US 20090104120A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2009/0104120 A1**

(43) **Pub. Date: Apr. 23, 2009**

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(54) **DLX1 CANCER MARKERS**

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(21) Appl. No.: **12/237,512**

(22) Filed: **Sep. 25, 2008**

Related U.S. Application Data

(60) Provisional application No. 60/974,864, filed on Sep. 25, 2007.

Publication Classification

(51) **Int. Cl.**

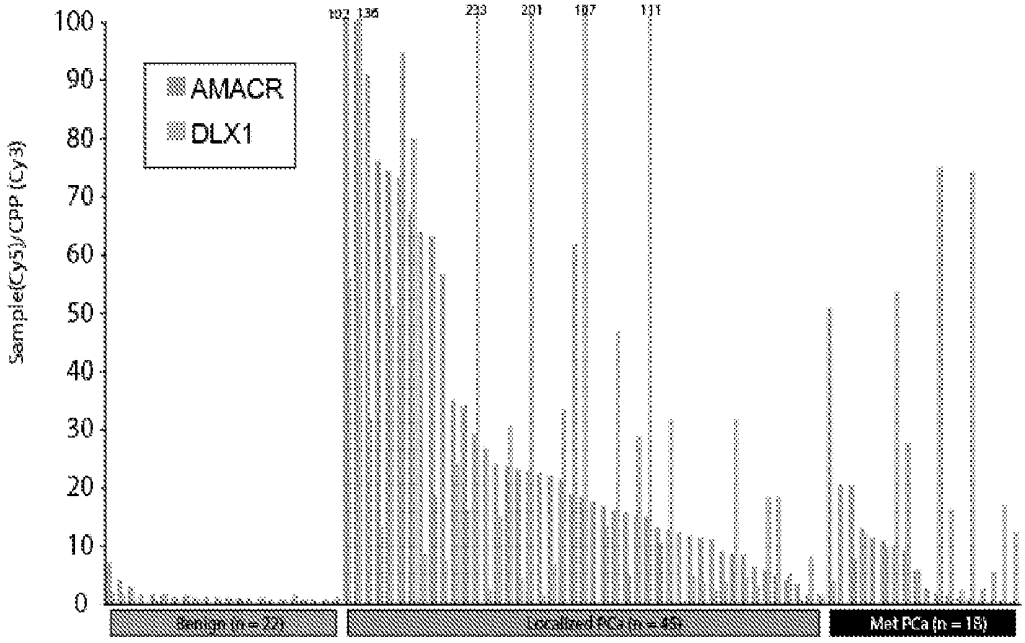
A61K 49/00	(2006.01)
G01N 33/53	(2006.01)
A61P 35/00	(2006.01)

(52) **U.S. Cl.** **424/9.2**; 435/7.1; 435/7.2

(57) **ABSTRACT**

This invention relates to compositions and methods for cancer diagnosis, research and therapy, including but not limited to, cancer markers. In particular, this invention relates to DLX1 cancer markers that are useful as diagnostic markers and clinical targets for prostate cancer.

Figure 1


AMACR
DLX1
Sample(Cy5)/CPP (Cy3)
100
90
80
70
60
50
40
30
20
10
0
102
136
233
201
187
111
Benign (n = 22)
Localized PCa (n = 45)
Met PCa (n = 18)

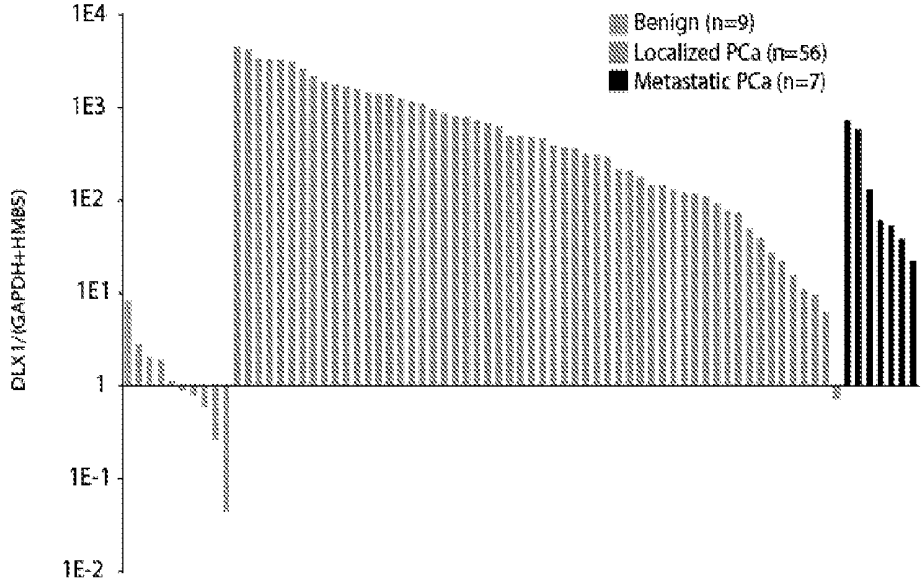

Benign (n=9)
Localized PCa (n=56)
Metastatic PCa (n=7)
DLX1/(GAPDH+HMBS)
1E4
1E3
1E2
1E1
1
1E-1
1E-2

Figure 2

DLX1 mRNA Splice Variant 1 (SEQ ID NO: 3)

1 aagctttgaa ccgagtttgg ggagctcagc agcatcatgc ttagactttt caaagagaca

61 aactccattt tcttatgaat ggaaagtgaa aacccctgtt ccgcttaaat tgggttcctt

121 cctgtcctga gaaacataga gacccccaaa agggaagcag aggagagaaa gtcccacacc

181 cagaccccgc gagaagagat gaccatgacc accatgccag aaagtctcaa cagccccgtg

241 tcgggcaagg cggtgtttat ggagtttggg ccgcccaacc agcaaatgtc tccttctccc

301 atgtcccacg ggcactactc catgcactgt ttacactcgg cgggccattc gcagcccgac

361 ggcgcctaca gctcagcctc gtccttctcc cgaccgctgg gctaccccta cgtcaactcg

421 gtcagcagcc acgcatccag cccctacatc agttcggtgc agtcctaccc gggcagcgcc

481 agcctcgccc agagccgcct ggaggaccca ggggcggact cggagaagag cacggtggtg

541 gaaggcggtg aagtgcgctt caatggcaag ggaaaaaaga tccgtaaacc caggacgatt

601 tattccagtt tgcagttgca ggctttgaac cggaggttcc agcaaactca gtacctagct

661 ctgccggaga gggcggagct cgcggcctct ttgggactca cacagactca ggtcaagatc

721 tggttccaaa acaagcgatc caagttcaag aagctgatga agcagggtgg ggcggctctg

781 gagggtagtg cgttggccaa cggtcgggcc ctgtctgctg gctccccacc cgtgccgccc

841 ggctggaacc ctaactcttc atccgggaag ggctcaggag gaaacgcggg ctcctatatc

901 cccagctaca catcgtggta cccttcagcg caccaagaag ctatgcagca accccaactt

961 atgtgaggtt gcccgcccgt ctccttcttg tctccccggc ccaggtccct cccgcctcca

1021 ggtccatcca tcccgtccgg aaaagaagga cccagaggga agaaggaaca gtggaggcgg

1081 gacgccctcc atctcctcgg agccccgcga ggtccggccc agcaacttcc cggcatccgc

1141 gctctagcct gaaccctggc ctgggccgag cagtggcagc agagagtggc ctcggaggga

1201 agccactgcc acctgagaca gcccaagcag caagataaac ccgctccacc cgacccgccg

1261 accttcagct ttgtgggact atcaggaaaa aacaaaacaa aaacaaaatg tagaaaaagc

1321 aaaagctctt ttctgtcctg tcagtctcct gtctcctttt gctctgtctg tgcgctggta

1381 aagtccaggt cctcatccgt ccgctgtcct cattctgcgg cctcagcaaa aagccacaag

1441 gtctgagcgg cccgggtcct gccgggctga ccatctccgg atcctgggac actctgcctg

1501 accatctgtg tagctggtgt gggaatctgg gggcattgga gggaggggtt tttatttatt

1561 gagaaatgga cttcgcctga ggctgtttgc caattcaggg ttctgctggg cgcaaggaac

1621 gcactgttca aacgcactgt ttactttaag cgcacgggga gaaacgaata aggaggacgt

1681 ggtgattttt aatttataca gtaacttttg tacttctctg gtatggagag tttggagccg

1741 aatgatttgc atttttttaca tgtccgacat tatttaataa ataattttta aaagaaaaga

1801 acgataaatg aagccaacat gattttctca tttcgggagg aactctgttg cttcgcctgg

1861 acaagaagga aaatgctgat ttcctccttg ggtagaaaga gggagcgagg gcaaatgggg

1921 agtagagaga aaacaggcga gaacaagcac tctaattcca gtgggcttta aaataagaca

1981 aaatcagctt tacaacaatc cctagaggct cgaccacaga ataatgccag tcaccaccct

2041 gaacgcacaa tctccagtgc aggatctaat gactgtacat attattgtta ttattattat

2101 tgttattatt gttgttctgt aaacatgttg cacaagctta gccttttgc gttctgttgt

2161 gtgtggctgt aaaaccccat gctttgtgaa atgagaatct tgacattttt cttgtgaaat

2221 ttggaaaatg tgatcaattg aaatcaactg tgttttgtgt tctctatgtc aaagtttagt

2281 tttatattga gaatgttaac ttattgcttt gtatcttggg aaaaaaactt tgtaaataag

2341 ttataaagtt tctttgagac agtaaaatta tgatttcttg aaaaaaaaaa aaaaaaaaaa

2401 aaa

Figure 3

DLX1 mRNA Splice Variant 2 (SEQ ID NO: 4)

