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EXPRESSION OF A GAGE/PAGE-LIKE PROTEIN IN BENIGN PROSTATIC HYPERPLASIA

RELATED APPLICATION

This application claims priority to U.S. Provisional Application 60/222,039, filed July 31, 2000, which is herein incorporated by reference in its entirety.

5 FIELD OF THE INVENTION

The invention relates generally to the changes in gene expression in prostate tissue removed from male patients with benign prostatic hyperplasia (BPH). The invention specifically relates to a human gene which is differentially expressed in BPH tissue compared to normal prostate tissue.

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BACKGROUND OF THE INVENTION

Benign Prostatic Hyperplasia (BPH)

BPH is the most common benign tumor in men aged >60 years. It is estimated that one in four men living to the age of 80 will require treatment for this disease. BPH is usually noted clinically after the age of 50, the incidence increasing with age, but as many as two thirds of men between the ages of 40 and 49 demonstrate histological evidence of the disease.

The anatomic location of the prostate at the bladder neck enveloping the urethra plays an important role in the pathology of BPH, including bladder outlet obstruction. Two prostate components are thought to play a role in bladder outlet obstruction. The first is the relative increase in prostate tissue mass. The second component is the prostatic smooth muscle tone.

The causative factors of BPH in man have been intensively studied (see Ziada *et al.*, *Urology* 53: 1-6, 1999). In general, the two most important factors appear to be aging and the presence of functional testes. Although these factors appear to be key to the development of BPH, both appear to be nonspecific.

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Molecular Changes in BPH

Little is known about the molecular changes in prostate cells associated with the

-2-

development and progression of BPH. It has been demonstrated that the expression levels of a number of individual genes are changed compared to normal prostate cells. These changes in gene expression include a decreased level of Wilm's tumor gene (WT-1) expression and increased expression of insulin growth factor II (IGF-II) (Dong *et al.*, *J Clin Endocrin Metab* 82: 2198-2203, 1997).

While the changes in the expression levels of a number of individual genes have been identified, the investigation of the global changes in gene expression has not been reported. Accordingly, there exists a need for the investigation of the changes in global gene expression levels as well as the need for the identification of new molecular markers associated with the development and progression of BPH. Furthermore, if intervention is expected to be successful in halting or slowing down BPH, means of accurately assessing the early manifestations of BPH need to be established. One way to accurately assess the early manifestations of BPH is to identify markers which are uniquely associated with disease progression. Likewise, the development of therapeutics to prevent or stop the progression of BPH relies on the identification of genes responsible for BPH development, progression and causation.

SUMMARY OF THE INVENTION

The present invention is based on the discovery of a new gene family that is differentially expressed in BPH tissue compared to normal prostate tissue and that may be distantly related to two families of genes encoding known prostate-associated or melanoma-associated genes.

The invention includes methods of diagnosing prostatic disease, in particular BPH, in a patient, comprising determining the level of expression of a protein or nucleic acid of the invention in a patient sample, wherein the differential expression of said protein or nucleic acid compared to normal prostate tissue is an indication of prostatic disease.

The present invention further provides methods of modulating the expression of a nucleic acid molecule encoding a protein of the invention, comprising: administering an effective amount of an agent which modulates the expression of a nucleic acid molecule encoding the protein. The invention also includes methods of screening for agents that modulate the level of or at least one activity of a protein of the invention, comprising: exposing cells which express the protein to the agent; and determining whether the agent

modulates the level of or at least one activity of a protein of the invention, thereby identifying an agent which modulates the level of or at least one activity of the protein.

The invention further provides methods of identifying an agent which modulates the expression of a nucleic acid molecule encoding a protein of the invention, comprising:
5 exposing cells which express the nucleic acid molecule to the agent; and determining whether the agent modulates expression of said nucleic acid molecule, thereby identifying an agent which modulates the expression of a nucleic acid molecule encoding the protein.

The invention further provides methods of identifying binding partners for a protein of the invention, comprising: exposing said protein to a potential binding partner; and
10 determining if the potential binding partner binds to said protein, thereby identifying binding partners for the protein.

The invention further includes methods of treating BPH, comprising administering to a patient in need thereof a therapeutically effective amount of an agent which modulates the expression or at least one activity of a protein of the invention as well as therapeutic or
15 pharmaceutical compositions comprising the agents.

The present invention further includes non-human transgenic animals comprising a nucleic acid molecule selected from the group consisting of an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2, an isolated nucleic acid molecule that encodes a fragment of at least 6 amino acids of SEQ ID NO: 2, an isolated nucleic acid
20 molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2 and an isolated nucleic acid molecule that encodes a protein that is expressed in BPH and that exhibits at least about 60% nucleotide sequence identity over the open reading frame of SEQ ID NO: 1. Non-human transgenic
25 animals modified to contain mutated nucleic acid molecules of the invention, such that expression of the encoded polypeptides of the invention is prevented, are also included in the invention.

The invention further includes expression vectors comprising: a nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; a nucleic acid molecule
30 that encodes a fragment of at least 6 amino acids of SEQ ID NO: 2; a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1; a nucleic acid

molecule which hybridizes to a complement of nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; or a nucleic acid molecule that encodes a protein that is expressed in BPH and that exhibits at least about 60% nucleotide sequence identity over the open-reading-frame of SEQ ID NO: 1.

5 The invention further includes antibodies or antigen-binding antibody fragments that recognize or specifically bind to a polypeptide or protein selected from the group consisting of a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, a polypeptide comprising a fragment of at least 6 amino acids of SEQ ID NO: 2, a polypeptide comprising conservative amino acid substitutions of SEQ ID NO: 2, naturally occurring amino acid
10 sequence variants of SEQ ID NO: 2, and a polypeptide exhibiting at least about 50% amino acid sequence identity with SEQ ID NO: 2. These antibodies or antigen-binding antibody fragments may be monoclonal or polyclonal antibodies or antibody fragments.

 The invention further includes compositions comprising a diluent and a polypeptide or protein selected from the group consisting of an isolated polypeptide comprising the amino
15 acid sequence of SEQ ID NO: 2, an isolated polypeptide comprising a fragment of at least 6 amino acids of SEQ ID NO: 2, an isolated polypeptide comprising conservative amino acid substitutions of SEQ ID NO: 2 and naturally occurring amino acid sequence variants of SEQ ID NO: 2, and an isolated polypeptide with an amino acid sequence having at least about
20 50%, 60%, 70% or 75% amino acid sequence identity with the sequence set forth in SEQ ID NO: 2, preferably at least about 80%, more preferably at least about 90%, and most preferably at least about 95% sequence identity with the sequence set forth in SEQ ID NO: 2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 The top part of Figure 1 shows the expression of JT463726 across a
25 panel of normal human prostate control and BPH samples, including BPH samples from men without symptoms ("Without"), BPH samples from men who were diagnosed with prostate cancer ("Cancer") and BPH samples from men with symptoms ("With"). In all cases, the subregion of the prostate analyzed in the normal and BPH patient samples was the transitional zone. Averages are shown with error bars representing the standard deviation.
30 JT463726 is strongly up-regulated in samples from symptomatic BPH patients and patients with BPH and cancer versus normal control patients. JT 463726 is also slightly up-regulated

in BPH patients without symptoms.

The lower part of Figure 1 shows an electronic Northern, in which the expression level of JT463726 was measured across a panel of normal tissues and diseased prostate tissues using the Affymetrix 42K human GeneChip set. For each tissue type, the mean +/-

5 SDM is shown as a horizontal bar graph for samples obtained from three or more normal individuals.

Figure 2 Figure 2 displays the results of second electronic Northern, in which the expression level of JT463726 was measured across a slightly different panel of normal

10 tissues and diseased prostate tissues using an Affymetrix microarray. For each tissue type, the number of samples tested is indicated in parentheses.

Figure 3 Figure 3 shows the results of semi-quantitative PCR for the expression of JT463726 (SEQ ID NO: 1) in various tissues. The samples in each in lane are as follows:

15 M) DNA markers; 1) heart; 2) brain; 3) leukocytes; 4) lung; 5) liver; 6) fetal brain; 7) kidney; 8) spleen; 9) placenta; 10) prostate, from BPH patient; 11) glomeruli; 12) osteoblast; N) negative control. The primer pair used was F9-30/R348-369. The samples in the top gel were reacted for 25 PCR cycles (U) and the samples in the bottom gel for 30 cycles (U).

Figure 4 Figure 4 shows the results of a Northern Assay of the expression level of JT463726 in various normal tissues. The samples in each in lane are as follows: M) RNA

20 markers; 1) brain; 2) colon; 3) heart; 4) kidney; 5) liver; 6) lung; 7) skeletal muscle; 8) placenta; 9) small intestine; 10) spleen; 11) stomach; 12) testis. The blot is an OriGene human mRNA blot, 2 ug mRNA/lane; the probe is the PCR product F9/R348. Hybridization

25 conditions are: Church-Gilbert buffer, overnight incubation, final wash in 0.5X SSC, 0.1% SDS at 65°C, film exposure 1 hr., -80°C.

