature* 321:522-525 (1986); Morrison et al., *Proc. Natl. Acad. Sci. U.S.A.*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyer et al., *Science* 239:1534-1536 (1988); Padlan, *Molec. Immun.* 28:489-498 (1991); Padlan, *Molec. Immunol.* 31(3):169-217 (1994); and Kettleborough, C.A. et al., *Protein Eng.* 4(7):773-83 (1991) each of which is incorporated herein by reference.

[0110] The phrase “complementarity determining region” refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. See, e.g., Chothia et al., *J. Mol. Biol.* 196:901-917 (1987); Kabat et al., U.S. Dept. of Health and Human Services NIH Publication No. 91-3242 (1991). The phrase “constant region” refers to the portion of the

antibody molecule that confers effector functions. In the present invention, mouse constant regions are substituted by human constant regions. The constant regions of the subject humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. One method of humanizing antibodies comprises aligning the non-human heavy and light chain sequences to human heavy and light chain sequences, selecting and replacing the non-human framework with a human framework based on such alignment, molecular modeling to predict the conformation of the humanized sequence and comparing to the conformation of the parent antibody. This process is followed by repeated back mutation of residues in the CDR region that disturb the structure of the CDRs until the predicted conformation of the humanized sequence model closely approximates the conformation of the non-human CDRs of the parent non-human antibody. Such humanized antibodies may be further derivatized to facilitate uptake and clearance, *e.g.* via Ashwell receptors. *See, e.g.*, U.S. Patent Nos. 5,530,101 and 5,585,089 which are incorporated herein by reference.

[0111] Humanized antibodies can also be produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/10741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin-encoding loci are substituted or inactivated. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

[0112] Using a transgenic animal described above, an immune response can be produced to a selected antigenic molecule, and antibody-producing cells can be removed

from the animal and used to produce hybridomas that secrete human monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein.

[0113] In the present invention, polypeptides of the invention and variants thereof are used to immunize a transgenic animal as described above. Monoclonal antibodies are made using methods known in the art, and the specificity of the antibodies is tested using isolated polypeptides. Methods for preparation of the human or primate polypeptide or an epitope thereof include, but are not limited to chemical synthesis, recombinant DNA techniques or isolation from biological samples. Chemical synthesis of a peptide can be performed, for example, by the classical Merrifield method of solid phase peptide synthesis (Merrifield, *J. Am. Chem. Soc.* 85:2149, 1963 which is incorporated by reference) or the FMOC strategy on a Rapid Automated Multiple Peptide Synthesis system (E. I. du Pont de Nemours Company, Wilmington, DE) (Caprino and Han, *J. Org. Chem.* 37:3404, 1972 which is incorporated by reference).

[0114] Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by subsequent boosts at appropriate intervals. The animals are bled and sera assayed against purified proteins usually by ELISA or by bioassay based upon the ability to block the action of proteins. When using avian species, e.g., chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared after the method of Milstein and Kohler by fusing splenocytes from immunized mice with continuously replicating tumor cells such as myeloma or lymphoma cells. (Milstein and Kohler, *Nature* 256:495-497, 1975; Gutfre and Milstein, *Methods in Enzymology: Immunochemical Techniques* 73:1-46, Langone and Banatis eds., Academic Press, 1981 which are incorporated by reference). The hybridoma cells so formed are then cloned by limiting dilution methods and supernatants assayed for antibody production by ELISA, RIA or bioassay.

[0115] The unique ability of antibodies to recognize and specifically bind to target proteins provides an approach for treating an overexpression of the protein. Thus, another

aspect of the present invention provides for a method for preventing or treating diseases involving overexpression of a polypeptide by treatment of a patient with specific antibodies to the protein.

[0116] Specific antibodies, either polyclonal or monoclonal, to the proteins can be produced by any suitable method known in the art as discussed above. For example, murine or human monoclonal antibodies can be produced by hybridoma technology or, alternatively, the polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to the proteins. Such antibodies can be from any class of antibodies including, but not limited to IgG, IgA, IgM, IgD, and IgE or in the case of avian species, IgY and from any subclass of antibodies.

[0117] In a preferred embodiment, oncogenes which encode secreted growth factors may be inhibited by raising antibodies against the secreted proteins of the present invention as described above. Without being bound by theory, antibodies used for treatment, bind and prevent the secreted protein from binding to its receptor, thereby inactivating the secreted protein.

[0118] In another preferred embodiment, the antibody is conjugated to a therapeutic moiety. In one aspect the therapeutic moiety is a small molecule that modulates the activity of the protein. In another aspect the therapeutic moiety modulates the activity of molecules associated with or in close proximity to the protein. The therapeutic moiety may inhibit enzymatic activity such as protease or protein kinase activity associated with cancer.

[0119] In a preferred embodiment, the polypeptides comprising one or more of the novel isoforms 2 and 3 of DKKL-1 splice products shown in Figures 4A-4B are purified or isolated after expression. Proteins may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and

reverse-phase HPLC chromatography, and chromatofocusing. For example, the protein may be purified using a standard antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., Protein Purification, Springer-Verlag, NY (1982). The degree of purification necessary will vary depending on the use of the protein. In some instances no purification will be necessary.

[0120] Once expressed and purified if necessary, the proteins and nucleic acids corresponding the novel isoforms 2 and 3 are useful in a number of applications. In one aspect, the expression levels of genes are determined for different cellular states in a cancer phenotype; that is, the expression levels of genes in normal tissue and in cancer tissue (and in some cases, for varying severities of lymphoma that relate to prognosis, as outlined below) are evaluated to provide expression profiles. An expression profile of a particular cell state or point of development is essentially a “fingerprint” of the state; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is unique to the state of the cell. By comparing expression profiles of cells in different states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. Then, diagnosis may be done or confirmed: does tissue from a particular patient have the gene expression profile of normal or cancer tissue.

[0121] “Differential expression,” or equivalents used herein, refers to both qualitative as well as quantitative differences in the temporal and/or cellular expression patterns of genes, within and among the cells. Thus, a differentially expressed gene can qualitatively have its expression altered, including an activation or inactivation, in, for example, normal versus cancer tissue. That is, genes may be turned on or turned off in a particular state, relative to another state. As is apparent to the skilled artisan, any comparison of two or more states can be made. Such a qualitatively regulated gene will exhibit an expression pattern within a state or cell type which is detectable by standard techniques in one such state or cell type, but is not detectable in both. Alternatively, the determination is quantitative in that expression is increased or decreased; that is, the expression of the gene is either up-regulated, resulting in an increased amount of transcript, or down-

regulated, resulting in a decreased amount of transcript. The degree to which expression differs need only be large enough to quantify via standard characterization techniques as outlined below, such as by use of Affymetrix GeneChip® expression arrays, Lockhart, Nature Biotechnology, 14:1675-1680 (1996), hereby expressly incorporated by reference. Other techniques include, but are not limited to, quantitative reverse transcriptase PCR, Northern analysis and RNase protection. As outlined above, preferably the change in expression (i.e. upregulation or downregulation) is at least about 50%, more preferably at least about 100%, more preferably at least about 150%, more preferably, at least about 200%, with from 300 to at least 1000% being especially preferred.

[0122] As will be appreciated by those in the art, this may be done by evaluation at either the gene transcript, or the protein level; that is, the amount of gene expression may be monitored using nucleic acid probes to the DNA or RNA equivalent of the gene transcript, and the quantification of gene expression levels, or, alternatively, the final gene product itself (protein) can be monitored, for example through the use of antibodies to the protein and standard immunoassays (ELISAs, etc.) or other techniques, including mass spectroscopy assays, 2D gel electrophoresis assays, etc.

[0123] In a preferred embodiment, gene expression monitoring is done and a number of other genes forming an expression profile including the novel isoforms 2 and 3 of DKKL-1, are monitored simultaneously. Multiple protein expression monitoring can be done as well. In this embodiment, the nucleic acid probes may be attached to biochips as outlined herein for the detection and quantification of the novel isoforms 2 and 3 of DKKL-1 sequences in a particular cell. The assays are done as is known in the art. In addition, while solid-phase assays are described, any number of solution based assays may be done as well.

Screening for Targeted Drugs

[0124] In one embodiment, any of the sequences described herein are used in drug screening assays. The DKKL-1 proteins, antibodies, nucleic acids, modified proteins corresponding to novel isoforms 2 and 3 and cells containing such sequences are used in drug screening assays or by evaluating the effect of drug candidates on a “gene expression profile” or expression profile of polypeptides. In one embodiment, the

expression profiles are used, preferably in conjunction with high throughput screening techniques to allow monitoring for expression profile genes after treatment with a candidate agent, Zlokarnik, et al., Science 279, 84-8 (1998), Heid, et al., Genome Res., 6:986-994 (1996).

[0125] Candidate bioactive agents are screened for the ability to modulate a gene. "Modulation" thus includes both an increase and a decrease in gene expression or activity. The preferred amount of modulation will depend on the original change of the gene expression in normal versus tumor tissue, with changes of at least 10%, preferably 50%, more preferably 100-300%, and in some embodiments 300-1000% or greater. Thus, if a gene exhibits a 4 fold increase in tumor compared to normal tissue, a decrease of about four fold is desired; a 10 fold decrease in tumor compared to normal tissue gives a 10 fold increase in expression for a candidate agent is desired, etc.

[0126] As will be appreciated by those in the art, this may be done by evaluation at either the gene or the protein level; that is, the amount of gene expression may be monitored using nucleic acid probes and the quantification of gene expression levels, or, alternatively, the level of the gene product itself can be monitored, for example through the use of antibodies to the protein and standard immunoassays. Alternatively, binding and bioactivity assays with the protein may be done as outlined below.

[0127] The term "candidate bioactive agent" or "drug candidate" or grammatical equivalents as used herein describes any molecule, e.g., protein, oligopeptide, small organic or inorganic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactive agents that are capable of directly or indirectly altering either the cancer phenotype, binding to and/or modulating the bioactivity of a protein, or the expression of a sequence, including both nucleic acid sequences and protein sequences. In a particularly preferred embodiment, the candidate agent suppresses a phenotype, for example to a normal tissue fingerprint. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

[0128] Candidate agents encompass numerous chemical classes, though typically they are organic or inorganic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 Daltons. Preferred small molecules are less than 2000, or less than 1500 or less than 1000 or less than 500 D. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Particularly preferred are peptides.