1 aagctttgaa ccgagtttgg ggagctcagc agcatcatgc ttagactttt caaagagaca

61 aactccattt tcttatgaat ggaaagtgaa aacccctgtt ccgcttaaat tgggttcctt

121 cctgtcctga gaaacataga gacccccaaa agggaagcag aggagagaaa gtcccacacc

181 cagaccccgc gagaagagat gaccatgacc accatgccag aaagtctcaa cagccccgtg

241 tcgggcaagg cggtgtttat ggagtttggg ccgcccaacc agcaaatgtc tccttctccc

301 atgtcccacg ggcactactc catgcactgt ttacactcgg cgggccattc gcagcccgac

361 ggcgcctaca gctcagcctc gtccttctcc cgaccgctgg gctaccccta cgtcaactcg

421 gtcagcagcc acgcatccag cccctacatc agttcggtgc agtcctaccc gggcagcgcc

481 agcctcgccc agagccgcct ggaggaccca ggtcaagatc tggttccaaa acaagcgatc

541 caagttcaag aagctgatga agcagggtgg ggcggctctg gagggtagtg cgttggccaa

601 cggtcgggcc ctgtctgctg gctccccacc cgtgccgccc ggctggaacc ctaactcttc

661 atccgggaag ggctcaggag gaaacgcggg ctcctatatc cccagctaca catcgtggta

721 cccttcagcg caccaagaag ctatgcagca accccaactt atgtgaggtt gcccgcccgt

781 ctccttcttg tctccccggc ccaggtccct cccgcctcca ggtccatcca tcccgtccgg

841 aaaagaagga cccagaggga agaaggaaca gtggaggcgg gacgccctcc atctcctcgg

901 agccccgcga ggtccggccc agcaacttcc cggcatccgc gctctagcct gaaccctggc

961 ctgggccgag cagtggcagc agagagtggc ctcggaggga agccactgcc acctgagaca

1021 gcccaagcag caagataaac ccgctccacc cgacccgccg accttcagct ttgtgggact

1081 atcaggaaaa aacaaaacaa aaacaaaatg tagaaaaagc aaaagctctt ttctgtcctg

1141 tcagtctcct gtctcctttt gctctgtctg tgcgctggta aagtccaggt cctcatccgt

1201 ccgctgtcct cattctgcgg cctcagcaaa aagccacaag gtctgagcgg cccgggtcct

1261 gccgggctga ccatctccgg atcctgggac actctgcctg accatctgtg tagctggtgt

1321 gggaatctgg gggcattgga gggagggggt tttatttatt gagaaatgga cttcgcctga

1381 ggctgtttgc caattcaggg ttctgctggg cgcaaggaac gcactgttca aacgcactgt

1441 ttactttaag cgcacgggga gaaacgaata aggaggacgt ggtgattttt aatttataca

1501 gtaacttttg tacttctctg gtatggagag tttggagccg aatgatttgc atttttttaca

1561 tgtccgacat tatttaataa ataatttta aaagaaaaga acgataaatg aagccaacat

1621 gattttctca tttcgggagg aactctgttg cttcgcctgg acaagaagga aaatgctgat

1681 ttcctccttg ggtagaaaga gggagcgagg gcaaatgggg agtagagaga aaacaggcga

1741 gaacaagcac tctaattcca gtgggcttta aaataagaca aaatcagctt tacaacaatc

1801 cctagaggct cgaccacaga ataatgccag tcaccaccct gaacgcacaa tctccagtgc

1861 aggatctaat gactgtacat attattgtta ttattattat tgttattatt gttgttctgt

1921 aaacatgttg cacaagctta gccttttttgc gttctgttgt gtgtggctgt aaaaccccat

1981 gctttgtgaa atgagaatct tgacattttt cttgtgaaat ttggaaaatg tgatcaattg

2041 aaatcaactg tgttttgtgt tctctatgtc aaagtttagt tttatattga gaatgttaac

2101 ttattgcttt gtatcttggg aaaaaaactt tgtaaataag ttataaagtt tctttgagac

2161 agtaaaatta tgatttcttg aaaaaaaaaa aaaaaaaaaa aaa

Figure 4

DLX1 polypeptide isoform 1 (SEQ ID NO:5)

1 mtmttmpesl nspvsgkavf mefgppnqqm spspmshghy smhclhsagh sqpdgayssa

61 ssfsrplgyp yvnsvsshas spyissvqsy pgsaslaqsr ledpgadsek stvveggevr

121 fngkgkkirk prtiysslql qalnrrfqqt qylalperae laaslgltqt qvkiwfqnkr

181 skfkklmkqg gaalegsala ngralsagsp pvppgwnpns ssgkgsggna gsyipsytsw

241 ypsahqeamq qpqlm

Figure 5

DLX1 polypeptide isoform 2 (SEQ ID NO:6)

1 mtmttmpesl nspvsgkavf mefgppnqqm spspmshghy smhclhsagh sqpdgayssa

61 ssfsrplgyp yvnsvsshas spyissvqsy pgsaslaqsr ledpgqdlvp kqaiqvqead

121 eagwggsgg

DLX1 CANCER MARKERS

FIELD OF THE INVENTION

[0001] This application claims priority to U.S. Provisional Application No. 60/974,864, filed Sep. 25, 2007, herein incorporated by reference in its entirety.

[0002] This invention relates to compositions and methods for cancer diagnosis, research and therapy, including but not limited to, cancer markers. In particular, this invention relates to DLX1 cancer markers that are useful as diagnostic markers and clinical targets for prostate cancer.

BACKGROUND OF THE INVENTION

[0003] Afflicting one out of nine men over age 65, prostate cancer (PCA) is a leading cause of male cancer-related death, second only to lung cancer (Abate-Shen and Shen, Genes Dev 14:2410 [2000]; Ruijter et al., Endocr Rev, 20:22 [1999]).

[0004] Prostate cancer is typically diagnosed with a digital rectal exam and/or prostate specific antigen (PSA) screening. An elevated serum PSA level can indicate the presence of PCA. PSA is used as a marker for prostate cancer because it is secreted only by prostate cells. A healthy prostate will produce a stable amount—typically below 4 nanograms per milliliter, or a PSA reading of "4" or less—whereas cancer cells produce escalating amounts that correspond with the severity of the cancer. A level between 4 and 10 may raise a doctor's suspicion that a patient has prostate cancer, while amounts above 50 may show that the tumor has spread elsewhere in the body.

[0005] When PSA or digital tests indicate a strong likelihood that cancer is present, a transrectal ultrasound (TRUS) is used to map the prostate and show any suspicious areas. Biopsies of various sectors of the prostate are used to determine if prostate cancer is present. Treatment options depend on the stage of the cancer. Men with a 10-year life expectancy or less who have a low Gleason number and whose tumor has not spread beyond the prostate are often treated with watchful waiting (no treatment). Treatment options for more aggressive cancers include surgical treatments such as radical prostatectomy (RP), in which the prostate is completely removed (with or without nerve sparing techniques) and radiation, applied through an external beam that directs the dose to the prostate from outside the body or via low-dose radioactive seeds that are implanted within the prostate to kill cancer cells locally. Anti-androgen hormone therapy is also used, alone or in conjunction with surgery or radiation. Hormone therapy uses luteinizing hormone-releasing hormones (LH-RH) analogs, which block the pituitary from producing hormones that stimulate testosterone production. Patients must have injections of LH-RH analogs for the rest of their lives.

[0006] While surgical and hormonal treatments are often effective for localized PCA, advanced disease remains essentially incurable. Androgen ablation is the most common therapy for advanced PCA, leading to massive apoptosis of androgen-dependent malignant cells and temporary tumor regression. In most cases, however, the tumor reemerges with a vengeance and can proliferate independent of androgen signals. The advent of prostate specific antigen (PSA) screening has led to earlier detection of PCA and significantly reduced PCA-associated fatalities. However, the impact of PSA screening on cancer-specific mortality is still unknown pending the results of prospective randomized screening studies (Etzioni et al., J. Natl. Cancer Inst., 91:1033 [1999]; Maattanen et al., Br. J. Cancer 79:1210 [1999]; Schroder et al., J. Natl. Cancer Inst., 90:1817 [1998]). A major limitation of the serum PSA test is a lack of prostate cancer sensitivity and specificity especially in the intermediate range of PSA detection (4-10 ng/ml). Elevated serum PSA levels are often detected in patients with non-malignant conditions such as benign prostatic hyperplasia (BPH) and prostatitis, and provide little information about the aggressiveness of the cancer detected. Coincident with increased serum PSA testing, there has been a dramatic increase in the number of prostate needle biopsies performed (Jacobsen et al., JAMA 274:1445 [1995]). This has resulted in a surge of equivocal prostate needle biopsies (Epstein and Potter J. Urol., 166:402 [2001]). Thus, development of additional serum and tissue biomarkers to supplement PSA screening is needed.

SUMMARY OF THE INVENTION

[0007] This invention relates to compositions and methods for cancer diagnosis, research and therapy, including but not limited to, cancer markers. In particular, this invention relates to DLX1 cancer markers that are useful as diagnostic markers and clinical targets for prostate cancer.

[0008] For example, in some embodiments, the present invention provides a method of diagnosing cancer, comprising: detecting the presence or absence of overexpression of DLX1 in a sample from a patient. In some embodiments, overexpression of DLX1 is indicative of cancer in the sample (e.g., prostate cancer). In some embodiments, the prostate cancer is localized prostate cancer or metastatic cancer. In some embodiments, the sample is a tumor sample, a cell sample, a blood sample, a serum sample, or a urine sample. In some embodiments, detecting overexpression of DLX1 comprises detecting overexpression of DLX1 mRNA (e.g., using a hybridization assay or an amplification assay (e.g., a quantitative PCR assay)). In some embodiments, detecting overexpression of DLX1 comprises detecting overexpression of DLX1 polypeptide (e.g., using an immunoassay).

[0009] The present invention further provides a method of screening compounds, comprising contacting a cell expressing DLX1 with a test compound and assaying the level of expression of DLX1 in the presence of the test compound. In some embodiments, the cell overexpresses DLX1. In some embodiments, contacting the cell expressing DLX1 with the test compound results in a decrease in expression of DLX1. In some embodiments, the cell is in vitro, ex vivo, or in vivo (e.g., in a non-human animal).

[0010] The present invention additionally provides a system, comprising a prostate cell sample, a reagent configured to detect overexpression of DLX1 (e.g., DLX1 mRNA or polypeptide). In some embodiments, the reagent is an antibody that specifically binds to DLX1 polypeptide, a nucleic acid probe that specifically hybridizes to DLX1, or a pair of amplification oligonucleotides that specifically amplify DLX1.

[0011] In further embodiments, the present invention provides a kit for detection of overexpression of DLX1 (e.g., DLX1 mRNA or polypeptide), comprising reagent configured to detect overexpression of DLX1 (e.g., DLX1 mRNA or polypeptide) and instructions for using the reagent to detect overexpression of DLX1. In some embodiments, the reagent is an antibody that specifically binds to DLX1 polypeptide, a nucleic acid probe that specifically hybridizes to DLX1, or a pair of amplification oligonucleotides that specifically amplify DLX1. In some embodiments, the kit further com-

prises any additional components necessary, sufficient, or useful for detection of overexpression of DLX1 (e.g., controls, buffers, etc.).

[0012] In yet other embodiments, the present invention provides methods of inhibiting DLX1 activity, comprising: contacting a cell expressing DLX1 with a compound that inhibits at least one activity of DLX1. In some embodiments, the compound inhibits expression or overexpression of DLX1. In some embodiments, the compound is an antibody that specifically binds to DLX1, an antisense oligonucleotide specific for DLX1, an siRNA specific for DLX1, or an miRNA specific for DLX1. In some embodiments, the cell is in vitro, ex vivo, or in vivo.

DESCRIPTION OF THE FIGURES

[0013] FIG. **1** shows over-expression of DLX1 in prostate cancer. A. Benign prostate (n=22), localized prostate cancers (PCa, n=45) and hormone refractory metastatic prostate cancers (n=18) were profiled on Agilent Whole Genome Oligo expression microarrays. B. Over-expression of DLX1 in prostate cancer was confirmed by qPCR in benign prostate (n=9), localized prostate cancers (n=56) and hormone refractory metastatic prostate cancers (n=7).