Figure 5 Figure 5 is a hydrophobicity plot of the protein encoded by the open reading frame of JT463726 (SEQ ID NO: 2). Analysis was done using the methods of

30 Goldman *et al.* and of Kyte-Doolittle.

-6-

Figure 6 Figure 6 shows an alignment of a JT463726 protein (SEQ ID NO: 2) and known GAGE and PAGE proteins.

Figure 7 Figure 7 shows the expression and location of a JT463726 protein in a comparison of four formalin-fixed, paraffin-embedded prostate tissue sections using anti-streptavidin biotinylated polyclonal antibodies and a fluorescence detection technique (described in Example 1). The specimens are as follows: (A) normal prostate; (B) BPH without symptoms; (C) BPH with symptoms; (D) BPH with symptoms, reacted with pre-immune serum.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. General Description

The present invention is based in part on the identification of a family of genes that are differentially expressed in human BPH tissue compared to normal human prostate tissue. This gene family corresponds to the human gene of SEQ ID NO: 1 that encodes a protein of 102 amino acids (JT463726). Genes that encode the human protein of SEQ ID NO: 2 may also be found in other animal species, particularly mammalian species.

The genes and proteins of the invention may be used as diagnostic agents or markers to detect BPH or the progression of BPH in a sample. They can also serve as a target for agents that modulate gene expression or activity. For example, agents may be identified that modulate biological processes associated with prostate growth, including the hyperplastic process of BPH.

The present invention is further based on the development of methods for isolating binding partners that bind to the proteins. Additionally, the proteins provide novel targets for the screening of synthetic small molecules and combinatorial or naturally occurring compound libraries to discover novel therapeutics to regulate prostate function.

II. Specific Embodiments

A. The Proteins Associated with BPH

The methods of the present invention employ or use proteins comprising or consisting of SEQ ID NO: 2, allelic variants of the proteins, and conservative amino acid substitutions of the proteins. As used herein, the “protein” or “polypeptide” refers, in part, to a protein that has the human amino acid sequence depicted in SEQ ID NO: 2. The terms also refer to naturally occurring allelic variants and proteins that have a slightly different amino acid sequence than that specifically recited above. Allelic variants, though possessing a slightly different amino acid sequence than those recited above, will still have the same or similar biological functions associated with these proteins.

As used herein, the family of proteins related to the human amino acid sequence of SEQ ID NO: 2 includes proteins that have been isolated from organisms in addition to humans. The methods used to identify and isolate other members of the family of proteins related to these proteins are described below.

The proteins used in the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated protein.

The proteins used in the present invention further include insertion, deletion or conservative amino acid substitution variants of the sequence set forth in SEQ ID NO: 2. As used herein, a conservative variant refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the protein. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the protein. For example, the overall charge, structure or hydrophobic/hydrophilic properties of the protein can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the protein.

Ordinarily, the allelic variants, the conservative substitution variants, and the members of the protein family, will have an amino acid sequence having at least about 50%,

60%, 70% or 75% amino acid sequence identity with the sequence set forth in SEQ ID NO: 2, more preferably at least about 80%, even more preferably at least about 90%, and most preferably at least about 95% sequence identity. Identity or homology with respect to such sequences is defined herein as the percentage of amino acid residues in the candidate
5 sequence that are identical with the known peptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity (see section B for the relevant parameters). Fusion proteins, or N-terminal, C-terminal or internal extensions, deletions, or insertions into the peptide sequence shall not be construed as affecting
10 homology.

Thus, the proteins used in the present invention include molecules having the amino acid sequence disclosed in SEQ ID NO: 2; fragments thereof having a consecutive sequence of at least about 3, 4, 5, 6, 10, 15, 20, 25, 30, 35 or more amino acid residues of these proteins; amino acid sequence variants wherein one or more amino acid residues has been
15 inserted N- or C-terminal to, or within, the disclosed coding sequence; and amino acid sequence variants of the disclosed sequence, or their fragments as defined above, that have been substituted by at least one residue. Such fragments, also referred to as peptides or polypeptides, may contain antigenic regions, functional regions of the protein identified as regions of the amino acid sequence which correspond to known protein domains, as well as
20 regions of pronounced hydrophilicity. The regions are all easily identifiable by using commonly available protein sequence analysis software such as MacVector (Oxford Molecular).

Contemplated variants further include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and the corresponding
25 proteins of other animal species, including but not limited to rabbit, mouse, rat, porcine, bovine, ovine, equine and non-human primate species, and the alleles or other naturally occurring variants of the family of proteins; and derivatives wherein the protein has been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as
30 an enzyme or radioisotope).

The present invention further provides compositions comprising a protein or

polypeptide of the invention and a diluent. Suitable diluents can be aqueous or non-aqueous solvents or a combination thereof, and can comprise additional components, for example water-soluble salts or glycerol, that contribute to the stability, solubility, activity, and/or storage of the protein or polypeptide.

As described below, members of the family of proteins can be used: (1) to identify agents which modulate the level of or at least one activity of the protein, (2) to identify binding partners for the protein, (3) as an antigen to raise polyclonal or monoclonal antibodies, (4) as a therapeutic agent or target and (5) as a diagnostic agent or marker of BPH and other hyperplastic diseases.

B. Nucleic Acid Molecules

The present invention utilizes nucleic acid molecules that encode the protein having SEQ ID NO: 2 and the related proteins herein described, preferably in isolated form. As used herein, "nucleic acid" is defined as RNA or DNA that encodes a protein or peptide as defined above, is complementary to a nucleic acid sequence encoding such peptides, hybridizes to the nucleic acid of SEQ ID NO: 1 and remains stably bound to it under appropriate stringency conditions, or encodes a polypeptide sharing at least about 50%, 60%, 70% or 75% sequence identity, preferably at least about 80%, more preferably at least about 85%, and most preferably at least about 90% or 95% or more identity with the peptide sequence of SEQ ID NO: 2 or exhibits at least about 60%, 70% or 75%, preferably at least about 80%, more preferably at least about 85%, and even more preferably at least about 90% or 95% or more nucleotide sequence identity over the open reading frame of SEQ ID NO: 1.

Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbones or including alternative bases, whether derived from natural sources or synthesized. Such hybridizing or complementary nucleic acids, however, are defined further as being novel and unobvious over any prior art nucleic acid including that which encodes, hybridizes under appropriate stringency conditions, or is complementary to nucleic acid encoding a protein according to the present invention.

Homology or identity at the nucleotide or amino acid sequence level is determined by **BLAST (Basic Local Alignment Search Tool)** analysis using the algorithm employed by the

programs **blastp**, **blastn**, **blastx**, **tblastn** and **tblastx** (Altschul S.F. *et al.* (1997) *Nucleic Acids Res* 25:3389-3402, and Karlin *et al.*, (1990) *Proc Natl Acad Sci USA* 87, 2264-2268, both fully incorporated by reference) which are tailored for sequence similarity searching. The approach used by the **BLAST** program is to first consider similar segments, with and without gaps, between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.*, *Nature Genetics* 6:119-129, 1994, which is fully incorporated by reference. The search parameters for **histogram**, **descriptions**, **alignments**, **expect** (*i.e.*, the statistical significance threshold for reporting matches against database sequences), **cutoff**, **matrix** and **filter** (low complexity) are at the default settings. The default scoring matrix used by **blastp**, **blastx**, **tblastn**, and **tblastx** is the **BLOSUM62** matrix (Henikoff *et al.*, *Proc Natl Acad Sci USA* 89:10915-10919, 1992, fully incorporated by reference), recommended for query sequences over 85 nucleotides or amino acids in length.

For **blastn**, the scoring matrix is set by the ratios of **M** (*i.e.*, the reward score for a pair of matching residues) to **N** (*i.e.*, the penalty score for mismatching residues), wherein the default values for **M** and **N** are 5 and -4, respectively. Four **blastn** parameters were adjusted as follows: **Q**=10 (gap creation penalty); **R**=10 (gap extension penalty); **wink**=1 (generates word hits at every **wink**th position along the query); and **gapw**=16 (sets the window width within which gapped alignments are generated). The equivalent **Blastp** parameter settings were **Q**=9; **R**=2; **wink**=1; and **gapw**=32. A **Bestfit** comparison between sequences, available in the GCG package version 10.0, uses DNA parameters **GAP**=50 (gap creation penalty) and **LEN**=3 (gap extension penalty) and the equivalent settings in protein comparisons are **GAP**=8 and **LEN**=2.

“Stringent conditions” are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C, or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is hybridization in 50% formamide, 5X SSC (0.75

M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal. Preferred molecules are those that hybridize under the above conditions to the complement of SEQ ID NO: 1 and which encode a functional protein. Even more preferred hybridizing molecules are those that hybridize under the above conditions to the complement strand of the open reading frame of SEQ ID NO: 1.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules encoding other polypeptides.

The present invention further utilizes fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of the entire protein coding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough to encode the functional region(s) of the protein. For instance, fragments which encode peptides corresponding to predicted antigenic regions may be prepared. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming (see the discussion in Section H).