[0129] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, or amidification to produce structural analogs.

[0130] In one embodiment, the candidate bioactive agents are proteins. By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline and norleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The side chains may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the

(S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradations.

[0131] In a preferred embodiment, the candidate bioactive agents are naturally occurring proteins or fragments of naturally occurring proteins. Thus, for example, cellular extracts containing proteins, or random or directed digests of proteinaceous cellular extracts, may be used. In this way libraries of prokaryotic and eukaryotic proteins may be made for screening in the methods of the invention. Particularly preferred in this embodiment are libraries of bacterial, fungal, viral, and mammalian proteins, with the latter being preferred, and human proteins being especially preferred.

[0132] In another preferred embodiment, the candidate bioactive agents are peptides of from about 5 to about 30 amino acids, with from about 5 to about 20 amino acids being preferred, and from about 7 to about 15 being particularly preferred. The peptides may be digests of naturally occurring proteins as is outlined above, random peptides, or “biased” random peptides. By “randomized” or grammatical equivalents herein is meant that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. Since generally these random peptides (or nucleic acids, discussed below) are chemically synthesized, they may incorporate any nucleotide or amino acid at any position. The synthetic process can be designed to generate randomized proteins or nucleic acids, to allow the formation of all or most of the possible combinations over the length of the sequence, thus forming a library of randomized candidate bioactive proteinaceous agents.

[0133] In one embodiment, the library is fully randomized, with no sequence preferences or constants at any position. In a preferred embodiment, the library is biased. That is, some positions within the sequence are either held constant, or are selected from a limited number of possibilities. For example, in a preferred embodiment, the nucleotides or amino acid residues are randomized within a defined class, for example, of hydrophobic amino acids, hydrophilic residues, sterically biased (either small or large) residues, towards the creation of nucleic acid binding domains, the creation of cysteines, for cross-linking, prolines for SH-3 domains, serines, threonines, tyrosines or histidines for phosphorylation sites, etc., or to purines, etc.

[0134] In one embodiment, the candidate bioactive agents are nucleic acids. As described generally for proteins, nucleic acid candidate bioactive agents may be naturally occurring nucleic acids, random nucleic acids, or “biased” random nucleic acids. In another embodiment, the candidate bioactive agents are organic chemical moieties, a wide variety of which are available in the literature.

[0135] The reactions outlined herein may be accomplished in a variety of ways, as will be appreciated by those in the art. Components of the reaction may be added simultaneously, or sequentially, in any order, with preferred embodiments outlined below. In addition, the reaction may include a variety of other reagents in the assays. These include reagents like salts, buffers, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal hybridization and detection, and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used, depending on the sample preparation methods and purity of the target. In addition, either solid phase or solution based (i.e., kinetic PCR) assays may be used.

[0136] Once the assay is run, the data are analyzed to determine the expression levels, and changes in expression levels as between states, of individual genes, forming a gene expression profile. In a preferred embodiment, as for the diagnosis and prognosis applications, having identified the differentially expressed gene(s) or mutated gene(s) important in any one state, screens can be run to test for alteration of the expression of the novel isoforms of DKKL-1 polynucleotides individually. That is, screening for modulation of regulation of expression of a single gene can be done. Thus, for example, in the case of target genes whose presence or absence is unique between two states, screening is done for modulators of the target gene expression.

[0137] In addition, screens can be done for novel genes that are induced in response to a candidate agent. After identifying a candidate agent based upon its ability to suppress a DKKL-1 expression and splicing pattern and creating a normal expression pattern a screen as described above can be performed to identify genes that are specifically modulated in response to the agent. Comparing expression profiles between normal tissue

and agent treated tissue reveals genes that are not expressed in normal tissue or diseased tissue, but are expressed in agent treated tissue.

Applications of the invention

[0138] In one embodiment, the invention provides methods for assessing the oncogenic potential of the novel splice variants of the DKKL1 gene in different tissues. The assessment can be performed in multiple different biological assays including transformation assay, colony formation assay, and nude mice studies.

[0139] Proteins encoded by the splice variants are purified from recombinant systems and used as immunogen for the generation of monoclonal antibody for therapeutic purposes.

[0140] The DKK family members are known to be secreted growth factors which act as either agonist or antagonist of the *wnt* signaling pathway. The invention provides physiological receptors of DKKL1. The invention further provides methods for regulating the effects of the different DKKL-1 splice variants in either *wnt* signaling or novel receptor signaling.

[0141] Signaling pathways induced by overexpression of the DKKL1 isoforms and the oncogenic phenotype associated with these signaling events are provided. Methods for use of such pathways for screening therapeutic entities that may influence the signaling potential of the DKKL1 oncogenes are provided.

[0142] Human cancer indication on DKKL1 by expression profiling on primary tumors is provided. Other potential oncogenic mechanisms, like DNA amplification of the loci and dys-regulation of the splicing events of the DKKL1 locus on different primary tumors are also provided as methods for detection of cancer states.

[0143] In one embodiment, a method of inhibiting cancer cell division is provided. In another embodiment, a method of inhibiting tumor growth is provided. In a further embodiment, methods of treating cells or individuals with cancer are provided.

[0144] The method comprises administration of a cancer inhibitor. In particular embodiments, the cancer inhibitor is an antisense molecule, a pharmaceutical composition, a therapeutic agent or small molecule, or a monoclonal, polyclonal,

chimeric or humanized antibody. In particular embodiments, a therapeutic agent is coupled with a an antibody, preferable a monoclonal antibody.

[0145] In other embodiments, methods for detection or diagnosis of cancer cells in an individual are provided. In particular embodiments, the diagnostic/detection agent is a small molecule that preferentially binds to a DKKL-1 isoform according to the invention. In one embodiment, the diagnostic/detection agent is an antibody, preferably a monoclonal antibody, preferably linked to a detectable agent.

[0146] In other embodiments of the invention, animal models and transgenic animals are provided, which find use in generating animal models of cancers, particularly lymphomas and carcinomas.

(a) Antisense molecules

[0147] In one embodiment, the cancer inhibitor is an antisense molecule. Antisense molecules as used herein include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences for cancer molecules. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment generally at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, (1988) and van der Krol et al., *BioTechniques* 6:958, (1988).

[0148] Antisense molecules can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides. These molecules function by specifically binding to matching sequences resulting in inhibition of peptide synthesis (Wu-Pong, Nov 1994, *BioPharm*, 20-33) either by steric blocking or by activating an RNase H enzyme. Antisense molecules can also alter protein synthesis by interfering with RNA processing or transport from the nucleus into the cytoplasm (Mukhopadhyay & Roth, 1996, *Crit. Rev. in Oncogenesis* 7, 151-190). In addition, binding of single stranded DNA to RNA can result in nuclease-mediated degradation of the heteroduplex (Wu-Pong, *supra*). Backbone modified DNA chemistry which have thus far been shown to act as substrates for RNase

H are phosphorothioates, phosphorodithioates, borontrifluoridates, and 2'-arabino and 2'-fluoro arabino-containing oligonucleotides.

[0149] Antisense molecules may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell. Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. It is understood that the use of antisense molecules or knock out and knock in models may also be used in screening assays as discussed above, in addition to methods of treatment.

(b) RNA Interference

[0150] RNA interference refers to the process of sequence-specific post transcriptional gene silencing in animals mediated by short interfering RNAs (siRNA) (Fire et al., *Nature*, 391, 806 (1998)). The corresponding process in plants is referred to as post transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L. (reviewed in Sharp, P.A., *RNA interference – 2001*, *Genes & Development* 15:485-490 (2001)).

[0151] Small interfering RNAs (siRNAs) are powerful sequence-specific reagents designed to suppress the expression of genes in cultured mammalian cells through a process known as RNA interference (RNAi). Elbashir, S.M. *et al. Nature* 411:494-498 (2001); Caplen, N.J. *et al. Proc. Natl. Acad. Sci. USA* 98:9742-9747 (2001); Harborth, J.

et al. J. Cell Sci. 114:4557-4565 (2001). The term "short interfering RNA" or "siRNA" refers to a double stranded nucleic acid molecule capable of RNA interference "RNAi", (see Kreutzer et al., WO 00/44895; Zernicka-Goetz et al. WO 01/36646; Fire, WO 99/32619; Mello and Fire, WO 01/29058). As used herein, siRNA molecules are limited to RNA molecules but further encompasses chemically modified nucleotides and non-nucleotides. siRNA gene-targeting experiments have been carried out by transient siRNA transfer into cells (achieved by such classic methods as liposome-mediated transfection, electroporation, or microinjection).

[0152] Molecules of siRNA are 21- to 23-nucleotide RNAs, with characteristic 2- to 3-nucleotide 3'-overhanging ends resembling the RNase III processing products of long double-stranded RNAs (dsRNAs) that normally initiate RNAi. When introduced into a cell, they assemble with yet-to-be-identified proteins of an endonuclease complex (RNA-induced silencing complex), which then guides target mRNA cleavage. As a consequence of degradation of the targeted mRNA, cells with a specific phenotype characteristic of suppression of the corresponding protein product are obtained. The small size of siRNAs, compared with traditional antisense molecules, prevents activation of the dsRNA-inducible interferon system present in mammalian cells. This avoids the nonspecific phenotypes normally produced by dsRNA larger than 30 base pairs in somatic cells.

[0153] Intracellular transcription of small RNA molecules is achieved by cloning the siRNA templates into RNA polymerase III (Pol III) transcription units, which normally encode the small nuclear RNA (snRNA) U6 or the human RNase P RNA H1. Two approaches have been developed for expressing siRNAs: in the first, sense and antisense strands constituting the siRNA duplex are transcribed by individual promoters (Lee, N.S. *et al. Nat. Biotechnol.* **20**, 500-505 (2002). Miyagishi, M. & Taira, K. *Nat. Biotechnol.* **20**, 497-500 (2002).); in the second, siRNAs are expressed as fold-back stem-loop structures that give rise to siRNAs after intracellular processing (Paul, C.P. *et al. Nat. Biotechnol.* **20**:505-508 (2002)). The endogenous expression of siRNAs from introduced DNA templates is thought to overcome some limitations of exogenous siRNA delivery, in particular the transient loss of phenotype. U6 and H1 RNA promoters are members of the type III class of Pol III promoters. (Paule, M.R. & White, R.J. *Nucleic Acids Res.* **28**, 1283-1298 (2000)).