[0014] FIG. **2** shows DLX1 mRNA transcript variant 1 (SEQ ID NO:3).

[0015] FIG. **3** shows DLX1 mRNA transcript variant 2 (SEQ ID NO:4).

[0016] FIG. **4** shows DLX1 polypeptide isoform 1 (SEQ ID NO:5).

[0017] FIG. **5** shows DLX1 polypeptide isoform 2 (SEQ ID NO:6).

DEFINITIONS

[0018] To facilitate an understanding of this disclosure, terms are defined below:

[0019] As used herein, the terms "detect", "detecting", or "detection" may describe either the general act of discovering or discerning or the specific observation of a detectably labeled composition.

[0020] As used herein, the term "siRNAs" refers to small interfering RNAs. In some embodiments, siRNAs comprise a duplex, or double-stranded region, of about 18-25 nucleotides long; often siRNAs contain from about two to four unpaired nucleotides at the 3' end of each strand. At least one strand of the duplex or double-stranded region of a siRNA is substantially homologous to, or substantially complementary to, a target RNA molecule. The strand complementary to a target RNA molecule is the "antisense strand;" the strand homologous to the target RNA molecule is the "sense strand," and is also complementary to the siRNA antisense strand. siRNAs may also contain additional sequences; non-limiting examples of such sequences include linking sequences, or loops, as well as stem and other folded structures. siRNAs appear to function as key intermediaries in triggering RNA interference in invertebrates and in vertebrates, and in triggering sequence-specific RNA degradation during posttranscriptional gene silencing in plants.

[0021] The term "RNA interference" or "RNAi" refers to the silencing or decreasing of gene expression by siRNAs. It is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by siRNA that is homologous in its duplex region to the sequence of the silenced gene. The gene may be endogenous or exogenous to the organism, present integrated into a chromosome or present in a transfection vector that is not integrated into the genome. The expression of the gene is either completely or partially inhibited. RNAi may also be considered to inhibit the function of a target RNA; the function of the target RNA may be complete or partial.

[0022] As used herein, the term "stage of cancer" refers to a qualitative or quantitative assessment of the level of advancement of a cancer. Criteria used to determine the stage of a cancer include, but are not limited to, the size of the tumor and the extent of metastases (e.g., localized or distant).

[0023] As used herein, the term "nucleic acid molecule" refers to any nucleic acid containing molecule, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl)uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

[0024] The term "gene" refers to a nucleic acid (e.g., DNA) sequence that comprises coding sequences necessary for the production of a polypeptide, precursor, or RNA (e.g., rRNA, tRNA). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (e.g., enzymatic activity, ligand binding, signal transduction, immunogenicity, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and the sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. Sequences located 5' of the coding region and present on the mRNA are referred to as 5' non-translated sequences. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

[0025] As used herein, the term "oligonucleotide," refers to a short length of single-stranded polynucleotide chain. Oligonucleotides are typically less than 200 residues long (e.g.,

between 15 and 100), however, as used herein, the term is also intended to encompass longer polynucleotide chains. Oligonucleotides are often referred to by their length. For example a 24 residue oligonucleotide is referred to as a "24-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

[0026] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[0027] The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is a nucleic acid molecule that at least partially inhibits a completely complementary nucleic acid molecule from hybridizing to a target nucleic acid is "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous nucleic acid molecule to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target that is substantially non-complementary (e.g., less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

[0028] When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe that can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above.

[0029] When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (i.e., it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

[0030] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule that contains pairing of complementary nucleic acids within its structure is said to be "self-hybridized."

[0031] As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Under "low stringency conditions" a nucleic acid sequence of interest will hybridize to its exact complement, sequences with single base mismatches, closely related sequences (e.g., sequences with 90% or greater homology), and sequences having only partial homology (e.g., sequences with 50-90% homology). Under "medium stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, sequences with single base mismatches, and closely relation sequences (e.g., 90% or greater homology). Under "high stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, and (depending on conditions such a temperature) sequences with single base mismatches. In other words, under conditions of high stringency the temperature can be raised so as to exclude hybridization to sequences with single base mismatches.

[0032] "High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42° C. in a solution consisting of 5×SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5×Denhardt's reagent and 100 μg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1×SSPE, 1.0% SDS at 42° C. when a probe of about 500 nucleotides in length is employed.

[0033] "Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42° C. in a solution consisting of 5×SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5×Denhardt's reagent and 100 μg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0×SSPE, 1.0% SDS at 42° C. when a probe of about 500 nucleotides in length is employed.

[0034] "Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42° C. in a solution consisting of 5×SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5×Denhardt's reagent [50×Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharamcia), 5 g BSA (Fraction V; Sigma)] and 100 μg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5×SSPE, 0.1% SDS at 42° C. when a probe of about 500 nucleotides in length is employed.

[0035] The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (e.g., increasing the temperature of the hybridization

and/or wash steps, the use of formamide in the hybridization solution, etc.) (see definition above for "stringency").

[0036] As used herein, the term "amplification oligonucleotide" refers to an oligonucleotide that hybridizes to a target nucleic acid, or its complement, and participates in a nucleic acid amplification reaction. An example of an amplification oligonucleotide is a "primer" that hybridizes to a template nucleic acid and contains a 3' OH end that is extended by a polymerase in an amplification process. Another example of an amplification oligonucleotide is an oligonucleotide that is not extended by a polymerase (e.g., because it has a 3' blocked end) but participates in or facilitates amplification. Amplification oligonucleotides may optionally include modified nucleotides or analogs, or additional nucleotides that participate in an amplification reaction but are not complementary to or contained in the target nucleic acid. Amplification oligonucleotides may contain a sequence that is not complementary to the target or template sequence. For example, the 5' region of a primer may include a promoter sequence that is non-complementary to the target nucleic acid (referred to as a "promoter-primer"). Those skilled in the art will understand that an amplification oligonucleotide that functions as a primer may be modified to include a 5' promoter sequence, and thus function as a promoter-primer. Similarly, a promoter-primer may be modified by removal of, or synthesis without, a promoter sequence and still function as a primer. A 3' blocked amplification oligonucleotide may provide a promoter sequence and serve as a template for polymerization (referred to as a "promoter-provider").

[0037] As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, that is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product that is complementary to a nucleic acid strand is induced, (i.e., in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

[0038] As used herein, the term "probe" refers to an oligonucleotide (i.e., a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, that is capable of hybridizing to at least a portion of another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the disclosed methods may be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (e.g., ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. The compositions and methods that use such compositions as disclosed herein are not limited to any particular detection system or label.

[0039] As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, crystals and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0040] This invention relates to compositions and methods for cancer diagnosis, research and therapy, including but not limited to, cancer markers. In particular, this invention relates to DLX1 cancer markers that are useful as diagnostic markers and clinical targets for prostate cancer.

[0041] Experiments described herein (See e.g., Example 1) analyzed 22 benign prostate tissues, 45 localized prostate cancers and 18 hormone refractory metastatic prostate cancers using Agilent Whole Genome Oligo arrays. Over-expression of the homeobox gene DLX1 was identified as a tissue biomarker of prostate cancer. The over-expression of DLX1 in localized and metastatic prostate cancer was confirmed using quantitative PCR (qPCR).

[0042] Accordingly, in some embodiments, the present invention provides diagnostic and therapeutic methods that utilize and target the over expression of DLX1.

I. DLX1

[0043] The highly conserved Dlx1, 2, 5 & 6 homeobox transcription factors also control the development of the basal ganglia and cortical local circuit neurons (Anderson et al., Science 1997, 278:474-476; Anderson et al., Neuron 1997, 19:27-37; Marin et al., J Neurosci 2000, 20:6063-6076; Panganiban et al., Development 2002, 129:4371-4386; Stuhmer et al., Cereb Cortex 2002, 12:75-85; Yun et al., Development 2002, 129:5029-5040. This gene family regulates the expression of glutamic acid decarboxylase [Anderson S, Mione M, Yun K, Rubenstein J L: Differential origins of neocortical projection and local circuit neurons: role of Dlx genes in neocortical interneuronogenesis. Cereb Cortex 1999, 9:646-654; Stuhmer et al., Development 2002, 129:245-252). These genes control the expression of Arx in cells derived from basal ganglia progenitor domains. Mice lacking Dlx1 have defects in subsets of cortical local circuit neurons that lead to their apoptosis and subsequent onset of epilepsy. Therefore, murine Dlx genes have a central role in controlling the development and function of forebrain GABAergic (inhibitory) neurons. The human DLX genes are organized as bigene clusters on chromosomes 2q31.1 (DLX1/2) and 7q21.3 (DLX5/6) and their expression is controlled by intra- and extragenic enhancers (Stock et al., Proc Natl Acad Sci USA 1996, 93:10858-10863; Ghanem et al., Genome Res 2003, 13:533-543; McGuinness et al., Genomics 1996, 35:473-485; Zerucha et al., J Neurosci 2000, 20:709-721. Dlx genes are important for the induction of GAD (Stuhmer et al., Development 129:245-252. 2002) as well as for their tangential migration to the cortex (Anderson et al., Science 278:474-476 1997). Dlx1 is required, in combination with Dlx2, for the generation of interneuron precursors in the MGE and their migration to the cortex (Anderson et al., 1997, supra; Panganiban et al., 2002 Development 129:4371-4386) and required

on its own for cell morphogenesis and survival of interneurons within the maturing cortex (Cobos et al., Nat Neurosci 8:1059-10682005).

[0044] DlX1 has recently been reported to have two splice variants of mRNA which result in two isoforms of DLX1 polypeptide. Accession numbers for DLX1 mRNA are NM_178120 (splice variant 1) and NM_001038493 (splice variant 2). Accession numbers for DLX1 polypeptide are NP_835221 (isoform 1) and NP_001033582 (isoform 2). DLX1 mRNA and polypeptide sequences are shown in FIGS. **2-5**.

II. Diagnostic Applications

[0045] In some embodiments, the present invention provides methods of diagnosing and/or characterizing prostate cancer based on the presence or absence of overexpression of DLX1. The disclosed overexpression of DLX1 provides RNA and protein based diagnostic methods that detect, either directly or indirectly, the overexpression of DLX1 mRNA or protein. The disclosed DLX1 overexpression also provides compositions useful for diagnostic purposes, such as oligonucleotide probes that specifically detect the overexpression of DLX1. Such compositions may be in the form of a kit.

[0046] The disclosed diagnostic methods may be qualitative or quantitative. Quantitative methods may, e.g., discriminate between the presence or absence of cancer or between indolent and aggressive cancers via a cutoff or threshold level where expression above that level provides information on the aggressiveness of the cancer which provides useful diagnostic and/or prognostic information to a treating physician or patient. Qualitative or quantitative diagnostic methods may include amplification of a target, signal or intermediary, such as by using a universal primer that amplifies a sequence that serves as an indicator for the presence or level of DLX1.