Fragments of the encoding nucleic acid molecules of the present invention (*i.e.*, synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding proteins of the invention, can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci *et al.* (*J Am Chem Soc* 103: 3185-3191, 1981) or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The encoding nucleic acid molecules of the present invention may further be modified so as to contain a detectable label for diagnostic and probe purposes. A variety of such labels

-12-

are known in the art and can readily be employed with the encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can readily employ any such label to obtain labeled variants of the nucleic acid molecules of the invention.

5 Modifications to the primary structure of the nucleic acid molecules by deletion, addition, or alteration of the amino acids incorporated into the encoded protein sequences during translation can be made without destroying the activity of the proteins. Such substitutions or other alterations result in proteins having an amino acid sequence encoded by a nucleic acid that may be used in the present invention.

10

C. Isolation of Other Related Nucleic Acid Molecules

As described above, the identification and characterization of the nucleic acid molecule having SEQ ID NO: 1 allows a skilled artisan to isolate nucleic acid molecules that encode other members of the protein family in addition to the sequences herein described.

15 Further, the presently disclosed nucleic acid molecules allow a skilled artisan to isolate nucleic acid molecules that encode other members of the family of proteins in addition to the proteins having SEQ ID NO: 2.

For instance, a skilled artisan can readily use the amino acid sequence of SEQ ID NO: 2 to generate antibody probes to screen expression libraries prepared from appropriate cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe a mammalian cDNA or genomic expression library, such as lambda gt10 library, to obtain the appropriate coding sequence for other members of the protein family. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or
20 expressed by constructions using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the coding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the protein family from any mammalian organism. Oligomers containing approximately 18-20 nucleotides (encoding
30 about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient

stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively clone an encoding nucleic acid molecule. A PCR denature-anneal-extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other encoding nucleic acid molecules.

Nucleic acid molecules encoding other members of the protein family may also be identified in existing genomic or other sequence information using any available computational method, including but not limited to: PSI-BLAST (Altschul, *et al.*, *Nucleic Acids Res* 25:3389-3402, 1997); PHI-BLAST (Zhang, *et al.*, *Nucleic Acids Res* 26:3986-3990, 1998), 3D-PSSM (Kelly *et al.*, *J Mol Biol* 299(2): 499-520, 2000); and other computational analysis methods (Shi *et al.*, *Biochem Biophys Res Commun* 262(1):132-8, 1999; Matsunami *et al.*, *Nature* 404(6778):601-4, 2000).

D. rDNA molecules Containing a Nucleic Acid Molecule

The present invention further utilizes recombinant DNA molecules (rDNAs) that contain a coding sequence. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in situ*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al.*, (Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). In the preferred rDNA molecules, a coding DNA sequence is operably linked to expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which one of the protein family encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, *e.g.*, protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of the structural gene included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other regulatory elements. Preferably, the inducible promoter is readily controlled, such as being responsive to a

-14-

nutrient in the host cell's medium.

In one embodiment, the vector containing a coding nucleic acid molecule will include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or bacteriophage promoter capable of directing the expression (transcription and translation) of the coding gene sequences in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from BioRad Laboratories, (Richmond, CA), pPL and pKK223 available from Pharmacia (Piscataway, NJ).

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, such as prostate cells, can also be used to form rDNA molecules that contain a coding sequence. Eukaryotic cell expression vectors, including viral vectors, are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors. Vectors may be modified to include prostate cell specific promoters if needed.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, *i.e.*, the neomycin phosphotransferase

(*neo*) gene. (Southern *et al.*, J Mol Anal Genet 1:327-341, 1982). Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by co-transfection of the host cell, and selected by culturing in the appropriate drug for the selectable marker.

5

E. Host Cells Containing an Exogenously Supplied Coding Nucleic Acid Molecule

The present methods may utilize host cells transformed with a nucleic acid molecule that encodes a protein of the present invention. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a protein of the invention are not
10 limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of the gene product. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human cell line.

Preferred eukaryotic host cells include prostate-derived cell lines, Chinese hamster
15 ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells (NIH/3T3) available from the ATCC as CRL 1658, baby hamster kidney cells (BHK), and the like eukaryotic tissue culture cell lines.

Any prokaryotic host can be used to express a rDNA molecule encoding a protein of the invention. The preferred prokaryotic host is *E. coli*.

20 Transformation of appropriate cell hosts with a rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed (see, for example, Cohen *et al.*, *Proc Natl Acad Sci USA* 69:2110, 1972; and Sambrook *et al.*, (Molecular Cloning: A
25 Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed (see, for example, Graham *et al.*, *Virol* 52:456, 1973; and Wigler *et al.*, *Proc Natl Acad Sci USA* 76:1373-1376, 1979).

30 Successfully transformed cells, *i.e.*, cells that contain a rDNA molecule of the present invention, can be identified by well known techniques including the selection for a selectable

-16-

marker. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern (*J Mol Biol* 98:503, 1975) or Berent *et al.*, *Biotech* 3:208, 1985) or the proteins produced from the cell assayed via an immunological method.

F. Production of Recombinant Proteins using a rDNA Molecule

The present invention further provides methods for producing a protein of the invention using nucleic acid molecules herein described. In general terms, the production of a recombinant form of a protein typically involves the following steps.

A nucleic acid molecule is first obtained that encodes a protein used in the methods of the invention, such as a nucleic acid molecule comprising, consisting essentially of or consisting of SEQ ID NO: 1, nucleotides 78-386 of SEQ ID NO: 1 or nucleotides 78-383 of SEQ ID NO: 1. If the encoding sequence is uninterrupted by introns, as are these open-reading-frames, it is directly suitable for expression in any host.

The nucleic acid molecule is then preferably placed in operable linkage with suitable control sequences, as described above, to form an expression unit containing the protein open reading frame. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the recombinant protein.

Optionally the recombinant protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in appropriate hosts. The construction of expression vectors that are operable in a variety of hosts is accomplished using appropriate replicons and control sequences, as set forth above. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with the nucleic acid molecules of the

invention to produce recombinant protein.

G. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for isolating and
5 identifying binding partners of proteins of the invention. In general, a protein of the
invention is mixed with a potential binding partner or an extract or fraction of a cell under
conditions that allow the association of potential binding partners with the protein of the
invention. After mixing, peptides, polypeptides, proteins or other molecules that have
become associated with a protein of the invention are separated from the mixture. The
10 binding partner that bound to the protein of the invention can then be removed and further
analyzed. To identify and isolate a binding partner, the entire protein, for instance a protein
comprising the entire amino acid sequence of SEQ ID NO: 2 can be used. Alternatively, a
fragment of the protein can be used.

As used herein, a cellular extract refers to a preparation or fraction which is made
15 from a lysed or disrupted cell. The preferred source of cellular extracts will be cells derived
from human prostate tissue or cells, for instance, biopsy tissue or tissue culture cells from
BPH. Alternatively, cellular extracts may be prepared from normal tissue or available cell
lines, particularly prostate derived cell lines.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted
20 using either physical or chemical disruption methods. Examples of physical disruption
methods include, but are not limited to, sonication and mechanical shearing. Examples of
chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A
skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain
extracts for use in the present methods.

25 Once an extract of a cell is prepared, the extract is mixed with the protein of the
invention under conditions in which association of the protein with the binding partner can
occur. A variety of conditions can be used, the most preferred being conditions that closely
resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity,
pH, temperature, and the concentration of cellular extract used, can be varied to optimize the
30 association of the protein with the binding partner.

-18-

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using a Far-Western assay according to the procedures of Takayama *et al.* (*Methods Mol Biol* 69:171-184, 1197) or Sauder *et al.* (*J Virol* 70(11):7713-7724, 1996) or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system or other *in vivo* protein-protein detection system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

H. Methods to Identify Agents that Modulate the Expression a Nucleic Acid

Encoding the Genes Associated BPH

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding a protein of the invention such as a protein having the amino acid sequence of SEQ ID NO: 2. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid of the invention, for instance a nucleic acid encoding the protein having the sequence of SEQ ID NO: 2, if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

In one assay format, cell lines that contain reporter gene fusions between nucleotides from within the open reading frame defined by nucleotides 78-386 of SEQ ID NO: 1 and/or the 5' and/or 3' regulatory elements and any assayable fusion partner may be prepared.

Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, *Anal Biochem* 188:245-254, 1190). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid of the invention.

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a protein of the invention, such as the protein having SEQ ID NO: 2. For instance, mRNA expression may be monitored directly by hybridization of probes to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.*, *supra*.

The preferred cells will be those derived from human prostate tissue, for instance, prostate biopsy tissue or cultured prostate cells from normal or BPH patients, for example BRF-55T cells (immortalized human prostate cells obtained from an individual with BPH (Iype *et al.*, "Establishment and characterization of immortalized human cell lines from prostatic carcinoma and benign prostatic hyperplasia," *Int J Oncol* 12:257-263, 1998). Alternatively, other available cells or cell lines may be used.

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and probe:non-target hybrids.

Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect

-20-

probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.*, supra, or Ausubel *et al.* (Current Protocols in Molecular Biology, Greene Publishing Co., NY, 1995).

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* and Ausubel *et al.* as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a silicon chip or a porous glass wafer. The solid support can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such solid supports and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up- or down-regulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID NO: 2 are identified.

Hybridization for qualitative and quantitative analysis of mRNAs may also be carried out by using a RNase Protection Assay (*i.e.*, RPA, see Ma *et al.*, *Methods* 10(3):273-278, 1996). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (*e.g.*, T7, T3 or SP6 RNA polymerase) is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for synthesis of a labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of isolated RNA (*i.e.*, total or fractionated mRNA) by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 µg/ml ribonuclease A and 2 µg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea/polyacrylamide gels for analysis.

In another assay format, cells or cell lines are first identified which express the gene

-21-

products of the invention physiologically. Cell and/or cell lines so identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and/or the cytosolic cascades. Such cell lines may be, but are not required to be, prostate derived. Further, such cells or cell lines may be transduced or transfected with an expression vehicle (*e.g.*, a plasmid or viral vector) construct comprising an operable non-translated 5' promoter-containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the instant gene products, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag or other detectable marker. Such a process is well known in the art (see Sambrook *et al.*, *supra*).

Cells or cell lines transduced or transfected as outlined above are then contacted with agents under appropriate conditions; for example, the agent in a pharmaceutically acceptable excipient is contacted with cells in an aqueous physiological buffer, such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and/or serum incubated at 37°C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the lysate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody to be further processed by immunological assay (*e.g.*, ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the agent-contacted sample will be compared with a control sample where only the excipient is contacted with the cells, and an increase or decrease in the immunologically generated signal from the agent-contacted sample compared to the control will be used to distinguish the effectiveness of the agent.

I. Methods to Identify Agents that Modulate the Level or at Least One Activity of the BPH Associated Proteins

Another embodiment of the present invention provides methods for identifying agents that modulate the level or at least one activity of a protein of the invention such as the protein

-22-

having the amino acid sequence of SEQ ID NO: 2. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

In one format, the relative amounts of a protein of the invention between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides, polypeptides or proteins of the invention if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co. (Rockford, IL), may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein (*Nature* 256:495-497, 1975) or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can

be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonal antibodies or the polyclonal antisera which contain the immunologically significant (antigen-binding) portion can be used
5 as antagonists, as well as the intact antibodies. Use of immunologically reactive (antigen-binding) antibody fragments, such as the Fab, Fab' or F(ab')₂ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or antigen-binding fragments may also be produced, using current
10 technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin, such as humanized antibodies.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent
15 is chosen randomly without considering the specific sequences involved in the association of a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the agent is
20 chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to or a derivative of any functional consensus site.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. Dominant negative proteins, DNAs encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells to affect function. "Mimic" used herein refers to the modification of a region or several regions of a peptide molecule to provide a structure
30 chemically different from the parent peptide but topographically and functionally similar to the parent peptide (see Grant G.A. in: Meyers (ed.) Molecular Biology and Biotechnology,

-24-

pp. 659-664, VCH Publishers, New York, 1995). A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA
5 encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with
10 critical positions of proteins of the invention. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies.

J. Uses for Agents that Modulate the Expression or at Least One Activity of the 15 Proteins

As provided in the Examples, the proteins and nucleic acids of the invention, such as the proteins having the amino acid sequence of SEQ ID NO: 2, are differentially expressed in BPH tissue. Agents that modulate or up- or down-regulate the expression of the protein or
20 agents, such as agonists or antagonists of at least one activity of the protein, may be used to modulate biological and pathologic processes associated with the protein's function and activity.

As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of a pathological or biological process mediated by a protein of the invention. The term "mammal" is defined as an individual belonging to the class Mammalia. The
25 invention is particularly useful in the treatment of human subjects.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression of a protein of the invention may be associated with prostate cell growth or hyperplasia. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For
30 instance, BPH may be prevented or disease progression modulated by the administration of agents which up- or down-regulate or modulate in some way the expression or at least one

activity of a protein of the invention.

The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with other known drugs. As used
5 herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or
10 concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides compositions containing one or more agents which modulate expression or at least one activity of a protein of the invention. While
15 individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1 to 100 $\mu\text{g/kg}$ body wt. The preferred dosages comprise 0.1 to 10 $\mu\text{g/kg}$ body wt. The most preferred dosages comprise 0.1 to 1 $\mu\text{g/kg}$ body wt.

In addition to the pharmacologically active agent, the compositions of the present
20 invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as
25 appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.
30 Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

5 Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

 In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents.

10 In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

15 **K. Transgenic Animals**

 Transgenic animals containing mutant, knock-out or modified genes corresponding to the cDNA sequences of SEQ ID NO: 1, or the open reading frame encoding the polypeptide sequence of SEQ ID NO: 2 or fragments thereof having a consecutive sequence of at least
20 about 3, 4, 5, 6, 10, 15, 20, 25, 30, 35 or more amino acid residues, are also included in the invention. Transgenic animals are genetically modified animals into which recombinant, exogenous or cloned genetic material has been experimentally transferred. Such genetic material is often referred to as a "transgene." The nucleic acid sequence of the transgene, in this case a form of SEQ ID NO: 1, may be integrated either at a locus of a genome where that
25 particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal.

 The term "germ cell line transgenic animal" refers to a transgenic animal in which the genetic alteration or genetic information was introduced into a germ line cell, thereby
30 conferring the ability of the transgenic animal to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then

they too are transgenic animals.

The alteration or genetic information may be foreign to the species of animal to which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (*see, e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.*, *Hypertension* 22(4):630-633, 1993; Brenin *et al.*, *Surg Oncol* 6(2): 99-110, 1997; Tuan (ed.), *Recombinant Gene Expression Protocols, Methods in Molecular Biology* No. 62, Humana Press, Thomas Jefferson University, PA, 1997).

A large number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.*, *Genetics* 143(4):1753-1760, 1996); or, are capable of generating a fully human antibody response (McCarthy, *The Lancet* 349(9049):405, 1997).

While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (*see, e.g.*, Kim *et al.*, *Mol Reprod Dev* 46(4):515-526, 1997; Houdebine, *Reprod Nutr Dev* 35(6):609-617, 1995; Petters, *Reprod Fertil Dev* 6(5):643-645, 1994; Schnieke *et al.*, *Science* 278(5346):2130-2133, 1997; and Amoah, *J Animal Science* 75(2):578-585, 1997).

The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of multiple nucleic

acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

5 L. Diagnostic Methods

As the genes and proteins of the invention are differentially expressed in BPH tissue compared to normal prostate tissue, the genes and proteins of the invention may be used to diagnose or monitor BPH, prostate function, or to track disease progression. One means of diagnosing BPH using the nucleic acid molecules or proteins of the invention involves
10 obtaining prostate tissue from living subjects. Obtaining tissue samples from living sources is problematic for tissues such as prostate. However, due to the nature of the treatment paradigms for BPH, biopsy may be necessary. When possible, urine, blood or peripheral lymphocyte samples may be used as the tissue sample in the assay. Commonly, in hyperplastic diseases, genes which are up-regulated in the affected tissue (for example the
15 prostate) are also up-regulated in lymphocytes, which may be isolated from whole blood.

The use of molecular biological tools has become routine in forensic technology. For example, nucleic acid probes may be used to determine the expression of a nucleic acid molecule comprising all or at least part of the sequence of SEQ ID NO: 1 in forensic/pathology specimens. Further, nucleic acid assays may be carried out by any means
20 of conducting a transcriptional profiling analysis. In addition to nucleic acid analysis, forensic methods of the invention may target the proteins of the invention, particularly a protein comprising SEQ ID NO: 2, to determine up- or down-regulation of the genes (Shiverick *et al.*, *Biochim Biophys Acta* 393(1):124-133, 1975).

Methods of the invention may involve treatment of tissues with collagenases or other
25 proteases to make the tissue amenable to cell lysis (Semenov *et al.*, *Biull Eksp Biol Med* 104(7):113-116, 1987). Further, it is possible to obtain biopsy samples from different regions of the prostate for analysis.

Assays to detect nucleic acid or protein molecules of the invention may be in any available format. Typical assays for nucleic acid molecules include hybridization or PCR
30 based formats. Typical assays for the detection of proteins, polypeptides or peptides of the invention include the use of antibody probes in any available format such as *in situ* binding

-29-

assays, etc. (see Harlow *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988). In preferred embodiments, assays are carried out with appropriate controls.

5 The above methods may also be used in other diagnostic protocols, including protocols and methods to detect disease states in other tissues or organs, for example, in tissues in which expression of the gene is detected (see Figures 1 and 2).