[0154] Co-expression of sense and antisense siRNAs mediate silencing of target genes, whereas expression of sense or antisense siRNA alone do not greatly affect target gene expression. Transfection of plasmid DNA, rather than synthetic siRNAs, may appear advantageous, considering the danger of RNase contamination and the costs of chemically synthesized siRNAs or siRNA transcription kits. Stable expression of siRNAs allows new gene therapy applications, such as treatment of persistent viral infections. Considering the high specificity of siRNAs, the approach also allows the targeting of disease-derived transcripts with point mutations, such as *RAS* or *TP53* oncogene transcripts, without alteration of the remaining wild-type allele. Finally, by high-throughput sequence analysis of the various genomes, the DNA-based methodology may also be a cost-effective alternative for automated genome-wide loss-of-function phenotypic analysis, especially when combined with miniaturized array-based phenotypic screens. (Ziauddin, J. & Sabatini, D.M. *Nature* 411:107-110 (2001)).

[0155] The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNA) (Berstein et al., 2001, *Nature*, 409:363 (2001)). Short interfering RNAs derived from dicer activity are typically about 21-23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21 and 22 nucleotide small temporal RNAs (stRNA) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., *Science*, 293, 834 (2001)). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir et al., *Genes Dev.*, 15, 188 (2001)).

[0156] This invention provides an expression system comprising an isolated nucleic acid molecule comprising a sequence capable of specifically hybridizing to the polynucleotide sequences of the novel DKKL-1 isoforms. In an embodiment, the nucleic acid molecule is capable of inhibiting the expression of the corresponding protein. A method of inhibiting expression of the novel DKKL-1 isoforms inside a cell by a vector-

directed expression of a short RNA which short RNA can fold in itself and create a double strand RNA having the novel DKKL-1 isoforms mRNA sequence identity and able to trigger posttranscriptional gene silencing, or RNA interference (RNAi), of the novel isoforms of the DKKL-1 gene inside the cell. In another method a short double strand RNA having the novel DKKL-1 isoform mRNA sequence identity is delivered inside the cell to trigger posttranscriptional gene silencing, or RNAi, of the novel DKKL-1 isoforms. In various embodiments, the nucleic acid molecule is at least a 7 mer, at least a 10 mer, or at least a 20 mer. In a further embodiment, the sequence is unique.

(c) Pharmaceutical Compositions

[0157] Pharmaceutical compositions encompassed by the present invention include as active agent, the polypeptides, polynucleotides, antisense oligonucleotides, or antibodies of the invention disclosed herein in a therapeutically effective amount. An "effective amount" is an amount sufficient to effect beneficial or desired results, including clinical results. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of an adenoviral vector is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state.

[0158] The compositions can be used to treat cancer as well as metastases of primary cancer. In addition, the pharmaceutical compositions can be used in conjunction with conventional methods of cancer treatment, *e.g.*, to sensitize tumors to radiation or conventional chemotherapy. The terms "treatment", "treating", "treat" and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, *i.e.*, arresting its development; or (c) relieving the disease symptom, *i.e.*, causing regression of the disease or symptom.

[0159] Where the pharmaceutical composition comprises an antibody that specifically binds to a gene product encoded by a differentially expressed polynucleotide, the antibody can be coupled to a drug for delivery to a treatment site or coupled to a detectable label to facilitate imaging of a site comprising cancer cells, such as prostate cancer cells. Methods for coupling antibodies to drugs and detectable labels are well known in the art, as are methods for imaging using detectable labels.

[0160] A "patient" for the purposes of the present invention includes both humans and other animals, particularly mammals, and organisms. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient is a mammal, and in the most preferred embodiment the patient is human.

[0161] The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. The effective amount for a given situation is determined by routine experimentation and is within the judgment of the clinician. For purposes of the present invention, an effective dose will generally be from about 0.01 mg/kg to about 5 mg/kg, or about 0.01 mg/kg to about 50 mg/kg or about 0.05 mg/kg to about 10 mg/kg of the compositions of the present invention in the individual to which it is administered.

[0162] A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus

particles. Such carriers are well known to those of ordinary skill in the art. Pharmaceutically acceptable carriers in therapeutic compositions can include liquids such as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier. Pharmaceutically acceptable salts can also be present in the pharmaceutical composition, e.g., mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington: The Science and Practice of Pharmacy* (1995) Alfonso Gennaro, Lippincott, Williams, & Wilkins.

[0163] The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents.

[0164] The pharmaceutical compositions of the present invention are preferably in a water soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic

acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0165] The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations.

[0166] The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a host, as previously described. The agents may be administered in a variety of ways, orally, parenterally e.g., subcutaneously, intraperitoneally, intravascularly, etc. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100% wt/vol. Once formulated, the compositions contemplated by the invention can be (1) administered directly to the subject (e.g., as polynucleotide, polypeptides, small molecule agonists or antagonists, and the like); or (2) delivered ex vivo, to cells derived from the subject (e.g., as in *ex vivo* gene therapy). Direct delivery of the compositions will generally be accomplished by parenteral injection, e.g., subcutaneously, intraperitoneally, intravenously or intramuscularly, intratumoral or to the interstitial space of a tissue. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment can be a single dose schedule or a multiple dose schedule.

[0167] Methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and described in *e.g.*, International Publication No. WO 93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells. Generally, delivery of nucleic acids for both ex vivo and in vitro applications can be accomplished by, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

[0168] The dose and the means of administration of the inventive pharmaceutical compositions are determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. For example, administration of polynucleotide therapeutic compositions agents includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. Preferably, the therapeutic polynucleotide composition contains an expression construct comprising a promoter operably linked to a polynucleotide of at least 12, 22, 25, 30, or 35 contiguous nt of the polynucleotide disclosed herein. Various methods can be used to administer the therapeutic composition directly to a specific site in the body. For example, a small metastatic lesion is located and the therapeutic composition injected several times in several different locations within the body of tumor. Alternatively, arteries that serve a tumor are identified, and the therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor. A tumor that has a necrotic center is aspirated and the composition injected directly into the now empty center of the tumor. An antisense composition is directly administered to the surface of the tumor, for example, by topical application of the composition. X-ray imaging is used to assist in certain of the above delivery methods.

[0169] Targeted delivery of therapeutic compositions containing an antisense polynucleotide, subgenomic polynucleotides, or antibodies to specific tissues can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis *et al.*, *Trends Biotechnol.* (1993) 11:202; Chiou *et al.*, *Gene Therapeutics: Methods And*

Applications Of Direct Gene Transfer (J.A. Wolff, ed.) (1994); Wu *et al.*, *J. Biol. Chem.* (1988) 263:621; Wu *et al.*, *J. Biol. Chem.* (1994) 269:542; Zenke *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1990) 87:3655; Wu *et al.*, *J. Biol. Chem.* (1991) 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. Factors such as method of action (e.g., for enhancing or inhibiting levels of the encoded gene product) and efficacy of transformation and expression are considerations that will affect the dosage required for ultimate efficacy of the antisense subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of antisense subgenomic polynucleotides or the same amounts re-administered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions of, for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

[0170] The therapeutic polynucleotides and polypeptides of the present invention can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, *Cancer Gene Therapy* (1994) 1:51; Kimura, *Human Gene Therapy* (1994) 5:845; Connelly, *Human Gene Therapy* (1995) 1:185; and Kaplitt, *Nature Genetics* (1994) 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

[0171] Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; USPN 5, 219,740; WO 93/11230; WO 93/10218; USPN 4,777,127; GB Patent No. 2,200,651; EP 0 345 242; and WO 91/02805), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine

encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, e.g., WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) 3:147 can also be employed.

[0172] Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, e.g., Curiel, *Hum. Gene Ther.* (1992) 3:147); ligand-linked DNA (see, e.g., Wu, *J. Biol. Chem.* (1989) 264:16985); eukaryotic cell delivery vehicles cells (see, e.g., USPN 5,814,482; WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and USPN 5,580,859. Liposomes that can act as gene delivery vehicles are described in USPN 5,422,120; WO 95/13796; WO 94/23697; WO 91/14445; and EP 0524968. Additional approaches are described in Philip, *Mol. Cell Biol.* (1994) 14:2411, and in Woffendin, *Proc. Natl. Acad. Sci.* (1994) 91:1581.

[0173] Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al.*, *Proc. Natl. Acad. Sci. USA* (1994) 91(24):11581. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials or use of ionizing radiation (see, e.g., USPN 5,206,152 and WO 92/11033). Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun (see, e.g., USPN 5,149,655); use of ionizing radiation for activating transferred gene (see, e.g., USPN 5,206,152 and WO 92/11033).

(d) Antibodies

[0174] In one embodiment, a cancer inhibitor is an antibody as discussed above. In one embodiment, the novel DKKL-1 isoform proteins of the present invention may be used to generate polyclonal and monoclonal antibodies to the proteins, which are useful as described herein. Similarly, the proteins can be coupled, using standard technology, to

affinity chromatography columns. These columns may then be used to purify antibodies against polypeptides of the novel DKKL-1 isoforms. In a preferred embodiment, the antibodies are generated to epitopes unique to a protein; that is, the antibodies show little or no cross-reactivity to other proteins. These antibodies find use in a number of applications. For example, the antibodies may be coupled to standard affinity chromatography columns and used to purify the novel DKKL-1 isoform proteins. The antibodies may also be used therapeutically as blocking polypeptides, as outlined above, since they will specifically bind to the protein.