[0047] The disclosed overexpression of DLX1 may be detected along with other markers in a multiplex or panel format. Markers are selected for their predictive value alone or in combination with the overexpression of DLX1. Exemplary prostate cancer markers include, but are not limited to: AMACR/P504S (U.S. Pat. No. 6,262,245); PCA3 (U.S. Pat. No. 7,008,765); PCGEM1 (U.S. Pat. No. 6,828,429); prostein/P501S, P503S, P504S, P509S, P510S, prostase/P703P, P710P (U.S. Publication No. 20030185830); and, those disclosed in U.S. Pat. Nos. 5,854,206 and 6,034,218, and U.S. Publication No. 20030175736, each of which is herein incorporated by reference in its entirety. Markers for other cancers, diseases, infections, and metabolic conditions are also contemplated for inclusion in a multiplex or panel format.

[0048] The diagnostic methods as disclosed herein may be modified with reference to data correlating overexpression of DLX1 with the presence, stage, aggressiveness or progression of the disease or the presence or risk of metastasis. The information provided by these diagnostic methods provide useful information to a physician who, based on that information, may choose an appropriate therapeutic treatment for a particular patient.

[0049] A. Sample

[0050] Any biological sample suspected of containing overexpression of DLX1 may be tested according to the disclosed methods. Such a sample may be tissue (e.g., prostate biopsy sample or tissue obtained by prostatectomy), blood, urine, semen, prostatic secretions or a fraction thereof (e.g., plasma, serum, urine supernatant, urine cell pellet or prostate cells), which may be obtained from a patient or other source of biological material, e.g., autopsy sample or forensic material. In preferred embodiments, a urine sample is collected immediately following an attentive digital rectal examination (DRE), which causes prostate cells from the prostate gland to shed into the urinary tract.

[0051] The sample may be processed to isolate or enrich the sample for DLX1 or cells that contain DLX1. A variety of well techniques that use standard laboratory practices may be used for this purpose, such as, e.g., centrifugation, immunocapture, cell lysis, and nucleic acid target capture (See, e.g., EP Pat. No. 1 409 727, herein incorporated by reference in its entirety).

[0052] B. RNA Detection

[0053] The disclosed overexpression of DLX1 may be detected as overexpression of mRNA using a variety of well known nucleic acid techniques that rely on standard laboratory methods, such as, e.g., nucleic acid sequencing, nucleic acid hybridization, and, nucleic acid amplification.

[0054] 1. Sequencing

[0055] Illustrative non-limiting examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. Those of ordinary skill in the art will recognize that because RNA is less stable in the cell and more prone to nuclease attack experimentally RNA is usually reverse transcribed to DNA before sequencing.

[0056] Chain terminator sequencing uses sequence-specific termination of a DNA synthesis reaction using modified nucleotide substrates. Extension is initiated at a specific site on the template DNA by using a short radioactive, or other labeled, oligonucleotide primer complementary to the template at that region. The oligonucleotide primer is extended using a DNA polymerase, standard four deoxynucleotide bases, and a low concentration of one chain terminating nucleotide, most commonly a di-deoxynucleotide. This reaction is repeated in four separate tubes with each of the bases taking turns as the di-deoxynucleotide. Limited incorporation of the chain terminating nucleotide by the DNA polymerase results in a series of related DNA fragments that are terminated only at positions where that particular di-deoxynucleotide is used. For each reaction tube, the fragments are size-separated by electrophoresis in a slab polyacrylamide gel or a capillary tube filled with a viscous polymer. The sequence is determined by reading which lane produces a visualized mark from the labeled primer as it is scanned from the top of the gel to the bottom.

[0057] Dye terminator sequencing alternatively labels the terminators. Complete sequencing can be performed in a single reaction by labeling each of the di-deoxynucleotide chain-terminators with a separate fluorescent dye, which fluoresces at a different wavelength.

[0058] 2. Hybridization

[0059] Illustrative non-limiting examples of nucleic acid hybridization techniques include, but are not limited to, in situ hybridization (ISH), microarray, and Southern or Northern blot.

[0060] In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand as a probe to localize a specific DNA or RNA sequence in a portion or section of tissue (in situ), or, if the tissue is small enough, the entire tissue (whole mount ISH). DNA ISH can be used to determine the structure of chromosomes. RNA ISH is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts. Sample cells and

tissues are usually treated to fix the target transcripts in place and to increase access of the probe. The probe hybridizes to the target sequence at elevated temperature, and then the excess probe is washed away. The probe that was labeled with either radio-, fluorescent- or antigen-labeled bases is localized and quantitated in the tissue using either autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity or the other non-radioactive labels, to simultaneously detect two or more transcripts.

[0061] 2.1 FISH

[0062] In some embodiments, overexpression of DLX1 is detected using fluorescence in situ hybridization (FISH). The preferred FISH assays use bacterial artificial chromosomes (BACs), which have been used extensively in the human genome sequencing project (see *Nature* 409: 953-958 (2001)) and clones containing specific BACs are widely available or can be made by using standard laboratory practices. Each BAC clone from the human genome has been given a reference name that unambiguously identifies it. These names can be used to find a corresponding GenBank sequence and to order copies of the clone from a distributor.

[0063] Probes are generally labeled with appropriate fluorescent or other markers and then used in hybridizations. Specific protocols are well known in the art and can be readily adapted for detecting DLX1 overexpression. Guidance regarding such methodology is provided in many references including: *In situ Hybridization: Medical Applications* (eds. G. R. Coulton and J. de Belleroche), Kluwer Academic Publishers, Boston (1992); *In situ Hybridization: In Neurobiology; Advances in Methodology* (eds. J. H. Eberwine, K. L. Valentino, and J. D. Barchas), Oxford University Press Inc., England (1994); *In situ Hybridization: A Practical Approach* (ed. D. G. Wilkinson), Oxford University Press Inc., England (1992)); Kuo, et al., *Am. J. Hum. Genet.* 49:112-119 (1991); Klinger, et al., *Am. J. Hum. Genet.* 51:55-65 (1992); and Ward, et al., *Am. J. Hum. Genet.* 52:854-865 (1993)). Patents providing guidance on such methodology include U.S. Pat. Nos. 5,225,326; 5,545,524; 6,121,489 and 6,573,043, and commercially available kits also provide protocols for performing FISH (e.+g., from Oncor, Inc., Gaithersburg, Md.). All of these references are hereby incorporated by reference in their entirety and may be used along with similar references in the art to establish procedural steps convenient for a particular laboratory.

[0064] 2.2 Microarrays

[0065] Different kinds of biological assays are called microarrays including, but not limited to: DNA microarrays (e.g., cDNA microarrays and oligonucleotide microarrays); protein microarrays; tissue microarrays; transfection or cell microarrays; chemical compound microarrays; and, antibody microarrays. A DNA microarray, commonly known as gene chip, DNA chip, or biochip, is a collection of microscopic DNA spots attached to a solid surface (e.g., glass, plastic or silicon chip) forming an array for the purpose of expression profiling or monitoring expression levels for thousands of genes simultaneously. The affixed DNA segments are known as probes, thousands of which can be used in a single DNA microarray. Microarrays can be used to identify disease genes by comparing gene expression in disease and normal cells. Microarrays can be fabricated using a variety of technologies, including but not limiting: printing with fine-pointed pins onto glass slides; photolithography using pre-made masks; photolithography using dynamic micromirror devices; ink-jet printing; or, electrochemistry on microelectrode arrays.

[0066] Southern and Northern blotting is used to detect specific DNA or RNA sequences, respectively. DNA or RNA extracted from a sample is fragmented, electrophoretically separated on a matrix gel, and transferred to a membrane filter. The filter bound DNA or RNA is subject to hybridization with a labeled probe complementary to the sequence of interest. Hybridized probe bound to the filter is detected. A variant of the procedure is the reverse Northern blot, in which the substrate nucleic acid that is affixed to the membrane is a collection of isolated DNA fragments and the probe is RNA extracted from a tissue and labeled.

[0067] 3. Amplification

[0068] DLX1 mRNA may be amplified prior to or simultaneous with detection. Illustrative non-limiting examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Those of ordinary skill in the art will recognize that certain amplification techniques (e.g., PCR) require that RNA be reversed transcribed to DNA prior to amplification (e.g., RT-PCR), whereas other amplification techniques directly amplify RNA (e.g., TMA and NASBA).

[0069] The polymerase chain reaction (U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159 and 4,965,188, each of which is herein incorporated by reference in its entirety), commonly referred to as PCR, uses multiple cycles of denaturation, annealing of primer pairs to opposite strands, and primer extension to exponentially increase copy numbers of a target nucleic acid sequence. In a variation called RT-PCR, reverse transcriptase (RT) is used to make a complementary DNA (cDNA) from mRNA, and the cDNA is then amplified by PCR to produce multiple copies of DNA. For other various permutations of PCR see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159; Mullis et al., *Meth. Enzymol.* 155: 335 (1987); and, Murakawa et al., *DNA* 7: 287 (1988), each of which is herein incorporated by reference in its entirety.

[0070] Transcription mediated amplification (U.S. Pat. Nos. 5,480,784 and 5,399,491, each of which is herein incorporated by reference in its entirety), commonly referred to as TMA, synthesizes multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH in which multiple RNA copies of the target sequence autocatalytically generate additional copies. See, e.g., U.S. Pat. Nos. 5,399,491 and 5,824,518, each of which is herein incorporated by reference in its entirety. In a variation described in U.S. Publ. No. 20060046265 (herein incorporated by reference in its entirety), TMA optionally incorporates the use of blocking moieties, terminating moieties, and other modifying moieties to improve TMA process sensitivity and accuracy.

[0071] The ligase chain reaction (Weiss, R., *Science* 254: 1292 (1991), herein incorporated by reference in its entirety), commonly referred to as LCR, uses two sets of complementary DNA oligonucleotides that hybridize to adjacent regions of the target nucleic acid. The DNA oligonucleotides are covalently linked by a DNA ligase in repeated cycles of thermal denaturation, hybridization and ligation to produce a detectable double-stranded ligated oligonucleotide product.

[0072] Strand displacement amplification (Walker, G. et al., *Proc. Natl. Acad. Sci. USA* 89: 392-396 (1992); U.S. Pat. Nos. 5,270,184 and 5,455,166, each of which is herein incorporated by reference in its entirety), commonly referred to as SDA, uses cycles of annealing pairs of primer sequences to opposite strands of a target sequence, primer extension in the presence of a dNTPαS to produce a duplex hemiphosphorothioated primer extension product, endonuclease-mediated nicking of a hemimodified restriction endonuclease recognition site, and polymerase-mediated primer extension from the 3' end of the nick to displace an existing strand and produce a strand for the next round of primer annealing, nicking and strand displacement, resulting in geometric amplification of product. Thermophilic SDA (tSDA) uses thermophilic endonucleases and polymerases at higher temperatures in essentially the same method (EP Pat. No. 0 684 315).