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following
10 working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1

15 Identification of Differentially Expressed BPH mRNA

Human tissue was obtained from the transitional zone of the prostate (the junction between the ejaculatory duct and the prostatic urethra) in biopsy samples from normal individuals and from patients with BPH or prostate cancer. BPH was defined histologically in all samples. Normal tissue and asymptomatic BPH samples came from individuals who
20 died of trauma and did not report symptoms. Because BPH is a disease associated with aging, two groups of normal individuals were identified, group 1, ages 20 or under, and group 2, ages 30-50. Patients having BPH with symptoms were defined as those with a need for frequent urination. In these patients, a radical prostatectomy had been performed. Prostate cancer patients provided age-matched tissue samples for symptomatic BPH patients, but were
25 without symptoms and without cancer in the transitional zone under histological examination.

Prostate tissue samples (> 500 mg) were excised, snap frozen in liquid nitrogen within 30 minutes of excision and stored at -80°C until extraction of mRNA. Microarray sample preparation was conducted with minor modifications, following the protocols set forth in the Affymetrix GeneChip® Expression Analysis Manual. Frozen tissue was ground to a powder
30 using a Spex Certiprep 6800 Freezer Mill. Total RNA was extracted from ground tissue or cultured cells with Trizol® (GibcoBRL) utilizing the manufacturer's protocol. The total RNA

-30-

yield for each tissue sample was 200-500 µg per 300 mg tissue weight. mRNA was isolated using the Oligotex mRNA Midi Kit® (Qiagen) followed by ethanol precipitation. Double stranded cDNA was generated from mRNA using the SuperScript Choice® system (GibcoBRL). First strand cDNA synthesis was primed with a T7-(dT24) oligonucleotide. The cDNA was phenol-chloroform extracted and ethanol precipitated to a final concentration of 1 µg/µl. From 2 µg of cDNA, cRNA was synthesized using Ambion's T7 MegaScript *in vitro* Transcription Kit®.

To biotin label the cRNA, nucleotides Bio-11-CTP and Bio-16-UTP (Enzo Diagnostics) were added into the reaction. Following a 37°C incubation for six hours, impurities were removed from the labeled cRNA following the RNAsEasyMini kit protocol (Qiagen). cRNA was fragmented (5' fragmentation buffer consisting of 200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc) for thirty-five minutes at 94°C. Following the Affymetrix protocol, 55 µg of fragmented cRNA was hybridized on a Human chip set and the HuGeneFL array for twenty-four hours at 60 rpm in a 45°C hybridization oven. The chips were washed and stained with Streptavidin Phycoerythrin (SAPE) (Molecular Probes) in Affymetrix fluidics stations. To amplify staining, SAPE solution was added twice with an anti-streptavidin biotinylated antibody (Vector Laboratories) staining step in between. Hybridization to the probe arrays was detected by fluorometric scanning (Hewlett Packard Gene Array Scanner). Data was analyzed using Affymetrix GeneChip version 3.3 and Expression Data Mining Tool (EDMT) software (version 1.1).

Differential expression of genes between the BPH and normal prostate samples (see Figure 1) was determined using an Affymetrix human GeneChip chip set by the following criteria: (1) For each gene, Affymetrix GeneChip average difference values were determined by standard Affymetrix EDT software algorithms, which also made "Absent" (=not detected), "Present" (=detected) or "Marginal" (=not clearly Absent or Present) calls for each GeneChip element; (2) all negative values (=Absent) were raised to a floor of +20 (positive 20) so that fold change calculations could be made where values were not already greater than or equal to +20; (3) median levels of expression were compared between the normal control group and the BPH with symptoms disease group to obtain greater than or equal to 3-fold up/down values; (4) The median value for the higher expressing group needed to be greater or equal to 200 average difference units in order to be considered for statistical

significance; (5) Genes passing the criteria of #1-4 were analyzed for statistical significance using a two-tailed T test and deemed statistically significant if $p < 0.05$.

The upper part of Figure 1 shows the expression of JT463726 across 25 human samples, including 10 normals, 5 BPH samples from individuals without symptoms, 5 BPH samples from individuals who were prostate cancer patients, and 5 BPH samples from patients with symptoms. A significant increase was observed (10.7-fold; $p = 0.0288$) in average JT463726 expression levels in tissue from BPH patients with symptoms compared to normal prostate tissue samples, indicating that up-regulation of JT463726 may be diagnostic for BPH in general and more strongly so for patients with symptoms and patients with prostate cancer.

The lower part of Figure 1 shows an electronic Northern in which the expression level of JT463726 was measured across a panel of normal tissues using the Affymetrix 42K human GeneChip set. For each tissue type, the mean $\pm$ SDM is shown as a horizontal bar for samples obtained from 3 or more normal individuals. Using a slightly different panel of normal tissues, as well as prostate samples from various BPH disease states, Figure 2 shows even more clearly that expression of JT463726 is highly restricted and tissue-specific. The highest expression levels of JT463726 are found in BPH + symptoms and BPH + cancer samples, with lower levels observed in uterus, endometrium, BPH without symptoms, normal prostate and myometrium samples.

Example 2

Cloning of Full Length Human cDNAs Corresponding to Differentially Expressed mRNA Species

The full length cDNA having SEQ ID NO: 1 was obtained by the oligo-pulling method. Briefly, a gene-specific oligo was designed based on the sequence of JT463726. The oligo was labeled with biotin and used to hybridize with 2 μ g of single-stranded plasmid DNA (cDNA recombinants) from a placental cDNA library following the procedures of Sambrook *et al.* The hybridized cDNAs were separated by streptavidin-conjugated beads and eluted by heating. The eluted cDNA was converted to double-stranded plasmid DNA and used to transform *E. coli* cells (DH10B), and the longest cDNA was screened. After positive selection was confirmed by PCR using gene-specific primers, the cDNA clone was subjected

to DNA sequencing.

The nucleotide sequence of the full-length human cDNA corresponding to the differentially regulated mRNA detected above is set forth in SEQ ID NO: 1. The corresponding amino acid sequence for the encoded protein is forth in SEQ ID NO: 2. The cDNA comprises 505 base pairs (491 base pairs and a polyA tail).

An open reading frame within the cDNA nucleotide sequence of SEQ ID NO: 1, at nucleotides 78-383 (78-386 including the stop codon), encodes a protein of 102 amino acids. The amino acid sequence corresponding to the protein encoded by SEQ ID NO: 1 is set forth in SEQ ID NO: 2.

SEQ ID NO: 1 shows homology to the JM27 gene, GenBank Accession No. AJ005894. Although a coding region is predicted for JM27, the predicted protein has not been isolated or characterized, and the orphan JM27 gene has not been associated with any function or biological pathway.

Figure 3 shows the results of the quantitative PCR analysis of expression levels of mRNA corresponding to SEQ ID NO: 1 in various human tissue samples. Real time PCR detection was accomplished by the use of the ABI PRISM 7700 Sequence Detection System. The 7700 measures the fluorescence intensity of the sample each cycle and is able to detect the presence of specific amplicons within the PCR reaction. Each sample was assayed for the level of GAPDH (as an endogenous reference) and mRNA corresponding to SEQ ID NO: 1. GAPDH detection was performed using Perkin Elmer part no. 402869 according to the manufacturer's directions. Primers were designed from SEQ ID NO: 1 using Primer Express, a program developed by PE to efficiently find primers and probes for specific sequences. These primers were used in conjunction with SYBR green (Molecular Probes), a nonspecific double-stranded DNA dye, to measure the expression level of mRNA corresponding to SEQ ID NO: 1, which was normalized to the GAPDH level in each sample.

Figure 4 shows the results of a Northern blot analysis of the expression level of JT463726 in various normal tissues. In figures 3 and 4, expression is seen only in placental tissue.

Figure 5 shows the results of a hydrophobicity analysis of the amino acid sequence of JT463726. The protein sequence appears to be largely hydrophilic, except for the region approximately between amino acids 20-30.

Figure 6 shows the similarity of SEQ ID NO: 2 at the amino acid sequence level between JT463726 and GAGE and PAGE proteins.

JT463726 contains regions of homology to a family of MAGE/GAGE-like proteins that have RGD motifs frequently found in cell adhesion proteins. As GAGE/MAGE antigens were reported previously to be targets for tumor-specific cytotoxic lymphocytes in melanoma (Brinkman *et al.*, *Cancer Res* 59:1445-1448, 1999), these results suggest that JT463726 may be related to prostate cancer progression. Overexpression of this molecule in symptomatic BPH and BPH with cancer (present data) and overexpression of PAGE-1 in prostate, tumorous prostate, uterine, cancerous uterine, fallopian tube and placental tissue samples (Brinkman *et al.*, *Proc Natl Acad Sci USA* 95:10757-10762, 1998), together with the fact that expression of both genes is remarkably tissue specific, lend further support to such an argument.

These data suggest that there are several commonalities between symptomatic BPH and BPH cancer. In fact, BPH in itself appears to be closely related to prostate cancer since both diseases involve overgrowth of epithelial cells. It has been suggested that tumor development, such as in prostate cancer, appears to proceed via a process in which a succession of genetic changes, each conferring a type of growth advantage, leads to the progressive conversion of normal human cells into cancer cells (Hanahan *et al.*, *Cell* 100:57-70, 2000). In contrast, BPH is rarely associated with genetic abnormalities and is an overgrowth of a more normal epithelium (De Marzo *et al.*, *Urology* 53(supp 3a):29-39, 1999).