[0175] Detection of specific binding of the antibody specific for the encoded cancer-associated polypeptide, when compared to a suitable control is an indication that encoded polypeptide is present in the sample. Suitable controls include a sample known not to contain the encoded the novel DKKL-1 isoform polypeptides or known not to contain elevated levels of the polypeptide; such as normal tissue, and a sample contacted with an antibody not specific for the encoded polypeptide, e.g., an anti-idiotypic antibody. A variety of methods to detect specific antibody-antigen interactions are known in the art and can be used in the method, including, but not limited to, standard immunohistological methods, immunoprecipitation, an enzyme immunoassay, and a radioimmunoassay. In general, the specific antibody will be detectably labeled, either directly or indirectly. Direct labels include radioisotopes; enzymes whose products are detectable (e.g., luciferase, β -galactosidase, and the like); fluorescent labels (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, and the like); fluorescence emitting metals, e.g., ^{152}Eu , or others of the lanthanide series, attached to the antibody through metal chelating groups such as EDTA; chemiluminescent compounds, e.g., luminol, isoluminol, acridinium salts, and the like; bioluminescent compounds, e.g., luciferin, aequorin (green fluorescent protein), and the like. The antibody may be attached (coupled) to an insoluble support, such as a polystyrene plate or a bead. Indirect labels include second antibodies specific for antibodies specific for the encoded polypeptide ("first specific antibody"), wherein the second antibody is labeled as described above; and members of specific binding pairs, e.g., biotin-avidin, and the like. The biological sample may be brought into contact with and immobilized on a solid support or carrier, such as nitrocellulose, that is capable of immobilizing cells, cell particles, or soluble proteins. The support may then be

washed with suitable buffers, followed by contacting with a detectably-labeled first specific antibody. Detection methods are known in the art and will be chosen as appropriate to the signal emitted by the detectable label. Detection is generally accomplished in comparison to suitable controls, and to appropriate standards.

[0176] In some embodiments, the methods are adapted for use *in vivo*, e.g., to locate or identify sites where cancer cells are present. In these embodiments, a detectably-labeled moiety, e.g., an antibody, which is specific for a cancer-associated polypeptide is administered to an individual (e.g., by injection), and labeled cells are located using standard imaging techniques, including, but not limited to, magnetic resonance imaging, computed tomography scanning, and the like. In this manner, cancer cells are differentially labeled.

(e) Detection and Diagnosis of Cancers

[0177] Without being bound by theory, it appears that the various the novel DKKL-1 isoform sequences are important in cancers. In one embodiment, the invention provides methods for identifying cells containing the novel splice form polynucleotides. As will be appreciated by those in the art, this may be done using any number of sequencing techniques.

[0178] In a preferred embodiment, the novel DKKL-1 isoform sequences are used as probes to determine the number of copies of the DKKL-1 gene in the genome. For example, some cancers exhibit chromosomal deletions or insertions, resulting in an alteration in the copy number of a gene.

[0179] The present invention provides methods of using the polynucleotides described herein for detecting cancer cells, facilitating diagnosis of cancer and the severity of a cancer (e.g., tumor grade, tumor burden, and the like) in a subject, facilitating a determination of the prognosis of a subject, and assessing the responsiveness of the subject to therapy (e.g., by providing a measure of therapeutic effect through, for example, assessing tumor burden during or following a chemotherapeutic regimen). Detection can be based on detection of a polynucleotide that is differentially expressed in a cancer cell, and/or detection of a polypeptide encoded by a polynucleotide that is

differentially expressed in a cancer cell. The detection methods of the invention can be conducted *in vitro* or *in vivo*, on isolated cells, or in whole tissues or a bodily fluid *e.g.*, blood, plasma, serum, urine, and the like).

[0180] In some embodiments, methods are provided for detecting a cancer cell by detecting expression in the cell of a transcript that is differentially expressed in a cancer cell. Any of a variety of known methods can be used for detection, including, but not limited to, detection of a transcript by hybridization with a polynucleotide that hybridizes to a polynucleotide that is differentially expressed in a prostate cancer cell; detection of a transcript by a polymerase chain reaction using specific oligonucleotide primers; *in situ* hybridization of a cell using as a probe a polynucleotide that hybridizes to a gene that is differentially expressed in a prostate cancer cell. The methods can be used to detect and/or measure mRNA levels of a gene that is differentially expressed in a cancer cell. In some embodiments, the methods comprise: a) contacting a sample with a polynucleotide that corresponds to a differentially expressed gene described herein under conditions that allow hybridization; and b) detecting hybridization, if any.

[0181] Detection of differential hybridization, when compared to a suitable control, is an indication of the presence in the sample of a polynucleotide that is differentially expressed in a cancer cell. Appropriate controls include, for example, a sample that is known not to contain a polynucleotide that is differentially expressed in a cancer cell, and use of a labeled polynucleotide of the same "sense" as the polynucleotide that is differentially expressed in the cancer cell. Conditions that allow hybridization are known in the art, and have been described in more detail above. Detection can also be accomplished by any known method, including, but not limited to, *in situ* hybridization, PCR (polymerase chain reaction), RT-PCR (reverse transcription-PCR), TMA, bDNA, and Nasbau and "Northern" or RNA blotting, or combinations of such techniques, using a suitably labeled polynucleotide. A variety of labels and labeling methods for polynucleotides are known in the art and can be used in the assay methods of the invention. Specificity of hybridization can be determined by comparison to appropriate controls.

[0182] Polynucleotides generally comprising at least 10 nt, at least 12nt or at least 15 contiguous nucleotides of a polynucleotide provided herein are used for a variety of purposes, such as probes for detection of and/or measurement of, transcription levels of a polynucleotide that is differentially expressed in a prostate cancer cell. As will be readily appreciated by the ordinarily skilled artisan, the probe can be detectably labeled and contacted with, for example, an array comprising immobilized polynucleotides obtained from a test sample (*e.g.*, mRNA). Alternatively, the probe can be immobilized on an array and the test sample detectably labeled. These and other variations of the methods of the invention are well within the skill in the art and are within the scope of the invention.

[0183] Nucleotide probes are used to detect expression of a gene corresponding to the provided polynucleotide. In Northern blots, mRNA is separated electrophoretically and contacted with a probe. A probe is detected as hybridizing to an mRNA species of a particular size. The amount of hybridization can be quantitated to determine relative amounts of expression, for example under a particular condition. Probes are used for *in situ* hybridization to cells to detect expression. Probes can also be used *in vivo* for diagnostic detection of hybridizing sequences. Probes are typically labeled with a radioactive isotope. Other types of detectable labels can be used such as chromophores, fluorophores, and enzymes. Other examples of nucleotide hybridization assays are described in WO92/02526 and USPN 5,124,246.

[0184] PCR is another means for detecting small amounts of target nucleic acids (see, *e.g.*, Mullis *et al.*, *Meth. Enzymol.* (1987) 155:335; USPN 4,683,195; and USPN 4,683,202). Two primer oligonucleotides that hybridize with the target nucleic acids are used to prime the reaction. The primers can be composed of sequence within or 3' and 5' to the polynucleotides disclosed herein. Alternatively, if the primers are 3' and 5' to these polynucleotides, they need not hybridize to them or the complements. After amplification of the target with a thermostable polymerase, the amplified target nucleic acids can be detected by methods known in the art, *e.g.*, Southern blot. mRNA or cDNA can also be detected by traditional blotting techniques (*e.g.*, Southern blot, Northern blot, etc.) described in Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual" (New York, Cold Spring Harbor Laboratory, 1989) (*e.g.*, without PCR amplification). In general, mRNA or cDNA generated from mRNA using a polymerase enzyme can be purified and

separated using gel electrophoresis, and transferred to a solid support, such as nitrocellulose. The solid support is exposed to a labeled probe, washed to remove any unhybridized probe, and duplexes containing the labeled probe are detected.

[0185] Methods using PCR amplification can be performed on the DNA from a single cell, although it is convenient to use at least about 10^5 cells. The use of the polymerase chain reaction is described in Saiki et al. (1985) *Science* 239:487, and a review of current techniques may be found in Sambrook, *et al.* Molecular Cloning: A Laboratory Manual, CSH Press 1989, pp. 14.2-14.33. A detectable label may be included in the amplification reaction. Suitable detectable labels include fluorochromes, (*e.g.* fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein, 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA)), radioactive labels, (*e.g.* ^{32}P , ^{35}S , ^3H , *etc.*), and the like. The label may be a two stage system, where the polynucleotides is conjugated to biotin, haptens, *etc.* having a high affinity binding partner, *e.g.* avidin, specific antibodies, *etc.*, where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

[0186] The detection methods can be provided as part of a kit. Thus, the invention further provides kits for detecting the presence and/or a level of a polynucleotide that is differentially expressed in a cancer cell (*e.g.*, by detection of an mRNA encoded by the differentially expressed gene of interest), and/or a polypeptide encoded thereby, in a biological sample. Procedures using these kits can be performed by clinical laboratories, experimental laboratories, medical practitioners, or private individuals. The kits of the invention for detecting a polypeptide encoded by a polynucleotide that is differentially expressed in a cancer cell may comprise a moiety that specifically binds the polypeptide, which may be an antibody that binds the polypeptide or fragment thereof. The kits of the invention used for detecting a polynucleotide that is differentially expressed in a prostate cancer cell may comprise a moiety that specifically hybridizes to such a polynucleotide. The kit may optionally provide additional components that are useful in the procedure,

including, but not limited to, buffers, developing reagents, labels, reacting surfaces, means for detection, control samples, standards, instructions, and interpretive information.

[0187] The present invention further relates to methods of detecting/diagnosing a neoplastic or preneoplastic condition in a mammal (for example, a human). "Diagnosis" as used herein generally includes determination of a subject's susceptibility to a disease or disorder, determination as to whether a subject is presently affected by a disease or disorder, prognosis of a subject affected by a disease or disorder (*e.g.*, identification of pre-metastatic or metastatic cancerous states, stages of cancer, or responsiveness of cancer to therapy), and therametrics (*e.g.*, monitoring a subject's condition to provide information as to the effect or efficacy of therapy).

[0188] The terms "treatment", "treating", "treat" and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, *i.e.*, arresting its development; or (c) relieving the disease symptom, *i.e.*, causing regression of the disease or symptom.

[0189] An "effective amount" is an amount sufficient to effect beneficial or desired results, including clinical results. An effective amount can be administered in one or more administrations.

[0190] A "cell sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for

certain components, such as proteins or polynucleotides. The term "cell sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[0191] As used herein, the terms "neoplastic cells", "neoplasia", "tumor", "tumor cells", "cancer" and "cancer cells", (used interchangeably) refer to cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation (i.e., de-regulated cell division). Neoplastic cells can be malignant or benign.

[0192] The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on. Examples of conditions that can be detected/diagnosed in accordance with these methods include cancers. Polynucleotides corresponding to genes that exhibit the appropriate expression pattern can be used to detect cancer in a subject. For a review of markers of cancer, see, *e.g.*, Hanahan et al. Cell 100:57-70 (2000).