[0073] Other amplification methods include, for example: nucleic acid sequence based amplification (U.S. Pat. No. 5,130,238, herein incorporated by reference in its entirety), commonly referred to as NASBA; one that uses an RNA replicase to amplify the probe molecule itself (Lizardi et al., *BioTechnol.* 6: 1197 (1988), herein incorporated by reference in its entirety), commonly referred to as Qβ replicase; a transcription based amplification method (Kwoh et al., *Proc. Natl. Acad. Sci. USA* 86:1173 (1989)); and, self-sustained sequence replication (Guatelli et al., *Proc. Natl. Acad. Sci. USA* 87: 1874 (1990), each of which is herein incorporated by reference in its entirety). For further discussion of known amplification methods see Persing, David H., "In Vitro Nucleic Acid Amplification Techniques" in *Diagnostic Medical Microbiology: Principles and Applications* (Persing et al., Eds.), pp. 51-87 (American Society for Microbiology, Washington, D.C. (1993)).

[0074] 4. Detection Methods

[0075] Non-amplified or amplified overexpression or expression of DLX1 can be detected by any conventional means. For example, DLX1 mRNA can be detected by hybridization with a detectably labeled probe and measurement of the resulting hybrids. Illustrative non-limiting examples of detection methods are described below.

[0076] One illustrative detection method, the Hybridization Protection Assay (HPA) involves hybridizing a chemiluminescent oligonucleotide probe (e.g., an acridinium ester-labeled (AE) probe) to the target sequence, selectively hydrolyzing the chemiluminescent label present on unhybridized probe, and measuring the chemiluminescence produced from the remaining probe in a luminometer. See, e.g., U.S. Pat. No. 5,283,174 and Norman C. Nelson et al., Nonisotopic Probing, Blotting, and Sequencing, ch. 17 (Larry J. Kricka ed., 2d ed. 1995, each of which is herein incorporated by reference in its entirety).

[0077] Another illustrative detection method provides for quantitative evaluation of the amplification process in real-time. Evaluation of an amplification process in "real-time" involves determining the amount of amplicon in the reaction mixture either continuously or periodically during the amplification reaction, and using the determined values to calculate the amount of target sequence initially present in the sample. A variety of methods for determining the amount of initial target sequence present in a sample based on real-time amplification are well known in the art. These include methods disclosed in U.S. Pat. Nos. 6,303,305 and 6,541,205, each of which is herein incorporated by reference in its entirety. Another method for determining the quantity of target sequence initially present in a sample, but which is not based on a real-time amplification, is disclosed in U.S. Pat. No. 5,710,029, herein incorporated by reference in its entirety.

[0078] Amplification products may be detected in real-time through the use of various self-hybridizing probes, most of which have a stem-loop structure. Such self-hybridizing probes are labeled so that they emit differently detectable signals, depending on whether the probes are in a self-hybridized state or an altered state through hybridization to a target sequence. By way of non-limiting example, "molecular torches" are a type of self-hybridizing probe that includes distinct regions of self-complementarity (referred to as "the target binding domain" and "the target closing domain") which are connected by a joining region (e.g., non-nucleotide linker) and which hybridize to each other under predetermined hybridization assay conditions. In a preferred embodiment, molecular torches contain single-stranded base regions in the target binding domain that are from 1 to about 20 bases in length and are accessible for hybridization to a target sequence present in an amplification reaction under strand displacement conditions. Under strand displacement conditions, hybridization of the two complementary regions, which may be fully or partially complementary, of the molecular torch is favored, except in the presence of the target sequence, which will bind to the single-stranded region present in the target binding domain and displace all or a portion of the target closing domain. The target binding domain and the target closing domain of a molecular torch include a detectable label or a pair of interacting labels (e.g., luminescent/quencher) positioned so that a different signal is produced when the molecular torch is self-hybridized than when the molecular torch is hybridized to the target sequence, thereby permitting detection of probe:target duplexes in a test sample in the presence of unhybridized molecular torches. Molecular torches and many types of interacting label pairs are known (e.g., U.S. Pat. No. 6,534,274, herein incorporated by reference in its entirety).

[0079] Another example of a detection probe having self-complementarity is a "molecular beacon" (see U.S. Pat. Nos. 5,925,517 and 6,150,097, herein incorporated by reference in entirety). Molecular beacons include nucleic acid molecules having a target complementary sequence, an affinity pair (or nucleic acid arms) holding the probe in a closed conformation in the absence of a target sequence present in an amplification reaction, and a label pair that interacts when the probe is in a closed conformation. Hybridization of the target sequence and the target complementary sequence separates the members of the affinity pair, thereby shifting the probe to an open conformation. The shift to the open conformation is detectable due to reduced interaction of the label pair, which may be, for example, a fluorophore and a quencher (e.g., DABCYL and EDANS).

[0080] Other self-hybridizing probes are well known to those of ordinary skill in the art. By way of non-limiting example, probe binding pairs having interacting labels (e.g., see U.S. Pat. No. 5,928,862, herein incorporated by reference in its entirety) may be adapted for use in the compositions and methods disclosed herein. Probe systems used to detect single nucleotide polymorphisms (SNPs) might also be used. Additional detection systems include "molecular switches," (e.g., see U.S. Publ. No. 20050042638, herein incorporated by reference in its entirety). Other probes, such as those comprising intercalating dyes and/or fluorochromes, are also useful for detection of amplification products in the methods

disclosed herein (e.g., see U.S. Pat. No. 5,814,447, herein incorporated by reference in its entirety).

[0081] C. Data Analysis

[0082] In some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay (e.g., the presence, absence, or amount of a given marker or markers) into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some preferred embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

[0083] Any method may be used that is capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information provides, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (e.g., a biopsy or a serum or urine sample) is obtained from a subject and submitted to a profiling service (e.g., clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (e.g., in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (e.g., a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (e.g., an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (i.e., expression data), specific for the diagnostic or prognostic information desired for the subject.

[0084] The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw expression data, the prepared format may represent a diagnosis or risk assessment (e.g., likelihood of cancer being present) for the subject, along with recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (e.g., at the point of care) or displayed to the clinician on a computer monitor.

[0085] In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

[0086] In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example, the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease.

[0087] D. In vivo Imaging

[0088] The overexpression of DLX1 disclosed herein may also be detected using in vivo imaging techniques, including but not limited to: radionuclide imaging; positron emission tomography (PET); computerized axial tomography, X-ray or magnetic resonance imaging method, fluorescence detection, and chemiluminescent detection. In some embodiments, in vivo imaging techniques are used to visualize the presence of or expression of cancer markers in an animal (e.g., a human or non-human mammal). For example, in some embodiments, cancer marker mRNA or protein is labeled using a labeled antibody specific for the cancer marker. A specifically bound and labeled antibody can be detected in an individual using an in vivo imaging method, including, but not limited to, radionuclide imaging, positron emission tomography, computerized axial tomography, X-ray or magnetic resonance imaging method, fluorescence detection, and chemiluminescent detection. Methods for generating antibodies to the disclosed cancer markers are described below.

[0089] The in vivo imaging methods that use the compositions disclosed herein that detect DLX1 overexpression or products derived from them are useful in the diagnosis of cancers, particularly prostate cancer, that express the cancer markers disclosed herein. In vivo imaging visualizes the presence of a marker indicative of the cancer, allowing diagnosis and/or prognosis without the use of an unpleasant biopsy. For example, the presence of a marker indicative of cancers likely to metastasize can be detected. The in vivo imaging methods can further be used to detect metastatic cancers in other parts of the body.

[0090] In some embodiments, reagents (e.g., antibodies) specific for the cancer markers (e.g., DLX1) are fluorescently labeled. The labeled antibodies are introduced into a subject (e.g., orally or parenterally). Fluorescently labeled antibodies are detected using any suitable method or system (e.g., see U.S. Pat. No. 6,198,107, herein incorporated by reference).

[0091] In other embodiments, antibodies are radioactively labeled. The use of antibodies for in vivo diagnosis is well known in the art, e.g., by using an antibody-based labeling system to image tumors (see Sumerdon et al., Nucl. Med. Biol 17:247-254 [1990], Griffin et al., J. Clin. Once. 9:631-640 [1991], and Lauffer, Magnetic Resonance in Medicine 22:339-342 [1991]). The label used with an antibody-based system will depend on the imaging modality chosen, for example, radioactive labels such as Indium-111, Technetium-99m, or Iodine-131 for use with planar scans or single photon emission computed tomography (SPECT), positron emitting labels such as Fluorine-19 for use with positron emission tomography (PET), and paramagnetic ions such as Gadolinium (III) or Manganese (II) for use with MRI.

[0092] Radioactive metals with half-lives ranging from 1 hour to 3.5 days are available for conjugation to antibodies, such as scandium-47 (3.5 days) gallium-67 (2.8 days), gallium-68 (68 minutes), technetiium-99m (6 hours), and indium-111 (3.2 days), of which gallium-67, technetium-99m, and indium-111 are preferable for gamma camera imaging, gallium-68 is preferable for positron emission tomography.

[0093] A useful method of labeling antibodies with such radiometals is by means of a bifunctional chelating agent, such as diethylenetriaminepentaacetic acid (DTPA), as described, for example, by Khaw et al. (Science 209:295 [1980]) for In-111 and Tc-99m, and by Scheinberg et al. (Science 215:1511 [1982]). Other chelating agents may also be used, but the 1-(p-carboxymethoxybenzyl)EDTA and the carboxycarbonic anhydride of DTPA are advantageous because their use permits conjugation without affecting the antibody's immunoreactivity substantially.

[0094] Another method for coupling DPTA to proteins is by use of the cyclic anhydride of DTPA, as described by Hnatowich et al. (Int. J. Appl. Radiat. Isot. 33:327 [1982]) for labeling of albumin with In-111, but which can be adapted for labeling of antibodies. A suitable method of labeling antibodies with Tc-99m is known (e.g., see Crockford et al., U.S. Pat. No. 4,323,546, herein incorporated by reference).

[0095] A preferred method of labeling immunoglobulins with Tc-99m is that described by Wong et al. (Int. J. Appl. Radiat. Isot., 29:251 [1978]) for plasma protein, and recently applied successfully by Wong et al. (J. Nucl. Med., 23:229 [1981]) for labeling antibodies.

[0096] In the case of the radiometals conjugated to the specific antibody, it is likewise desirable to introduce as high a proportion of the radiolabel as possible into the antibody molecule without destroying its immunospecificity. A further improvement may be achieved by effecting radiolabeling in the presence of the specific cancer marker, to insure that the antigen binding site on the antibody is protected. The antigen is separated after labeling.