Figure 7 shows the expression and location of the JT463726 protein via immunohistochemical staining, using the same samples as in the gene expression studies and an anti-peptide antibody specific for JT463726. A synthetic peptide, CPGQREGTPPIEERKVE (amino acid residues 43-60 of SEQ ID NO: 2), was used for the production of polyclonal antibodies in rabbits. The antibodies were affinity purified using the synthetic peptide. JT463726 protein is over expressed in symptomatic BPH (C) when compared to either asymptomatic BPH (B), normal prostate (A) or pre-immune serum (D). Within the prostate tissue, however, expression of this protein is confined to stromal cells, with no expression in the epithelial (glandular) component.

Because BPH with symptoms is associated with hypertrophic growth of the prostate

and/or low grade, chronic inflammation, up-regulation of JT463726 DNA or of protein products derived from JT463726 is likely to be of diagnostic significance in disease states involving hypertrophic growth and/or inflammatory responses in tissues in which JT463726 is found, and nucleotides or protein products derived from JT463726 are potential targets for therapeutic intervention.

Example 3

Detection of JT463726 mRNA for BPH Disease Screening

The expression level of mRNA corresponding to SEQ ID NO: 1 is determined in prostate tissue biopsy samples, in urine or possibly in lymphocytes from blood samples, as described in Example 1 and Figure 1, *i.e.*, by screening mRNA samples on a GeneChip, or as described in Example 2 and Figure 3, *i.e.*, by semi-quantitative PCR analysis using the fluorescent detection system. Alternatively, samples from non-prostate hyperplastic tissues in malignant or non-malignant states may also be analyzed. Tissue samples from patients with BPH with symptoms and from normal subjects may be used as positive and negative controls. Using standard assay methods, *e.g.*, an Affymetrix GeneChip set, a level of expression of JT463726 statistically significantly higher than that of the normal control is indicative of BPH or a likelihood of developing BPH.

Alternatively, because BPH with symptoms involves a chronic inflammatory response, the up-regulation of JT463726 in prostate tissue could specifically identify cases in which inflammation is occurring, which would have additional diagnostic and prognostic implications and utility.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents, patent applications and publications referred to in this application are herein incorporated by reference in their entirety.

-35-

What Is Claimed:

1. A method of diagnosing prostatic disease in a patient comprising assaying for the expression of JT463726 protein or nucleic acid in a sample, wherein the differential expression of said protein or nucleic acid compared to normal prostate tissue is an indication of prostatic disease.
- 5 2. A method of claim 1, wherein the sample is prostate biopsy tissue.
3. A method claim 1, wherein the sample is a body fluid.
- 10 4. A method of claim 3, wherein the body fluid is selected from the group consisting of urine, blood and peripheral lymphocytes.
5. A method of claim 1, wherein the assay comprises a transcriptional profiling (TP) assay.
- 15 6. A method of claim 5, wherein said TP assay is selected from the group consisting of a hybridization assay, a RNase protection assay, RT-PCR, Northern blot, differential display, TAQMAN and a chip hybridization assay
- 20 7. A method of claim 1, wherein the presence or amount of the protein is assayed.
8. A method of claim 7, wherein the protein is assayed by binding to an antibody.
9. A method of any one of claims 1-7, wherein the prostatic disease is benign prostatic hyperplasia (BPH).
- 25 10. A method of screening for agents that modulate the expression of a nucleic acid molecule encoding JT463726, comprising the steps of:
 - a) exposing cells that express the nucleic acid molecule to the agent; and

-36-

b) determining whether JT463726 expression is modulated in the presence of the agent.

11. A method of screening for agents that modulate at least one activity of JT463726 protein, comprising the steps of:

a) exposing cells that express the protein to the agent; and

b) determining whether at least one activity of JT463726 is modulated in the presence of the agent.

12. A method of treating BPH comprising administering to a patient in need thereof a therapeutically effective amount of an agent which modulates the expression or at least one activity of JT463726.

13. An agent for the treatment of BPH identified by the method of any one of claims 10 or 11.

14. A pharmaceutical composition comprising at least one agent of claim 13 and a pharmaceutically acceptable carrier.

15. The pharmaceutical composition of claim 14, further comprising a second agent which modulates the onset or progression of BPH.

16. A method of treating BPH, comprising administering to a patient in need thereof a therapeutically effective amount of an agent or a composition of any one of claims 14 or 15.

17. A non-human transgenic animal comprising a nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; (b) an isolated nucleic acid molecule that encodes a fragment of at least 6 amino acids of SEQ ID NO: 2; (c) an isolated nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1; (d) an isolated nucleic acid molecule which hybridizes to a nucleic acid molecule that encodes the amino acid sequence

-37-

of SEQ ID NO: 2; and (e) an isolated nucleic acid molecule that encodes a protein that is expressed in BPH and that exhibits at least about 60% nucleotide sequence identity over the open-reading-frame of SEQ ID NO: 1.

5 18. A non-human transgenic animal of claim 17 which does not express a JT463726 protein.

19. An expression vector comprising: (a) a nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; (b) a nucleic acid molecule that encodes a fragment of at
10 least 6 amino acids of SEQ ID NO: 2; (c) a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1; (d) a nucleic acid molecule which hybridizes to a nucleic acid molecule that encodes the amino acid sequence of SEQ ID NO: 2; or (e) a nucleic acid molecule that encodes a protein that is expressed in BPH and that exhibits at least about 60% nucleotide sequence identity over the open-reading-frame of SEQ
15 ID NO: 1.

20. An antibody that recognizes or specifically binds to a polypeptide or protein selected from the group consisting of a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, a polypeptide comprising a fragment of at least 6 amino acids of SEQ ID NO: 2, a
20 polypeptide comprising conservative amino acid substitutions of SEQ ID NO: 2, naturally occurring amino acid sequence variants of SEQ ID NO: 2, and a polypeptide exhibiting at least about 50% amino acid sequence identity with SEQ ID NO: 2.

21. A composition comprising a diluent and a polypeptide or protein selected from the
25 group consisting of an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 2, an isolated polypeptide comprising a fragment of at least 6 amino acids of SEQ ID NO: 2, an isolated polypeptide comprising conservative amino acid substitutions of SEQ ID NO: 2, naturally occurring amino acid sequence variants of SEQ ID NO: 2, and an isolated polypeptide exhibiting at least about 50% amino acid sequence identity with SEQ ID NO: 2.