[0193] Biological samples suitable for use in this method include biological fluids such as serum, plasma, pleural effusions, urine and cerebro-spinal fluid, CSF, tissue samples (e.g., mammary tumor or prostate tissue slices) can also be used in the method of the invention, including samples derived from biopsies. Cell cultures or cell extracts derived, for example, from tissue biopsies can also be used.

[0194] The compound is preferably a binding protein, e.g., an antibody, polyclonal or monoclonal, or antigen binding fragment thereof, which can be labeled with a detectable marker (e.g., fluorophore, chromophore or isotope, etc). Where appropriate, the compound can be attached to a solid support such as a bead, plate, filter, resin, etc. Determination of formation of the complex can be effected by contacting the complex with a further compound (e.g., an antibody) that specifically binds to the first compound (or complex). Like the first compound, the further compound can be attached to a solid support and/or can be labeled with a detectable marker.

[0195] The identification of elevated levels of the novel DKKL-1 isoform polypeptides in accordance with the present invention makes possible the identification of subjects (patients) that are likely to benefit from adjuvant therapy. For example, a biological sample from a post primary therapy subject (e.g., subject having undergone surgery) can be screened for the presence of circulating protein, the presence of elevated levels of the protein, determined by studies of normal populations, being indicative of residual tumor tissue. Similarly, tissue from the cut site of a surgically removed tumor can be examined (e.g., by immunofluorescence), the presence of elevated levels of product (relative to the surrounding tissue) being indicative of incomplete removal of the tumor. The ability to identify such subjects makes it possible to tailor therapy to the needs of the particular subject. Subjects undergoing non-surgical therapy, e.g., chemotherapy or radiation therapy, can also be monitored, the presence in samples from such subjects of elevated levels of the protein being indicative of the need for continued treatment. Staging of the disease (for example, for purposes of optimizing treatment regimens) can also be effected, for example, by biopsy.

[0196] Certain aspects of the present invention are described in greater detail in the non-limiting examples that follow.

EXAMPLES

[0197] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all and only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric.

Example 1: Detection of novel splice forms of DKKL-1

[0198] Expression products of the human DKKL1 gene were amplified and cloned from Origene “Multiple-choice first-strand cDNA CH-1011- testis” – a gene expression library of the human testis tissue using gene specific primers designed against publicly available sequences of DKKL1: NM_014419 and AF177398. In addition to the known isoform 1, two novel splice variants of the human DKKL1 gene were identified by aberrant size and sequenced.

[0199] Alignments were performed using the Celera (hCG16206 and hCT_7238) sequences of DKKL-1 as shown in Figure 1. A diagram of alignment in terms of complexity is shown in Figure 2 using the coding sequence of the Celera hCT_7238.

Example 2: Characterization of novel splice forms of DKKL-1

[0200] Nucleotide sequence alignment of the novel isoforms 2 and 3 of DKKL-1 splice products with the coding sequence of the Celera hCT_7238 is shown in Figures 3A-3E. Sagres clones 379-stop, 379-R6, 379-R7, 379-R3 and 379-RS2 represent the known normal splice pattern of isoform 1. The coding sequences were aligned with the sequences of Sagres clones starting at position -4 before the start codon and ending at the stop codon for DKKL-1. A novel isoform 2 comprises the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E and lacks exon 4. The novel splice junction of isoform 2 spans nucleotides 329-330 of the DKKL-1 coding sequence. A novel isoform 3 comprises the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E and lacks exons 3 and 4. The novel splice junction of isoform 3 spans positions 188 and 189 of the DKKL-1 coding sequence.

[0201] Polypeptide sequence alignment of the novel isoforms 2 and 3 of DKKL-1 splice products with normal isoform 1 and Celera hCT_7238 is shown in Figures 4A-4B. The novel isoform 2 has a novel splice junction spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B. The novel isoform 3 comprises the novel splice junction spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B.

[0202] All three splice variants (isoform 1, 2 and 3) were secreted when overexpressed and localized in the plasma membrane of some cancer cell lines that were tested. This behavior is similar to known human Dickkopf (dkk) proteins.

Example 3: Detection of elevated levels of cDNA associated with cancer using arrays.

[0203] cDNA sequences representing the novel DKKL-1 isoforms to be screened for differential expression in cancer are assayed by hybridization on polynucleotide arrays. The cDNA sequences include cDNA clones isolated from cell lines or tissues of interest. cDNAs are spotted onto reflective slides (Amersham) according to methods well known in the art at a density of 9,216 spots per slide representing 4,068 sequences (including controls) spotted in duplicate, with approximately 0.8 μ l of an approximately 200ng/ μ l solution of cDNA.

[0204] PCR products of selected cDNA clones corresponding to the gene products of interest are prepared in a 50% DMSO solution. These PCR products are spotted onto Amersham aluminum microarray slides at a density of 9216 clones per array using a Molecular Dynamics Generation III spotting robot. Clones are spotted in duplicate, for a total of 4608 different sequences per chip.

[0205] cDNA probes are prepared from total RNA obtained by laser capture microdissection (LCM, Arcturus Engineering Inc., Mountain View, CA) of tumor tissue samples and normal tissue samples isolated from patients.

[0206] Total RNA is first reverse transcribed into cDNA using a primer containing a T7 RNA polymerase promoter, followed by second strand DNA synthesis. cDNA is then transcribed *in vitro* to produce antisense RNA using the T7 promoter-mediated expression (see, *e.g.*, Luo *et al.* (1999) *Nature Med* 5:117-122), and the antisense RNA is then converted into cDNA. The second set of cDNAs are again transcribed *in vitro*, using the T7 promoter, to provide antisense RNA. This antisense RNA is then fluorescently labeled, or the RNA is again converted into cDNA, allowing for a third round of T7-mediated amplification to produce more antisense RNA. Thus the procedure provides for two or three rounds of *in vitro* transcription to produce the final RNA used for fluorescent

labeling. Probes are labeled by making fluorescently labeled cDNA from the RNA starting material. Fluorescently labeled cDNAs prepared from the tumor RNA sample are compared to fluorescently labeled cDNAs prepared from normal cell RNA sample. For example, the cDNA probes from the normal cells are labeled with Cy3 fluorescent dye (green) and the cDNA probes prepared from suspected cancer cells are labeled with Cy5 fluorescent dye (red).

[0207] The differential expression assay is performed by mixing equal amounts of probes from tumor cells and normal cells of the same patient. The arrays are prehybridized by incubation for about 2 hrs at 60°C in 5x SSC, 0.2% SDS, 1 mM EDTA, and then washing three times in water and twice in isopropanol. Following prehybridization of the array, the probe mixture is then hybridized to the array under conditions of high stringency (overnight at 42°C in 50% formamide, 5X SSC, and 0.2% SDS. After hybridization, the array is washed at 55°C three times as follows: 1) first wash in 1X SSC/0.2% SDS; 2) second wash in 0.1X SSC/0.2% SDS; and 3) third wash in 0.1X SSC.

[0208] The arrays are then scanned for green and red fluorescence using a Molecular Dynamics Generation III dual color laser-scanner/detector. The images are processed using BioDiscovery Autogene software, and the data from each scan set normalized. The experiment is repeated, this time labeling the two probes with the opposite color in order to perform the assay in both “color directions.” Each experiment is sometimes repeated with two more slides (one in each color direction). The data from each scan is normalized, and the level of fluorescence for each sequence on the array expressed as a ratio of the geometric mean of 8 replicate spots/genes from the four arrays or 4 replicate spots/gene from 2 arrays or some other permutation.

[0209] Normalization: The objective of normalization is to generate a cDNA library in which all transcripts expressed in a particular cell type or tissue are equally represented (S.M. Weissman, Mol Biol. Med. 4(3):133-143 (1987); Patanjali, et al., Proc. Natl. Acad. Sci. USA 88(5):1943-1947 (1991)), and therefore isolation of as few as 30,000 recombinant clones in an optimally normalized library may represent the entire gene expression repertoire of a cell, estimated to number 10,000 per cell.

[0210] Total RNA is extracted from harvested cells using RNeasy™ Protect Kit (Qiagen, Valencia, CA), following manufacturer's recommended procedures. RNA is quantified using RiboGreen™ RNA quantification kit (Molecular Probes, Inc. Eugene, OR). One µg of total RNA is reverse transcribed and PCR amplified using SMART™ PCR cDNA synthesis kit (ClonTech, Palo Alto, CA). The cDNA products are size-selected by agarose gel electrophoresis using standard procedures (Sambrook, J.T., et al. *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, NY). The cDNA is extracted using Bio 101 GeneClean® II kit (Qbiogene, Carlsbad, CA). Normalization of the cDNA is carried out using kinetics of hybridization principles: 1.0 µg of cDNA is denatured by heat at 100° C for 10 minutes, then incubated at 42° C for 42 hours in the presence of 120 mM NaCl, 10 mM Tris.HCl (pH=8.0), 5 mM EDTA.Na⁺ and 50% formamide. Single-stranded cDNA ("normalized") is purified by hydroxyapatite chromatography (#130-0520, BioRad, Hercules, CA) following the manufacturer's recommended procedures, amplified and converted to double-stranded cDNA by three cycles of PCR amplification, and cloned into plasmid vectors using standard procedures (Sambrook, J.T., et al. *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, NY). All primers/adaptors used in the normalization and cloning process are provided by the manufacturer in the SMART™ PCR cDNA synthesis kit (ClonTech, Palo Alto, CA). Supercompetent cells (XL-2 Blue Ultracompetent Cells, Stratagene, California) are transfected with the normalized cDNA libraries, plated on solid media and grown overnight at 36° C.