[0097] In still further embodiments, in vivo biophotonic imaging (Xenogen, Almeda, Calif.) is used for in vivo imaging. This real-time in vivo imaging utilizes luciferase, an enzyme that catalyzes a light-emitting reaction. The luciferase gene is incorporated into cells, microorganisms, and animals (e.g., to produce a fusion protein with a cancer marker associated with DLX1 overexpression) so that when the cancer marker is active a light emission occurs which is captured as an image and analyzed by using a CCD camera and appropriate software.

[0098] E. Compositions & Kits

[0099] Compositions for use in the disclosed diagnostic methods include, but are not limited to, probes, amplification oligonucleotides, and antibodies. In some embodiments, the compositions detect a product only when overexpression of DLX1 is present. These compositions include, but are not limited to: a single labeled probe comprising a sequence that hybridizes to DLX1 and can be quantitated and a pair of amplification oligonucleotides for quantitative PCR detection of DLX1 overexpression, and an antibody for detection of the overexpression of DLX1 polypeptide.

[0100] Any of these compositions, alone or in combination with other compositions disclosed herein or well known in the art, may be provided in the form of a kit. For example, the single labeled probe and pair of amplification oligonucleotides may be provided in a kit for the amplification and detection of DLX1 overexpression. Kits may further comprise appropriate controls and/or detection reagents. Any one or more reagents that find use in any of the methods described herein may be provided in the kit.

[0101] The probe and antibody compositions may also be provided in the form of an array.

III. Antibodies

[0102] DLX1 polypeptides, which include fragments, derivatives and analogs thereof, which may be used as immunogens to produce antibodies useful for diagnostic and therapeutic applications. Such antibodies may be polyclonal or monoclonal, chimeric, humanized, single chain or Fab fragments, which may be labeled or unlabeled, all of which may be produced by using well known procedures and standard laboratory practices. See, e.g., Burns, ed., *Immunochemical Protocols,* 3rd ed., Humana Press (2005); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988); Kozbor et al., *Immunology Today* 4: 72 (1983); Köhler and Milstein, *Nature* 256: 495 (1975).

[0103] For example, for preparation of a monoclonal antibody, protein, as such, or together with a suitable carrier or diluent is administered to an animal (e.g., a mammal) under conditions that permit the production of antibodies. For enhancing the antibody production capability, complete or incomplete Freund's adjuvant may be administered. Normally, the protein is administered once every 2 weeks to 6 weeks, in total, about 2 times to about 10 times. Animals suitable for use in such methods include, but are not limited to, primates, rabbits, dogs, guinea pigs, mice, rats, sheep, goats, etc.

[0104] For preparing monoclonal antibody-producing cells, an individual animal whose antibody titer has been confirmed (e.g., a mouse) is selected, and 2 days to 5 days after the final immunization, its spleen or lymph node is harvested and antibody-producing cells contained therein are fused with myeloma cells to prepare the desired monoclonal antibody producer hybridoma. Measurement of the antibody titer in antiserum can be carried out, for example, by reacting the labeled protein, as described hereinafter and antiserum and then measuring the activity of the labeling agent bound to the antibody. The cell fusion can be carried out according to known methods, for example, the method described by Koehler and Milstein (Nature 256:495 [1975]). As a fusion promoter, for example, polyethylene glycol (PEG) or Sendai virus (HVJ), preferably PEG is used.

[0105] Examples of myeloma cells include NS-1, P3U1, SP2/0, AP-1 and the like. The proportion of the number of antibody producer cells (spleen cells) and the number of myeloma cells to be used is preferably about 1:1 to about 20:1. PEG (preferably PEG 1000-PEG 6000) is preferably added in concentration of about 10% to about 80%. Cell fusion can be carried out efficiently by incubating a mixture of both cells at about 20° C. to about 40° C., preferably about 30° C. to about 37° C. for about 1 minute to 10 minutes.

[0106] Various methods may be used for screening for a hybridoma producing the antibody (e.g., against DLX1). For example, where a supernatant of the hybridoma is added to a solid phase (e.g., microplate) to which antibody is adsorbed directly or together with a carrier and then an anti-immunoglobulin antibody (if mouse cells are used in cell fusion, anti-mouse immunoglobulin antibody is used) or Protein A labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase. Alternately, a supernatant of the hybridoma is added to a solid phase to which an anti-immunoglobulin antibody or Protein A is adsorbed and then the protein labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the protein bound to the solid phase.

[0107] Selection of the monoclonal antibody can be carried out according to any known method or its modification. Normally, a medium for animal cells to which HAT (hypoxanthine, aminopterin, thymidine) are added is employed. Any

selection and growth medium can be employed as long as the hybridoma can grow. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku) and the like can be used. Normally, the cultivation is carried out at 20° C. to 40° C., preferably 37° C. for about 5 days to 3 weeks, preferably 1 week to 2 weeks under about 5% CO2 gas. The antibody titer of the supernatant of a hybridoma culture can be measured according to the same manner as described above with respect to the antibody titer of the anti-protein in the antiserum.

[0108] Separation and purification of a monoclonal antibody (e.g., against DLX1) can be carried out according to the same manner as those of conventional polyclonal antibodies such as separation and purification of immunoglobulins, for example, salting-out, alcoholic precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method wherein only an antibody is collected with an active adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody.

[0109] Polyclonal antibodies may be prepared by any known method or modifications of these methods including obtaining antibodies from patients. For example, a complex of an immunogen (an antigen against the protein) and a carrier protein is prepared and an animal is immunized by the complex according to the same manner as that described with respect to the above monoclonal antibody preparation. A material containing the antibody against is recovered from the immunized animal and the antibody is separated and purified.

[0110] As to the complex of the immunogen and the carrier protein to be used for immunization of an animal, any carrier protein and any mixing proportion of the carrier and a hapten can be employed as long as an antibody against the hapten, which is crosslinked on the carrier and used for immunization, is produced efficiently. For example, bovine serum albumin, bovine cycloglobulin, keyhole limpet hemocyanin, etc. may be coupled to an hapten in a weight ratio of about 0.1 part to about 20 parts, preferably, about 1 part to about 5 parts per 1 part of the hapten.

[0111] In addition, various condensing agents can be used for coupling of a hapten and a carrier. For example, glutaraldehyde, carbodiimide, maleimide activated ester, activated ester reagents containing thiol group or dithiopyridyl group, and the like find use with the present invention. The condensation product as such or together with a suitable carrier or diluent is administered to a site of an animal that permits the antibody production. For enhancing the antibody production capability, complete or incomplete Freund's adjuvant may be administered. Normally, the protein is administered once every 2 weeks to 6 weeks, in total, about 3 times to about 10 times.

[0112] The polyclonal antibody is recovered from blood, ascites and the like, of an animal immunized by the above method. The antibody titer in the antiserum can be measured according to the same manner as that described above with respect to the supernatant of the hybridoma culture. Separation and purification of the antibody can be carried out according to the same separation and purification method of immunoglobulin as that described with respect to the above monoclonal antibody.

IV. Drug Screening Applications

[0113] In some embodiments, the disclosed compositions and methods are used in drug screening assays (e.g., to screen for anticancer drugs). These screening methods use cancer markers that include those associated with DLX1 overexpression. For example, an embodiment may screen for compounds that alter (e.g., decrease) the expression of DLX1 or associated signaling molecules. Compounds or agents to be screened for may interfere with transcription (e.g., by interacting with a promoter region), may interfere with mRNA produced from DLX1 (e.g., by RNA interference, antisense technologies, etc.), or may interfere with pathways that are upstream or downstream of the biological activity of DLX1. In some embodiments, candidate compounds are antisense or interfering RNA agents (e.g., oligonucleotides) directed against cancer markers. In other embodiments, candidate compounds are antibodies or small molecules that specifically bind to a cancer marker regulator or expression product associated with DLX1 and inhibit its biological function.

[0114] In some embodiments, candidate compounds are evaluated for their ability to alter cancer marker expression by contacting a compound with a cell expressing a cancer marker and then assaying for the effect of the candidate compounds on expression. In some embodiments, the effect of candidate compounds on expression of a cancer marker gene is assayed for by detecting the level of cancer marker mRNA expressed by the cell. mRNA expression can be detected by any suitable method. In other embodiments, the effect of candidate compounds on expression of cancer marker genes is assayed by measuring the level of polypeptide encoded by the cancer markers. The level of polypeptide expressed can be measured using any suitable method, including but not limited to, those disclosed herein.

[0115] The test compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone, which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckennann et al., J. Med. Chem. 37: 2678-85 [1994]); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are preferred for use with peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) Anticancer Drug Des. 12:145).

[0116] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al., Proc. Natl. Acad. Sci. U.S.A. 90:6909 [1993]; Erb et al., Proc. Nad. Acad. Sci. USA 91:11422 [1994]; Zuckermann et al., J. Med. Chem. 37:2678 [1994]; Cho et al., Science 261:1303 [1993]; Carrell et al., Angew. Chem. Int. Ed. Engl. 33.2059 [1994]; Carell et al., Angew. Chem. Int. Ed. Engl. 33:2061 [1994]; and Gallop et al., J. Med. Chem. 37:1233 [1994].

[0117] Libraries of compounds may be presented in solution (e.g., Houghten, Biotechniques 13:412-421 [1992]), or on beads (Lam, Nature 354:82-84 [1991]), chips (Fodor, Nature 364:555-556 [1993]), bacteria or spores (U.S. Pat. No. 5,223,409; herein incorporated by reference), plasmids (Cull et al., Proc. Nad. Acad. Sci. USA 89:18651869 [1992]) or on phage (Scott and Smith, Science 249:386-390 [1990]; Devlin

Science 249:404-406 [1990]; Cwirla et al., Proc. Natl. Acad. Sci. 87:6378-6382 [1990]; Felici, J. Mol. Biol. 222:301 [1991]).

V. Therapeutic Applications

[0118] Some embodiments provide therapies for cancer (e.g., prostate cancer). Preferred therapy embodiments target DLX1 directly or indirectly.

[0119] A. RNA Interference and Antisense Therapies

[0120] In some embodiments, nucleic acid based therapeutics target the expression of DLX1. Some embodiments employ compositions comprising oligomeric antisense or RNAi compounds, particularly oligonucleotides (e.g., those identified in the drug screening methods described above), for use in modulating the function of nucleic acid molecules encoding DLX1, ultimately modulating the amount of DLX1 expressed.

1. RNA Interference (RNAi)

[0121] In some embodiments, RNAi is used to inhibit expression of DLX1. RNAi represents an evolutionary conserved cellular defense for controlling the expression of foreign genes in most eukaryotes, including humans. RNAi is typically triggered by double-stranded RNA (dsRNA) and causes sequence-specific mRNA degradation of single-stranded target RNAs homologous in response to dsRNA. The mediators of mRNA degradation are small interfering RNA duplexes (siRNAs), which are normally produced from long dsRNA by enzymatic cleavage in the cell. siRNAs are generally approximately twenty-one nucleotides in length (e.g. 21-23 nucleotides in length), and have a base-paired structure characterized by two nucleotide 3'-overhangs. Following the introduction of a small RNA, or RNAi, into the cell, it is believed the sequence is delivered to an enzyme complex called RISC(RNA-induced silencing complex). RISC recognizes the target and cleaves it with an endonuclease. It is noted that if larger RNA sequences are delivered to a cell, RNase III enzyme (Dicer) converts longer dsRNA into 21-23 nt ds siRNA fragments. In some embodiments, RNAi oligonucleotides are designed to target DLX1.