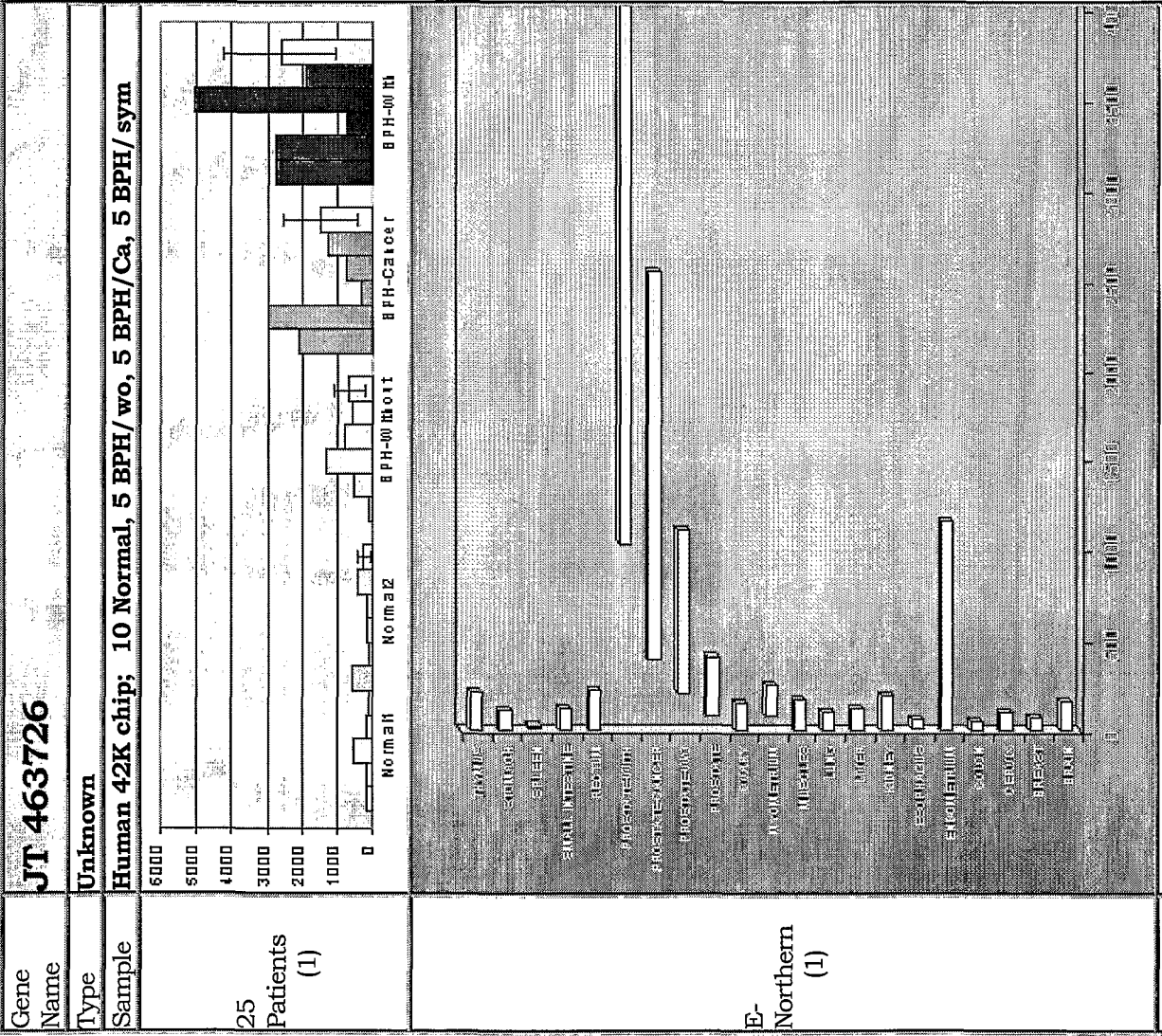
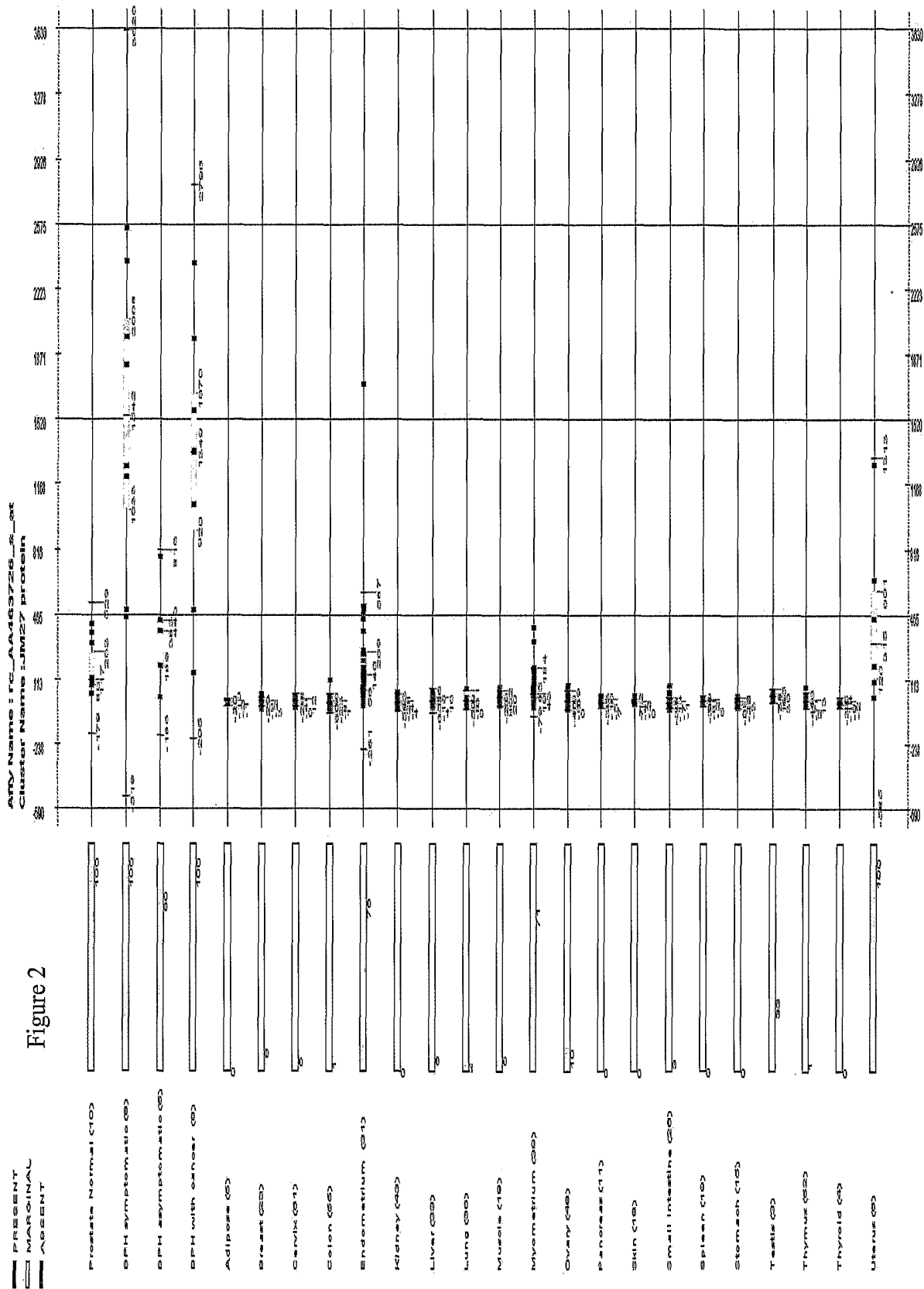