[0211] The sequences of 10,000 recombinants per normalized library are analyzed by capillary sequencing using the ABI PRISM 3700 DNA Analyzer (Applied Biosystems, California). To determine the representation of transcripts in a library, BLAST analysis is performed on the clone sequences to assign transcript identity to each isolated clone, i.e., the sequences of the isolated polynucleotides are first masked to eliminate low complexity sequences using the XBLAST masking program (Claverie "Effective Large-Scale Sequence Similarity Searches," Computer Methods for Macromolecular Sequence Analysis, Doolittle, ed., Meth. Enzymol. 266:212-227 Academic Press, NY, NY (1996); see particularly Claverie, in "Automated DNA Sequencing and Analysis Techniques" Adams *et al.*, eds., Chap. 36, p. 267 Academic Press, San Diego, 1994 and Claverie *et al.*

Comput. Chem. (1993) 17:191). Generally, masking does not influence the final search results, except to eliminate sequences of relative little interest due to their low complexity, and to eliminate multiple “hits” based on similarity to repetitive regions common to multiple sequences, e.g., Alu repeats. The remaining sequences are then used in a BLASTN vs. GenBank search. The sequences are also used as query sequence in a BLASTX vs. NRP (non-redundant proteins) database search.

[0212] Automated sequencing reactions are performed using a Perkin-Elmer PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit containing AmpliTaq DNA Polymerase, FS, according to the manufacturer's directions. The reactions are cycled on a GeneAmp PCR System 9600 as per manufacturer's instructions, except that they are annealed at 20° C. or 30° C. for one minute. Sequencing reactions are ethanol precipitated, pellets are resuspended in 8 microliters of loading buffer, 1.5 microliters is loaded on a sequencing gel, and the data is collected by an ABI PRISM 3700 DNA Sequencer. (Applied Biosystems, Foster City, CA).

[0213] The number of times a sequence is represented in a library is determined by performing sequence identity analysis on the cloned cDNA sequences and assigning transcript identity to each isolated clone. First, each sequence is checked to determine if it is a bacterial, ribosomal, or mitochondrial contaminant. Such sequences are excluded from the subsequent analysis. Second, sequence artifacts, such as vector and repetitive elements, are masked and/or removed from each sequence.

[0214] The remaining sequences are compared via BLAST (Altschul et. al, J. Mol. Biol., 215:40, 1990) to GenBank and EST databases for gene identification and are compared with each other via FastA (Pearson & Lipman, PNAS, 85:2444, 1988) to calculate the frequency of cDNA appearance in the normalized cDNA library. The sequences are also searched against the GenBank and GeneSeq nucleotide databases using the BLASTN program (BLASTN 1.3MP: Altschul et al., J. Mol. Bio. 215:403, 1990). Fourth, the sequences are analyzed against a non-redundant protein (NRP) database with the BLASTX program (BLASTX 1.3MP: Altschul et al., supra). This protein database is a combination of the Swiss-Prot, PIR, and NCBI GenPept protein databases. The BLASTX program is run using the default BLOSUM-62 substitution

matrix with the filter parameter: "xnu+seg". The score cutoff utilized is 75. Assembly of overlapping clones into contigs is done using the program Sequencher (Gene Codes Corp.; Ann Arbor, Mich.). The assembled contigs are analyzed using the programs in the GCG package (Genetic Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711) Suite Version 10.1.

Example 4: Detection novel DKKL-1 isoforms in Human Cancer Cells and Tissues.

[0215] DNA from human cancer tissues, human colon, normal human tissues and from other human cell lines are extracted following the procedure of Delli Bovi et al. (1986, Cancer Res. 46:6333-6338). The DNA is resuspended in a solution containing 0.05 M Tris HCl buffer, pH 7.8, and 0.1 mM EDTA, and the amount of DNA recovered is determined by microfluorometry using Hoechst 33258 dye. Cesarone, C. et al., Anal Biochem 100:188-197 (1979).

[0216] Polymerase chain reaction (PCR) is performed using Taq polymerase following the conditions recommended by the manufacturer (Perkin Elmer Cetus) with regard to buffer, Mg^{2+} , and nucleotide concentrations. Thermocycling is performed in a DNA cycler by denaturation at 94° C. for 3 min. followed by either 35 or 50 cycles of 94° C. for 1.5 min., 50° C. for 2 min. and 72° C. for 3 min. The ability of the PCR to amplify the selected regions of the gene is tested by using a cloned polynucleotide(s) as a positive template(s). Optimal Mg^{2+} , primer concentrations and requirements for the different cycling temperatures are determined with these templates. The master mix recommended by the manufacturer is used. To detect possible contamination of the master mix components, reactions without template are routinely tested.

[0217] Southern blotting and hybridization are performed as described by Southern, E. M., (J. Mol. Biol. 98:503-517, 1975), using the cloned sequences labeled by the random primer procedure (Feinberg, A. P., et al., 1983, Anal. Biochem. 132:6-13).

Prehybridization and hybridization are performed in a solution containing 6xSSPE, 5% Denhardt's, 0.5% SDS, 50% formamide, 100 µg/ml denaturated salmon testis DNA, incubated for 18 hrs at 42° C., followed by washings with 2xSSC and 0.5% SDS at room temperature and at 37° C. and finally in 0.1xSSC with 0.5% SDS at 68° C. for 30 min

(Sambrook et al., 1989, in "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Lab. Press). For paraffin-embedded tissue sections the conditions described by Wright and Manos (1990, in "PCR Protocols", Innis et al., eds., Academic Press, pp. 153-158) are followed using primers designed to detect a 250 bp sequence.

Example 5: Expression of cloned polynucleotides in host cells.

[0218] To study the protein products of the novel DKKL-1 isoforms, restriction fragments from isoform 2 or 3 cDNA are cloned into the expression vector pMT2 (Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press pp 16.17-16.22 (1989)) and transfected into COS cells grown in DMEM supplemented with 10% FCS. Transfections are performed employing calcium phosphate techniques (Sambrook, et al (1989) pp. 16.32-16.40, supra) and cell lysates are prepared forty-eight hours after transfection from both transfected and untransfected COS cells. Lysates are subjected to analysis by immunoblotting using anti-peptide antibody.

[0219] In immunoblotting experiments, preparation of cell lysates and electrophoresis are performed according to standard procedures. Protein concentration is determined using BioRad protein assay solutions. After semi-dry electrophoretic transfer to nitrocellulose, the membranes are blocked in 500 mM NaCl, 20 mM Tris, pH 7.5, 0.05% Tween-20 (TTBS) with 5% dry milk. After washing in TTBS and incubation with secondary antibodies (Amersham), enhanced chemiluminescence (ECL) protocols (Amersham) are performed as described by the manufacturer to facilitate detection.

Example 6: Generation of antibodies against polypeptides.

[0220] Polypeptides encoded by the novel isoforms are synthesized or isolated from bacterial or other (e.g., yeast, baculovirus) expression systems and conjugated to rabbit serum albumin (RSA) with m-maleimido benzoic acid N-hydroxysuccinimide ester (MBS) (Pierce, Rockford, Ill.). Immunization protocols with these peptides are performed according to standard methods. Initially, a pre-bleed of the rabbits is performed prior to immunization. The first immunization includes Freund's complete adjuvant and 500 µg conjugated peptide or 100 µg purified peptide. All subsequent immunizations, performed

four weeks after the previous injection, include Freund's incomplete adjuvant with the same amount of protein. Bleeds are conducted seven to ten days after the immunizations.

[0221] For affinity purification of the antibodies, the corresponding polypeptide is conjugated to RSA with MBS, and coupled to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). Antiserum is diluted 10-fold in 10 mM Tris-HCl, pH 7.5, and incubated overnight with the affinity matrix. After washing, bound antibodies are eluted from the resin with 100 mM glycine, pH 2.5.

Example 7: Generation of monoclonal antibodies against a novel DKKL-1 isoform polypeptide

[0222] A non-denaturing adjuvant (Ribi, R730, Corixa, Hamilton MT) is rehydrated to 4ml in phosphate buffered saline. 100µl of this rehydrated adjuvant is then diluted with 400µl of Hank's Balanced Salt Solution and this is then gently mixed with the cell pellet used for immunization. Approximately 500 µg conjugated peptide or 100 µg purified peptide and Freund's complete are injected into Balb/c mice via foot-pad, once a week. After 6 weeks of weekly injection, a drop of blood is drawn from the tail of each immunized animal to test the titer of antibodies against polypeptides using FACS analysis. When the titer reaches at least 1:2000, the mice are sacrificed in a CO₂ chamber followed by cervical dislocation. Lymph nodes are harvested for hybridoma preparation. Lymphocytes from mice with the highest titer are fused with the mouse myeloma line X63-Ag8.653 using 35% polyethylene glycol 4000. On day 10 following the fusion, the hybridoma supernatants are screened for the presence of specific monoclonal antibodies by fluorescence activated cell sorting (FACS). Conditioned medium from each hybridoma is incubated for 30 minutes with a combined aliquot of PC3, Colo-205, LnCap, or Panc-1 cells. After incubation, the cell samples are washed, resuspended in 0.1 ml diluent and incubated with 1 µg/ml of FITC conjugated F(ab')₂ fragment of goat anti-mouse IgG for 30 min at 4⁰C. The cells are washed, resuspended in 0.5 ml FACS diluent and analyzed using a FACScan cell analyzer (Becton Dickinson; San Jose, CA). Hybridoma clones are selected for further expansion, cloning, and characterization based on their binding to the surface of one or more of cell lines which express the polypeptide as assessed by FACS.

Example 8: ELISA assay for Detecting DKKL-1 isoform antigens.

[0223] To test blood samples for antibodies that bind specifically to recombinantly produced antigens encoded by the novel splice forms of DKKL-1, the following procedure is employed. After a recombinant protein is purified, the recombinant protein is diluted in PBS to a concentration of 5 µg/ml (500 ng/100 µl). 100 microliters of the diluted antigen solution is added to each well of a 96-well Immulon 1 plate (Dynatech Laboratories, Chantilly, Va.), and the plate is then incubated for 1 hour at room temperature, or overnight at 4° C., and washed 3 times with 0.05% Tween 20 in PBS. Blocking to reduce nonspecific binding of antibodies is accomplished by adding to each well 200 µl of a 1% solution of bovine serum albumin in PBS/Tween 20 and incubation for 1 hour. After aspiration of the blocking solution, 100 µl of the primary antibody solution (anticoagulated whole blood, plasma, or serum), diluted in the range of 1/16 to 1/2048 in blocking solution, is added and incubated for 1 hour at room temperature or overnight at 4° C. The wells are then washed 3 times, and 100 µl of goat anti-human IgG antibody conjugated to horseradish peroxidase (Organon Teknika, Durham, N.C.), diluted 1/500 or 1/1000 in PBS/Tween 20, 100 µl of *o*-phenylenediamine dihydrochloride (OPD, Sigma) solution is added to each well and incubated for 5-15 minutes. The OPD solution is prepared by dissolving a 5 mg OPD tablet in 50 ml 1% methanol in H₂O and adding 50 µl 30% H₂O₂ immediately before use. The reaction is stopped by adding 25 µl of 4M H₂SO₄. Absorbances are read at 490 nm in a microplate reader (Bio-Rad).