[0122] Chemically synthesized siRNAs have become powerful reagents for genome-wide analysis of mammalian gene function in cultured somatic cells. Beyond their value for validation of gene function, siRNAs also hold great potential as gene-specific therapeutic agents (Tuschl and Borkhardt, Molecular Intervent. 2002; 2(3):158-67, herein incorporated by reference).

[0123] The transfection of siRNAs into animal cells results in the potent, long-lasting post-transcriptional silencing of specific genes (Caplen et al, Proc Natl Acad Sci U.S.A. 2001; 98: 9742-7; Elbashir et al., Nature. 2001; 411:494-8; Elbashir et al., Genes Dev. 2001; 15: 188-200; and Elbashir et al., EMBO J. 2001; 20: 6877-88, all of which are herein incorporated by reference). Methods and compositions for performing RNAi with siRNAs are known (e.g., see U.S. Pat. No. 6,506,559, herein incorporated by reference). siRNAs are extraordinarily effective at lowering the amounts of targeted RNA, and by extension proteins, frequently to undetectable levels. The silencing effect can last several months, and is extraordinarily specific, because one nucleotide mismatch between the target RNA and the central region of the siRNA is frequently sufficient to prevent silencing (Brummelkamp et al, Science 2002; 296:550-3; and Holen et al, Nucleic Acids Res. 2002; 30:1757-66, both of which are herein incorporated by reference).

[0124] An important factor in the design of siRNAs is the presence of accessible sites for siRNA binding. Bahoia et al., (J. Biol. Chem., 2003; 278: 15991-15997; herein incorporated by reference) describe the use of a type of DNA array called a scanning array to find accessible sites in mRNAs for designing effective siRNAs. These arrays comprise oligonucleotides ranging in size from monomers to a certain maximum, usually Corners, synthesized using a physical barrier (mask) by stepwise addition of each base in the sequence. Thus the arrays represent a full oligonucleotide complement of a region of the target gene. Hybridization of the target mRNA to these arrays provides an exhaustive accessibility profile of this region of the target mRNA. Such data are useful in the design of antisense oligonucleotides (ranging from 7 mers to 25 mers), where it is important to achieve a compromise between oligonucleotide length and binding affinity, to retain efficacy and target specificity (Sohail et al, Nucleic Acids Res., 2001; 29(10): 2041-2045). Additional methods and concerns for selecting siRNAs are described for example, in WO 05054270, WO05038054A1, WO03070966A2, J Mol. Biol. 2005 May 13; 348(4):883-93, J Mol. Biol. 2005 May 13; 348(4):871-81, and Nucleic Acids Res. 2003 Aug. 1; 31(15):4417-24, each of which is herein incorporated by reference in its entirety. In addition, software (e.g., the MWG online siMAX siRNA design tool) is commercially or publicly available for use in the selection of siRNAs.

2. Antisense

[0125] In other embodiments, expression of DLX1 is inhibited using antisense compounds that specifically hybridize with one or more nucleic acids encoding DLX1. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds that specifically hybridize to it is generally referred to as "antisense." The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity that may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of DLX1. Herein, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. For example, expression may be inhibited to potentially prevent tumor proliferation.

[0126] Antisense methods preferably target specific nucleic acids. "Targeting" an antisense compound to a particular nucleic acid usually refers to a multistep process that begins with identification of a nucleic acid sequence whose function is to be modulated. This may be, e.g., a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. Herein, the target is a nucleic acid molecule encoding DLX1. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Herein, a preferred intragenic

site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). Eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially used for translation initiation in a particular cell type or tissue, or under a particular set of conditions. Herein, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA molecule transcribed from a gene encoding DLX1, regardless of the sequence(s) of such codons.

[0127] Translation termination codon (or "stop codon") of a gene may have one of three sequences (i.e., 5'-UAA, 5'-UAG and 5'-UGA; the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

[0128] The open reading frame (ORF) or "coding region," which refers to the region between the translation initiation codon and the translation termination codon, is also a region that may be targeted effectively. Other target regions include the 5' untranslated region (5' UTR), referring to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3' UTR), referring to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The cap region may also be a preferred target region.

[0129] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," that are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites (i.e., intron-exon junctions) may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

[0130] In some embodiments, target sites for antisense inhibition are identified using commercially available software programs (e.g., Biognostik, Gottingen, Germany; SysArris Software, Bangalore, India; Antisense Research Group, University of Liverpool, Liverpool, England; GeneTrove, Carlsbad, Calif.). In other embodiments, target sites for antisense inhibition are identified using the accessible site method described in PCT Publ. No. WO0198537A2, herein incorporated by reference.

[0131] Once one or more target sites have been identified, oligonucleotides are chosen that are sufficiently complementary to the target (i.e., hybridize sufficiently well and with sufficient specificity) to give the desired effect. For example, in preferred embodiments, antisense oligonucleotides are targeted to or near the start codon associated with DLX1.

[0132] In the context of this invention, "hybridization," with respect to antisense compositions and methods, means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds. It is understood that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired (i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed).

[0133] Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with specificity, can be used to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway.

[0134] The specificity and sensitivity of antisense is also applied for therapeutic uses. For example, antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides are useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues, and animals, especially humans.

[0135] While antisense oligonucleotides are preferred, other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics may be used, such as are described below. Preferred antisense compounds comprise from about 8 to about 30 nucleobases (i.e., from about 8 to about 30 linked bases), although both longer and shorter sequences may be used. Particularly preferred antisense com-

pounds are antisense oligonucleotides, even more preferably those comprising from about 12 to about 25 nucleobases.

[0136] Specific examples of preferred antisense compounds include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined herein, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[0137] Other embodiments include pharmaceutical compositions and formulations that include the antisense compounds as described herein.

[0138] B. Gene Therapy

[0139] Embodiments may use any genetic manipulation to modulate the expression of DLX1 cancer markers described herein. Examples of genetic manipulation include, but are not limited to, gene knockout (such as by removing DLX1 from the chromosome using, e.g., by recombination), expression of antisense constructs with or without inducible promoters, and the like. Delivery of nucleic acid construct to cells in vitro or in vivo may be conducted using any suitable method. A suitable method is one that introduces the nucleic acid construct into the cell such that the desired event occurs (e.g., expression of an antisense construct). Genetic therapy may also be used to deliver siRNA or other interfering molecules that are expressed in vivo (e.g., upon stimulation by an inducible promoter (e.g., an androgen-responsive promoter)).

[0140] Introduction of molecules carrying genetic information into cells is achieved by any of various methods including, but not limited to, directed injection of naked DNA constructs, bombardment with gold particles loaded with said constructs, and macromolecule mediated gene transfer using, for example, liposomes, biopolymers, and the like. Preferred methods use gene delivery vehicles derived from viruses, including, but not limited to, adenoviruses, retroviruses, vaccinia viruses, and adeno-associated viruses. Because of the higher efficiency as compared to retroviruses, vectors derived from adenoviruses are the preferred gene delivery vehicles for transferring nucleic acid molecules into host cells in vivo. Adenoviral vectors and their use in gene transfer are well known (e.g., see PCT publications WO 00/12738 and WO 00/09675 and U.S. Pat. Nos. 6,033,908, 6,019,978, 6,001,557, 5,994,132, 5,994,128, 5,994,106, 5,981,225, 5,885,808, 5,872,154, 5,830,730, and 5,824,544, each of which is herein incorporated by reference in its entirety). Such vectors and methods have been shown to provide very efficient in vivo gene transfer into a variety of solid tumors in animal models and into human solid tumor xenografts in immune-deficient mice.

[0141] Vectors may be administered to subject in a variety of well known ways, e.g., administered into tumors or tissue associated with tumors by using direct injection or administration via the blood or lymphatic circulation (See e.g., PCT publication 99/02685 herein incorporated by reference in its entirety). Exemplary dose levels of adenoviral vector are preferably 10^8 to 10^{11} vector particles added to the perfusate.

[0142] C. Antibody or Small Molecule Therapies

[0143] Some embodiments are or use antibodies and/or small molecules that target prostate tumors that express DLX1. In some embodiments, the therapeutic regimen is selected based on a diagnostic result and uses a suitable antibody (e.g., monoclonal, polyclonal, or synthetic) in the therapeutic methods. In preferred embodiments, the antibodies used for cancer therapy are humanized antibodies. Methods for humanizing antibodies are well known (See e.g., U.S. Pat. Nos. 6,180,370, 5,585,089, 6,054,297, and 5,565,332; each of which is herein incorporated by reference).

[0144] In some embodiments, the therapeutic antibodies comprise an antibody generated against DLX1, wherein the antibody is conjugated to a cytotoxic agent. In such embodiments, a tumor specific therapeutic agent is generated that does not target normal cells, thus reducing many of the detrimental side effects of traditional chemotherapy. For certain applications, it is envisioned that the therapeutic agents will be pharmacologic agents that will serve as useful agents for attachment to antibodies, particularly cytotoxic or otherwise anticellular agents having the ability to kill or suppress the growth or cell division of tumor cells. Embodiments may use any pharmacologic agent that can be conjugated to an antibody, and delivered in active form. Exemplary anticellular agents include chemotherapeutic agents, radioisotopes, and cytotoxins. Such therapeutic antibodies may include a variety of cytotoxic moieties, including but not limited to, radioactive isotopes (e.g., iodine-131, iodine-123, technicium-99m, indium-111, rhenium-188, rhenium-186, gallium-67, copper-67, yttrium-90, iodine-125 or astatine-211), hormones such as a steroid, antimetabolites such as cytosines (e.g., arabinoside, fluorouracil, methotrexate or aminopterin; an anthracycline; mitomycin C), vinca alkaloids (e.g., demecolcine; etoposide; mithramycin), and antitumor alkylating agent such as chlorambucil or melphalan. Other embodiments may include agents such as a coagulant, a cytokine, growth factor, bacterial endotoxin or the lipid A moiety of bacterial endotoxin. For example, in some embodiments, therapeutic agents will include plant-, fungus- or bacteria-derived toxin, such as an A chain toxins, a ribosome inactivating protein, α-sarcin, aspergillin, restrictocin, a ribonuclease, diphtheria toxin or pseudomonas exotoxin, to mention just a few examples. In some preferred embodiments, deglycosylated ricin A chain is used.