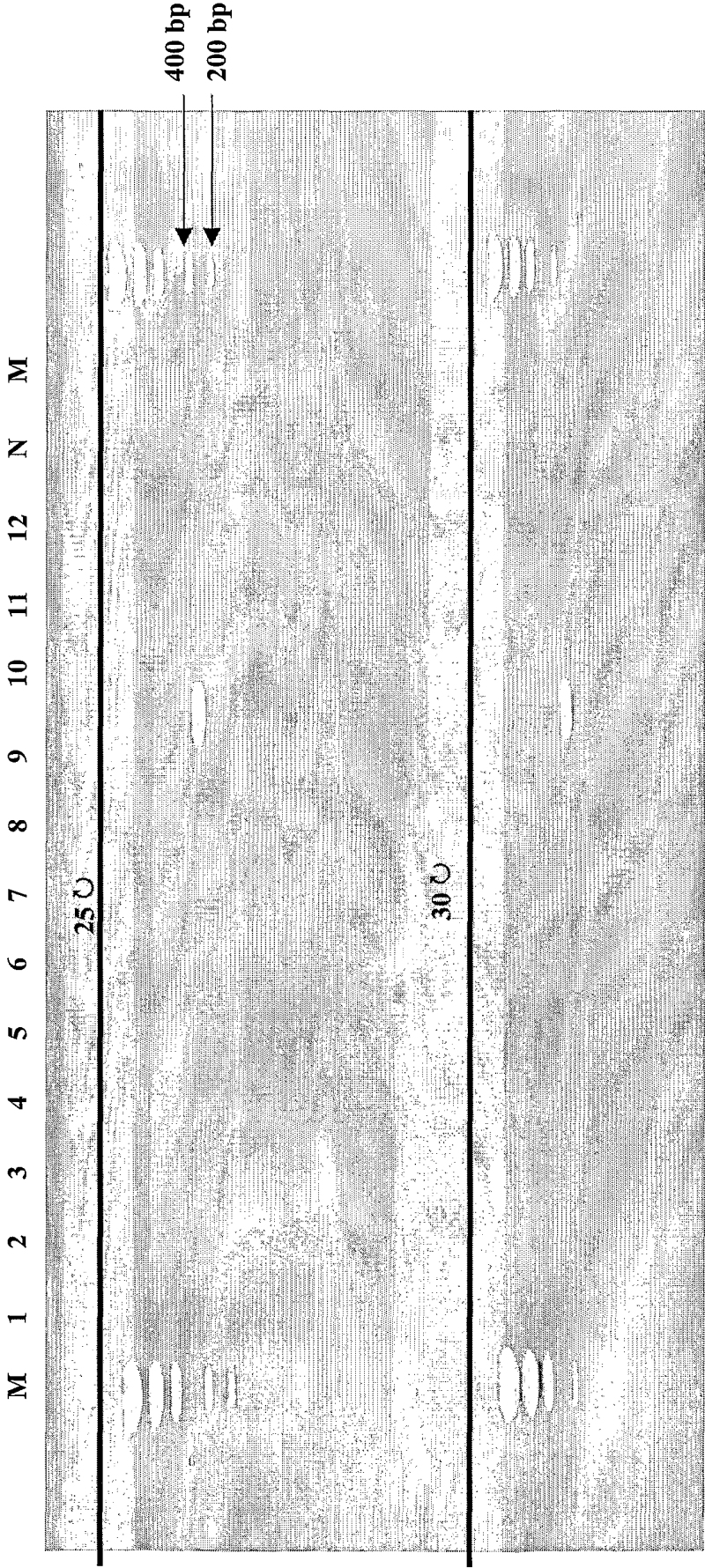
Figure 1

1-WA; 1639028-1



1-WA; 1639028-1

Figure 3



1-WA; 1639028-1

Figure 4

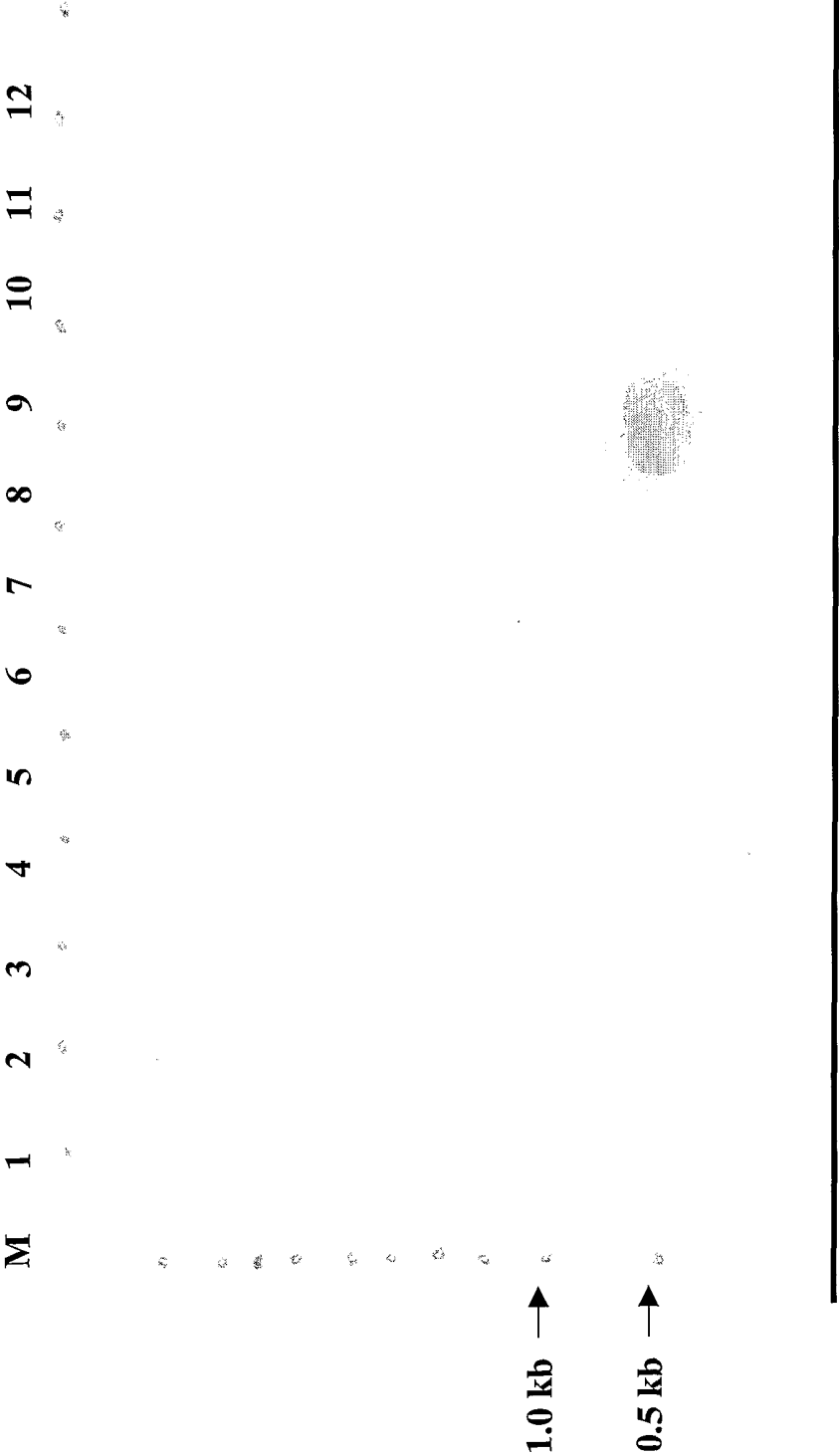
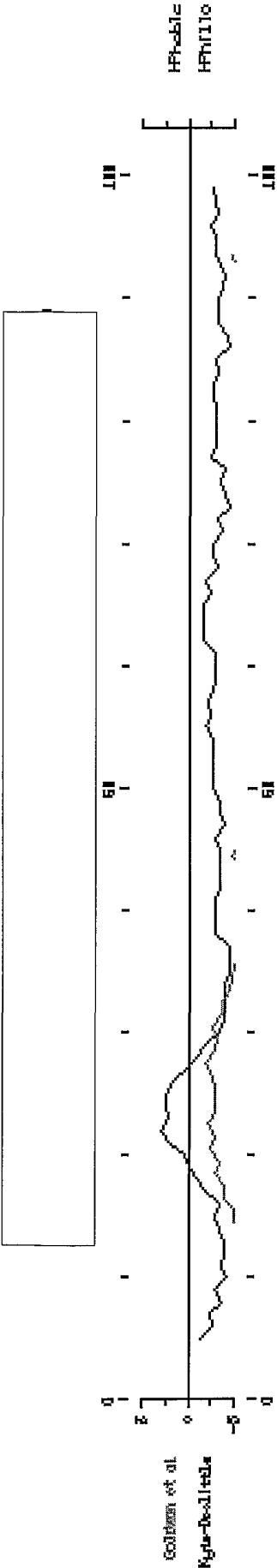


Figure 5



CLUSTAL W (1.74) multiple sequence alignment

Figure 6

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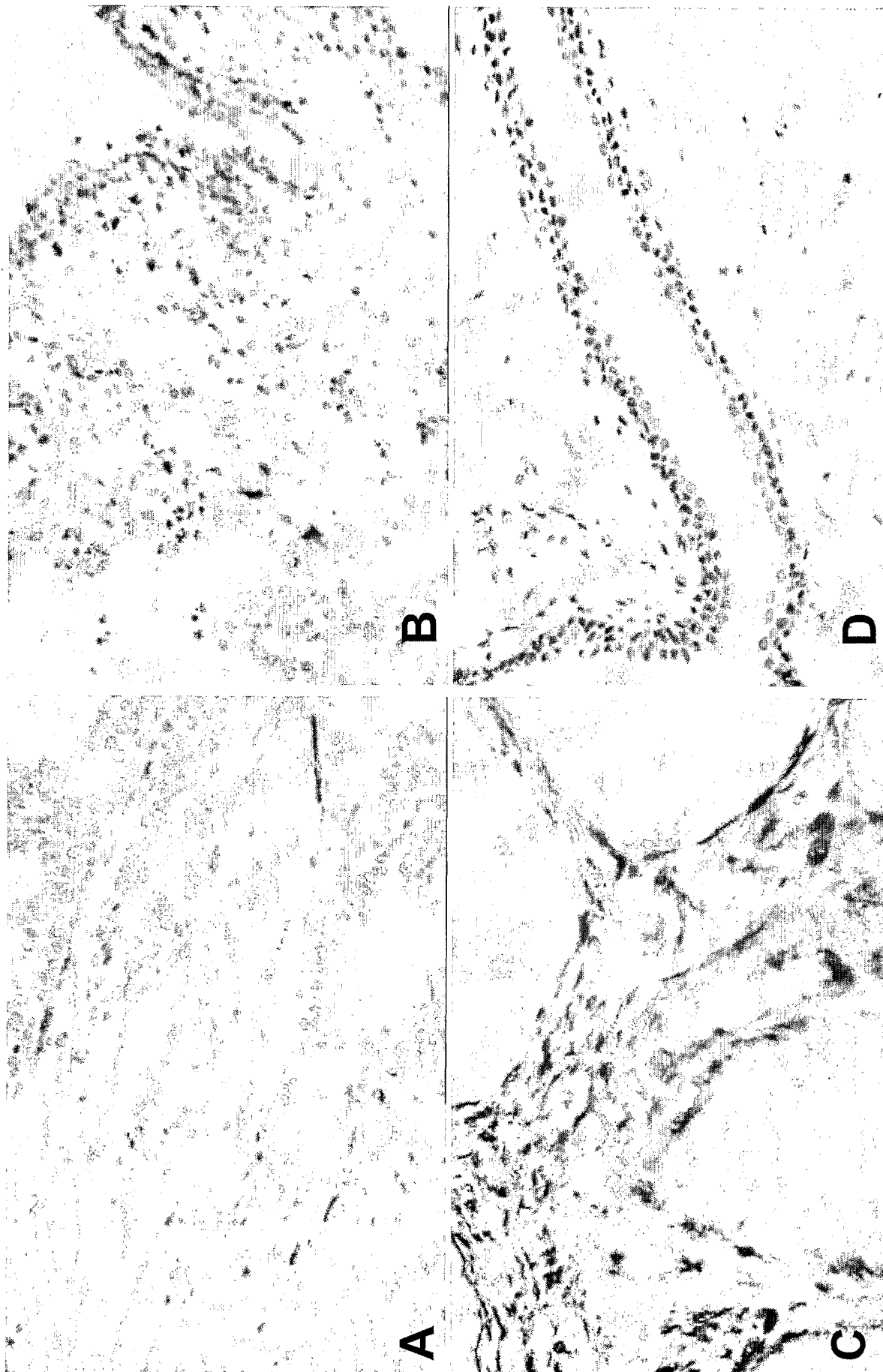


Figure 7

1-WA; 1639028-1

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