Example 9: Generation of transgenic animals expressing polypeptides as a means for testing therapeutics.

[0224] Novel DKKL-1 isoform nucleic acids are used to generate genetically modified non-human animals, or site specific gene modifications thereof, in cell lines, for the study of function or regulation of prostate tumor-related genes, or to create animal models of diseases, including prostate cancer. The term "transgenic" is intended to encompass genetically modified animals having an exogenous gene that is stably transmitted in the host cells where the gene may be altered in sequence to produce a modified protein, or having an exogenous LTR promoter operably linked to a reporter gene. Transgenic animals may be made through a nucleic acid construct randomly integrated into the

genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, YACs, and the like. Of interest are transgenic mammals, e.g. cows, pigs, goats, horses, etc., and particularly rodents, e.g. rats, mice, etc.

[0225] The modified cells or animals are useful in the study of gene function and regulation. Specific constructs of interest include, but are not limited to, antisense constructs to block gene expression, expression of dominant negative gene mutations, and over-expression of a gene. Expression of a gene or variants thereof in cells or tissues where it is not normally expressed or at abnormal times of development is provided. In addition, by providing expression of proteins derived from DKKL-1 polynucleotides in cells in which it is otherwise not normally produced, changes in cellular behavior can be induced.

[0226] DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and negative selection are included. For various techniques for transfecting mammalian cells, see Keown et al., *Methods in Enzymology* 185:527-537 (1990).

[0227] For embryonic stem (ES) cells, an ES cell line is employed, or embryonic cells are obtained freshly from a host, e.g. mouse, rat, guinea pig, etc. Such cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of appropriate growth factors, such as leukemia inhibiting factor (LIF). When ES cells are transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of integration of the construct. Those colonies that are positive may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting chimeric animals screened for cells bearing the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected.

[0228] The chimeric animals are screened for the presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs are maintained as allogeneic or congenic grafts or transplants, or in in vitro culture. The transgenic animals may be any non-human mammal, such as laboratory animals, domestic animals, etc. The transgenic animals are used in functional studies, drug screening, etc., e.g. to determine the effect of a candidate drug on prostate cancer, to test potential therapeutics or treatment regimens, etc.

[0229] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0230] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising at least 10 contiguous nucleotides of a polynucleotide sequence selected from the group consisting of: (a) nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement.
2. A host cell comprising a recombinant nucleic acid of claim 1.
3. An expression vector comprising the isolated nucleic acid according to claim 1.
4. A host cell comprising the expression vector of claim 3.
5. The polynucleotide according to claim 1, wherein said polynucleotide, or its complement or a fragment thereof, further comprises a detectable label.
6. The polynucleotide according to claim 1, wherein said polynucleotide, or its complement or a fragment thereof, is attached to a solid support.
7. The polynucleotide according to claim 1, wherein said polynucleotide, or its complement or a fragment thereof, is prepared at least in part by chemical synthesis.
8. The polynucleotide according to claim 1, wherein said polynucleotide, or its complement or a fragment thereof, is an antisense fragment.
9. The polynucleotide according to claim 1, wherein said polynucleotide, or its complement or a fragment thereof, is single stranded.
10. The polynucleotide according to claim 1, wherein said polynucleotide, or its complement or a fragment thereof, is double stranded.
11. The polynucleotide according to claim 1, comprising at least 15 contiguous nucleotides.
12. The polynucleotide according to claim 1, comprising at least 20 contiguous nucleotides.

13. An isolated polypeptide, encoded within an open reading frame of a DKKL1 sequence selected from the group consisting of: (a) nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement.
14. An isolated polypeptide, encoded within an open reading frame of a DKKL1 sequence selected from the group consisting of: (a) at least 4 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B, and (b) at least 4 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B.
15. The polypeptide of claim 14, wherein said polypeptide comprises the amino acid sequence of an epitope of the amino acid sequence of a DKKL1 polypeptide selected from the group consisting of: (a) at least 4 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B, and (b) at least 4 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B.
16. The polypeptide of claim 14, wherein said polypeptide or fragment thereof is attached to a solid support.
17. An isolated antibody or antigen binding fragment thereof, that binds to a polypeptide according to any one of claims 13-16.
18. The isolated antibody or antigen binding fragment thereof according the claim 17, wherein said antibody or fragment thereof is attached to a solid support.
19. The isolated antibody or antigen binding fragment thereof according the claim 17, wherein said antibody is a monoclonal antibody.
20. The isolated antibody or antigen binding fragment thereof according the claim 17, wherein said antibody is a polyclonal antibody.

21. The isolated antibody or antigen binding fragment thereof according the claim 17, wherein said antibody or fragment thereof further comprises a detectable label.

22. An isolated antibody that binds to a polypeptide, or antigen binding fragment thereof, according to any of claims 13-16, prepared by a method comprising the steps of: (i) immunizing a host animal with a composition comprising said polypeptide, or antigen binding fragment thereof, and (ii) collecting cells from said host expressing antibodies against the antigen or antigen binding fragment thereof.

23. The monoclonal antibody according to claim 19, wherein the monoclonal antibody is prepared by a process comprising:

(a) providing a hybridoma capable of producing the monoclonal antibody; and

(b) culturing the hybridoma under conditions that provide for the production of the monoclonal antibody by the hybridoma.

24. A hybridoma that produces the monoclonal antibody according to claim 19.

25. The antibody according to claim 17, wherein the antibody is a humanized antibody.

26. A kit for detecting cancer cells comprising the antibody according to claim 17.

27. A kit for detecting cancer cells comprising the monoclonal antibody according to claim 19.

28. A kit for diagnosing the presence of cancer in a test sample, said kit comprising at least one polynucleotide that selectively hybridizes to a DKKL1 polynucleotide sequence selected from the group consisting of: (a) nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement.

29. A method of screening for anticancer activity comprising:

(a) providing a cell that expresses a DKKL1 gene encoded by a nucleic acid sequence selected from the group consisting of: (a) nucleotides spanning positions 329

and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement;

(b) contacting a tissue sample derived from a cancer cell with an anticancer drug candidate; and

(c) monitoring an effect of the anticancer drug candidate on an expression of the DKKL1 polynucleotide in the tissue sample.

30. The method of screening for anticancer activity according to claim 29, further comprising:

(d) comparing the level of expression in the absence of said drug candidate to the level of expression in the presence of the drug candidate.

31. A method for detecting cancer associated with expression of a polypeptide in a test cell sample, comprising the steps of:

(i) detecting a level of expression of at least one DKKL1 polypeptide selected from the group consisting of: (a) at least 4 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B, and (b) at least 4 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B; and

(ii) comparing the level of expression of the polypeptide in the test sample with a level of expression of polypeptide in a normal cell sample, wherein an altered level of expression of the polypeptide in the test cell sample relative to the level of polypeptide expression in the normal cell sample is indicative of the presence of cancer in the test cell sample.

32. A method for detecting cancer associated with the presence of an antibody in a test serum sample, comprising the steps of:

(i) detecting a level of an antibody against an antigenic polypeptide selected from the group consisting of detecting a level of expression of at least one DKKL1 polypeptide

selected from the group consisting of: (a) at least 4 consecutive residues spanning positions 108 and 109 of the polypeptide sequences of clones 379-R8 and 379-RS3 shown in Figures 4A-4B, and (b) at least 4 consecutive residues spanning positions 61 and 62 of the polypeptide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 4A-4B; and

(ii) comparing said level of said antibody in the test sample with a level of said antibody in the control sample, wherein an altered level of antibody in said test sample relative to the level of antibody in the control sample is indicative of the presence of cancer in the test serum sample.

33. A method for screening for a bioactive agent capable of modulating the activity of a DKKL1 protein, wherein said protein is encoded by a nucleic acid comprising a nucleic acid sequence selected from the group consisting of (a) nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement, the method comprising:

- a) combining the DKKL1 protein and a candidate bioactive agent; and
- b) determining the effect of the candidate agent on the bioactivity of the protein.

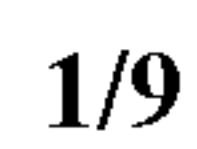
34. The method of screening for the bioactive agent according to claim 33, wherein the bioactive agent affects the expression of the DKKL1 protein.

35. A method for treating cancers comprising administering to a patient an inhibitor of a DKKL1 protein, wherein said protein is encoded by a nucleic acid comprising a nucleic acid sequence selected from the group consisting of: (a) nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement.

36. The method for treating cancers according to claim 35, wherein the inhibitor of a DKKL1 protein binds to the DKKL1 protein.

37. A method for inhibiting expression of a DKKL1 gene in a cell comprising:
- contacting a cell expressing a DKKL1 gene with a double stranded RNA comprising a sequence capable of hybridizing to a DKKL1 mRNA corresponding to the polynucleotide sequences of (a) nucleotides spanning positions 329 and 330 of the nucleotide sequences of clones 379-R8 and 379-RS3 shown in Figures 3A-3E, or its complement, and (b) consecutive nucleotides spanning positions 188 and 189 of the nucleotide sequences of clones 379-R4, 379-R5, 379-R2, 379-RS7 and 379-RS4 shown in Figures 3A-3E, or its complement, in an amount sufficient to elicit RNA interference; and
- inhibiting expression of the DKKL1 gene in the cell.
38. The method of claim 37, wherein the double stranded RNA is provided by introducing a short interfering RNA (siRNA) into the cell by a method selected from the group consisting of transfection, electroporation, and microinjection.
39. The method of claim 37, wherein the double stranded RNA is provided by introducing a short interfering RNA (siRNA) into the cell by an expression vector.