[0145] In any event, it is proposed that agents such as these may, if desired, be successfully conjugated to an antibody, in a manner that will allow their targeting, internalization, release or presentation to blood components at the site of the targeted tumor cells as required using known conjugation technology (See, e.g., Ghose et al., Methods Enzymol., 93:280 [1983]).

[0146] For example, some embodiments provide immunotoxins targeted against DLX1. Immunotoxins are conjugates of a specific targeting agent typically a tumor-directed antibody or fragment, with a cytotoxic agent, such as a toxin moiety. The targeting agent directs the toxin to, and thereby selectively kills, cells carrying the targeted antigen. In some embodiments, therapeutic antibodies employ crosslinkers that provide high in vivo stability (Thorpe et al., Cancer Res., 48:6396 [1988]).

[0147] In other embodiments, particularly those involving treatment of solid tumors, antibodies are designed to have a cytotoxic or otherwise anticellular effect against the tumor vasculature, by suppressing the growth or cell division of the vascular endothelial cells. This attack is intended to lead to a tumor-localized vascular collapse, depriving the tumor cells, particularly those tumor cells distal of the vasculature, of oxygen and nutrients, ultimately leading to cell death and tumor necrosis.

[0148] In preferred embodiments, antibody based therapeutics are formulated as pharmaceutical compositions as described herein. In preferred embodiments, administration of an antibody composition that targets a moiety associated with DLX1 results in a measurable decrease in cancer (e.g., decrease or elimination of tumor).

VI. Transgenic Animals

[0149] Embodiments include generation of transgenic animals comprising an exogenous cancer marker gene that is identical to or representative of DLX1 as described herein, which includes mutants and variants thereof (e.g., truncations or single nucleotide polymorphisms). In preferred embodiments, the transgenic animal displays an altered phenotype (e.g., increased or decreased presence of markers associated with DLX1) as compared to wild-type animals. Methods for analyzing the presence or absence of such phenotypes include but are not limited to, those disclosed herein. In some preferred embodiments, the transgenic animals further display an increased or decreased growth of tumors or evidence of cancer.

[0150] Such transgenic animals are useful in drug (e.g., cancer therapy) screens. In some embodiments, test compounds (e.g., a drug that is suspected of being useful to treat cancer) and control compounds (e.g., a placebo) are administered to the transgenic animals and the control animals and the effects evaluated.

[0151] The transgenic animals can be generated via a variety of methods. In some embodiments, embryonal cells at various developmental stages are used to introduce transgenes for the production of transgenic animals. Different methods are used depending on the stage of development of the embryonal cell. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter that allows reproducible injection of 1-2 picoliters (pl) of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host genome before the first cleavage (Brinster et al., Proc. Natl. Acad. Sci. USA 82:4438-4442 [1985]). As a consequence, all cells of the transgenic non-human animal carry the incorporated transgene. This is reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells harbor the transgene based on standard Mendelian genetics. Methods for making transgenics are well known (e.g., see U.S. Pat. No. 4,873,191, which is herein incorporated by reference in its entirety).

[0152] In other embodiments, retroviral infection is used to introduce transgenes into a non-human animal. In some embodiments, the retroviral vector is used to transfect oocytes by injecting the retroviral vector into the perivitelline space of the oocyte (U.S. Pat. No. 6,080,912, incorporated herein by reference). In other embodiments, the developing non-human embryo can be cultured in vitro to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Janenich, Proc. Natl. Acad. Sci. USA 73:1260 [1976]). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan et al., in *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [1986]). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner et al., Proc. Natl. Acad. Sci. USA 82:6927 [1985]). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Stewart, et al, EMBO J., 6:383 [1987]). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner et al., Nature 298:623 [1982]). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of cells that form the transgenic animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome that generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germline, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner et al., supra [1982]). Additional means of using retroviruses or retroviral vectors to create transgenic animals known to the art involve the micro-injection of retroviral particles or mitomycin C-treated cells producing retrovirus into the perivitelline space of fertilized eggs or early embryos (PCT International Application WO 90/08832 [1990], and Haskell and Bowen, Mol. Reprod. Dev., 40:386 [1995]).

[0153] In other embodiments, the transgene is introduced into embryonic stem cells and the transfected stem cells are used to form an embryo. ES cells are obtained by culturing pre-implantation embryos in vitro under appropriate conditions (Evans et al., Nature 292:154 [1981]; Bradley et al., Nature 309:255 [1984]; Gossler et al., Proc. Acad. Sci. USA 83:9065 [1986]; and Robertson et al., Nature 322:445 [1986]). Transgenes can be efficiently introduced into the ES cells by DNA transfection by a variety of methods known to the art including calcium phosphate co-precipitation, protoplast or spheroplast fusion, lipofection and DEAE-dextran-mediated transfection. Transgenes may also be introduced into ES cells by retrovirus-mediated transduction or by micro-injection. Such transfected ES cells can thereafter colonize an embryo following their introduction into the blastocoel of a blastocyst-stage embryo and contribute to the germ line of the resulting chimeric animal (for review, See, Jaenisch, Science 240:1468 [1988]). Prior to the introduction of transfected ES cells into the blastocoel, the transfected ES cells may be subjected to various selection protocols to enrich for ES cells which have integrated the transgene assuming that the transgene provides a means for such selection. Alternatively, the polymerase chain reaction may be used to screen for ES cells that have integrated the transgene. This technique obviates the need for growth of the transfected ES cells under appropriate selective conditions prior to transfer into the blastocoel.

[0154] In still other embodiments, homologous recombination is used to knock-out gene function or create deletion mutants (e.g., truncation mutants), using well known methods (see U.S. Pat. No. 5,614,396, incorporated herein by reference).

EXPERIMENTAL

[0155] The following examples are provided to demonstrate and illustrate certain preferred embodiments and aspects of the compositions and methods disclosed herein, but are not to be construed as limiting the scope of the claimed invention.

Example 1

DLX1 Cancer Markers

[0156] This Example describes the characterization of DLX1 expression in prostate cancer.

A. Methods

Expression Profiling

[0157] Expression profiling of benign prostate tissues, localized prostate cancers, and hormone refractory metastatic prostate cancer samples were performed using the Agilent Whole Human Genome Oligo Microarray (Santa Clara, Calif.). Total RNA isolated using Trizol was purified using the Qiagen RNAeasy Micro kit (Valencia, Calif.). One μg of total RNA was converted to cDNA and labeled according to the manufacturer's protocol (Agilent). Hybridizations were performed for 16 hrs at 65° C., and arrays were scanned on an Agilent DNA microarray scanner. For all hybridizations, prostate tissue samples were labeled with Cy5 and hybridized against a commercially available (Clontech) pool of benign prostate tissues (CPP), which was labeled with Cy3. Images were analyzed and data extracted using Agilent Feature Extraction Software 9.1.3.1, with linear and lowess normalization performed for each array.

Quantitative PCR (QPCR)

[0158] Quantitative PCR (QPCR) was performed using Power SYBR Green Mastermix (Applied Biosystems, Foster City, Calif.) on an Applied Biosystems 7300 Real Time PCR system as described (Tomlins et al., Science 310, 644-8 (2005); Tomlins et al., Cancer Res 66, 3396-400 (2006)). Oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, Iowa). HMBS and GAPDH3 primers were as described. Primers used to amplify DLX1 are as follows:

```
DLX1-f:
5'- GCGGCCTCTTTGGGACTCACAC-3'   (SEQ ID NO:1)

DLX1-r:
5'- GGCCAACGCACTACCCTCCAGA-3'   (SEQ ID NO:2)
```

B. Results

[0159] Results are shown in FIG. **1**. FIG. **1** shows the over-expression of DLX1 in prostate cancer. FIG. **1**A shows benign prostate (n=22), localized prostate cancers (PCa, n=45) and hormone refractory metastatic prostate cancers (n=18) profiled on Agilent Whole Genome Oligo expression microarrays. All samples (Cy5) were hybridized competitively with a commercially available (Clontech) pool of benign prostate tissues (CPP, Cy3). Cy5/Cy3 ratios are shown for DLX1 and AMACR. Values for samples with ratios Cy5/Cy3 ratios greater than 100 are indicated. FIG. **1**B shows over-expression of DLX1 in prostate cancer as confirmed by qPCR in benign prostate (n=9), localized prostate cancers (n=56) and hormone refractory metastatic prostate cancers (n=7). Ratios of DLX1 to the average of the housekeeping genes HMBS and GAPDH are shown.

[0160] All publications, patents, patent applications and sequences identified by accession numbers mentioned in the above specification are herein incorporated by reference in their entirety. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Modifications and variations of the described compositions and methods of the invention that do not significantly change the functional features of the compositions and methods described herein are intended to be within the scope of the following claims.

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Gly
```

We claim:

1. A method of diagnosing cancer, comprising: detecting the presence of absence of overexpression of DLX1 in a sample from a patient.

2. The method of claim **1**, wherein overexpression of DLX1 is indicative of cancer in said sample.

3. The method of claim **2**, wherein said cancer is prostate cancer.

4. The method of claim **1**, wherein said sample is selected from the group consisting of a tumor sample, a cell sample, a blood sample, a serum sample, or a urine sample.

5. The method of claim **1**, wherein said detecting overexpression of DLX1 comprising detecting overexpression of DLX1 mRNA.

6. The method of claim **5**, wherein said detecting overexpression of DLX1 mRNA comprises performing a detection technique selected from the group consisting of hybridization assay, and an amplification assay.

7. The method of claim **6**, wherein said amplification assay comprises a quantitative PCR assay.

8. The method of claim **1**, wherein said detecting overexpression of DLX1 comprises detecting overexpression of DLX1 polypeptide.

9. The method of claim **8**, wherein said detecting overexpression of DLX1 polypeptide comprises an immunoassay.

10. A method of screening compounds, comprising contacting a cell expressing DLX1 with a test compound and assaying the level of expression of DLX1 in the presence of the test compound.

11. The method of claim **10**, wherein said cell overexpresses DLX1.

12. The method of claim **10**, wherein said contacting said cell expressing DLX1 with said test compound results in a decrease in expression of DLX1.

13. The method of claim **10**, wherein said cell is in vitro.

14. The method of claim **10**, wherein said cell is ex vivo.

15. The method of claim **10**, wherein said cell is in vivo.

16. The method of claim **15**, wherein said cell is in a non-human animal.

* * * * *

专利名称(译)	dlx1癌症标记		
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申请号	US12/237512	申请日	2008-09-25
[标]申请(专利权)人(译)	密歇根大学		
申请(专利权)人(译)	密歇根大学董事会		
当前申请(专利权)人(译)	密歇根大学董事会		
[标]发明人	CHINNAIYAN ARUL M TOMLINS SCOTT A		
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IPC分类号	A61K49/00 G01N33/53 A61P35/00		
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摘要(译)

本发明涉及用于癌症诊断，研究和治疗的组合物和方法，包括但不限于癌症标志物。特别地，本发明涉及DLX1癌症标志物，其可用作前列腺癌的诊断标志物和临床靶标。

Figure 1