Alignment of celera DKKL1 transcript with novel splice variants



transcript alignment for novel isoforms of DKKL1

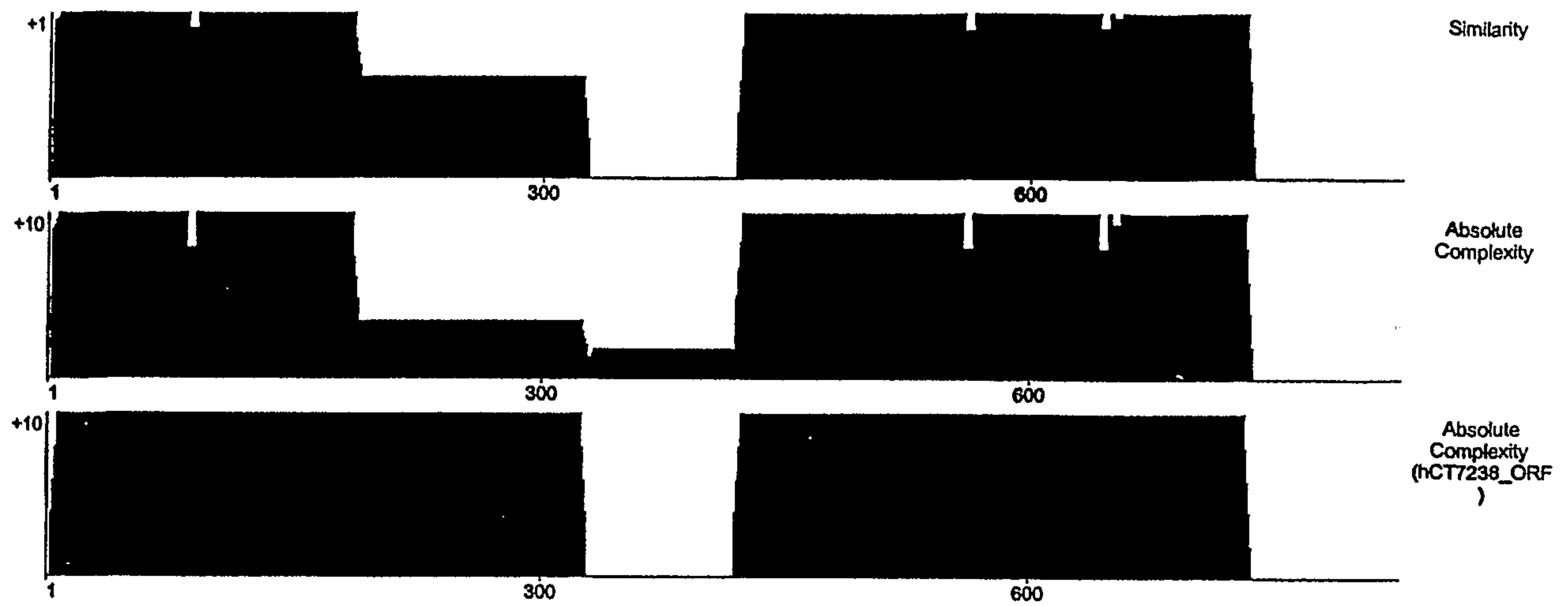


FIG. 2

transcript alignment for novel isoforms of DKKL1

		Section 1											
		(1)	1	10	20	30	40	50	64				
hCT7238 ORF	(1)	----ATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
sars0379-stop	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R6	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone 379-R7	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R3	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS2	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone 379-R8	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS3	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379 R4	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R5	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R2	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS7	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS4	(1)	ATGGATGGGAGAAGCCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
		Section 2											
		(65)	65	70	80	90	100	110	128				
hCT7238 ORF	(61)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
sars0379-stop	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R6	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone 379-R7	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R3	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS2	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone 379-R8	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS3	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379 R4	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R5	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R2	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS7	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS4	(65)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
		Section 3											
		(129)	129	140	150	160	170	180	192				
hCT7238 ORF	(125)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
sars0379-stop	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R6	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone 379-R7	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R3	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS2	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone 379-R8	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS3	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379 R4	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R5	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-R2	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS7	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											
clone-379-RS4	(129)	CTCTCTACCCCTGCTGATGCGGCTCCCGAGCTGCCCCCGCAAGGCGGGCATCTGCTGCTCCTGCTGCTGCTGCTC											

FIG. 3A

Section 9

[illegible]

transcript alignment for novel isoforms of DKKL1

Section 10								
	(577)	577	590	600	610	620	630	640
hCT7238 ORF (573)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
sars0379-stop (577)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-R6 (577)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone 379-R7 (577)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-R3 (577)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-RS2 (577)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone 379-R8 (484)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-RS3 (484)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379 R4 (343)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-R5 (343)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-R2 (343)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-RS7 (343)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
clone-379-RS4 (343)		CGAGAAGCGACACCGCCTGCAGGCCATCCGGGATGGACTCCGCAAGGGGACCCACAAGGACGTC						
Section 11								
	(641)	641	650	660	670	680	690	704
hCT7238 ORF (637)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
sars0379-stop (641)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R6 (641)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone 379-R7 (641)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R3 (641)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS2 (641)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone 379-R8 (548)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS3 (548)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379 R4 (407)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R5 (407)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R2 (407)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS7 (407)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS4 (407)		CTAAGAGAGCGGAGCGAGACCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
Section 12								
	(705)	705	710	720	730	740	750	768
hCT7238 ORF (701)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT				GGGTGGGGACCGGGGAGCACCTGCCTGTAGCCCCC		
sars0379-stop (705)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R6 (705)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone 379-R7 (705)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R3 (705)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS2 (705)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone 379-R8 (612)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS3 (612)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379 R4 (471)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R5 (471)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-R2 (471)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS7 (471)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						
clone-379-RS4 (471)		AGATCCGCAAGCGCGCTGCTGCAAGTCAGGCGCTCCGCGCGAAGACACCACTTACTCT						

FIG. 3D

transcript alignment for novel isoforms of DKKL1

Section 13

	(769)	769	780	790	800	810	823
hCT7238 ORF (765)		ATCAGACCCTGCCCCAAGCACC	CATATGGAAATAAAGTTCTTTCTTACATCTAACA				
sgrs0379-stop (734)		-----	-----	-----	-----	-----	-----
clone-379-R6 (734)		-----	-----	-----	-----	-----	-----
clone 379-R7 (734)		-----	-----	-----	-----	-----	-----
clone-379-R3 (734)		-----	-----	-----	-----	-----	-----
clone-379-RS2 (734)		-----	-----	-----	-----	-----	-----
clone 379-R8 (641)		-----	-----	-----	-----	-----	-----
clone-379-RS3 (641)		-----	-----	-----	-----	-----	-----
clone-379 R4 (500)		-----	-----	-----	-----	-----	-----
clone-379-R5 (500)		-----	-----	-----	-----	-----	-----
clone-379-R2 (500)		-----	-----	-----	-----	-----	-----
clone-379-RS7 (500)		-----	-----	-----	-----	-----	-----
clone-379-RS4 (500)		-----	-----	-----	-----	-----	-----

FIG. 3E

protein alignment for novel isoforms of DKKL1

Section 1

	(1)	1	10	20	30	48
hCP35274.2	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of DKKL1-pending chris	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone 379-R6	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone 379-R7	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-R3	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-RS2	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone 379-R8	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-RS3	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379 R4	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-R5	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-R2	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-RS7	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			
Translation of clone-379-RS4	(1)	MGEASPPAPARRHLLVLLLLSTLVPS	AAAPTHDADAQESSIGITGL			

Section 2

	(49)	49	60	70	80	96
hCP35274.2	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of DKKL1-pending chris	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone 379-R6	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone 379-R7	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-R3	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-RS2	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone 379-R8	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-RS3	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379 R4	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-R5	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-R2	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-RS7	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			
Translation of clone-379-RS4	(49)	OSTLOCFSRRETK	SNLIRGIDSLFSAVMEKGLRGNHIXENORHLE			

Section 3

	(97)	97	110	120	130	144
hCP35274.2	(97)	INTLSHLEQEX	RTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of DKKL1-pending chris	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone 379-R6	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone 379-R7	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone-379-R3	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone-379-RS2	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone 379-R8	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone-379-RS3	(97)	INTLSHLEQEX	MTDNKTGEVLISENVVASIQPAEGSFEGDLK			
Translation of clone-379 R4	(62)	-----	-----	-----	-----	-----
Translation of clone-379-R5	(62)	-----	-----	-----	-----	-----
Translation of clone-379-R2	(62)	-----	-----	-----	-----	-----
Translation of clone-379-RS7	(62)	-----	-----	-----	-----	-----
Translation of clone-379-RS4	(62)	-----	-----	-----	-----	-----

FIG. 4A

protein alignment for novel isoforms of DKKL1

Section 4

	(145)	145	150	160	170	180	192
hCP35274.2	(145)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of DKKL1-pending chris	(145)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone 379-R6	(145)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone 379-R7	(145)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-R3	(145)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-RS2	(145)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone 379-R8	(114)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-RS3	(114)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379 R4	(67)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-R5	(67)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-R2	(67)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-RS7	(67)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	
Translation of clone-379-RS4	(67)	EKEALVPTOKATDSFHT	ELHPRVAEW	TIKLP	RRRSHODALEG	SHWLSE	

Section 5

	(193)	193	200	210	220	230	240
hCP35274.2	(193)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of DKKL1-pending chris	(193)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone 379-R6	(193)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone 379-R7	(193)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-R3	(193)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-RS2	(193)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone 379-R8	(162)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-RS3	(162)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379 R4	(115)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-R5	(115)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-R2	(115)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-RS7	(115)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		
Translation of clone-379-RS4	(115)	KRHRLOAIFDGLRKGTHKDV	LE	ETESSSHSRISPRKPHLY	ITRPSR		

Section 6

	(241)	241B
hCP35274.2	(241)	QI-
Translation of DKKL1-pending chris	(241)	QI-
Translation of clone 379-R6	(241)	QI-
Translation of clone 379-R7	(241)	QI-
Translation of clone-379-R3	(241)	QI-
Translation of clone-379-RS2	(241)	QI-
Translation of clone 379-R8	(210)	QI-
Translation of clone-379-RS3	(210)	QI-
Translation of clone-379 R4	(163)	QI-
Translation of clone-379-R5	(163)	QI-
Translation of clone-379-R2	(163)	QI-
Translation of clone-379-RS7	(163)	QI-
Translation of clone-379-RS4	(163)	QI-

FIG. 4B