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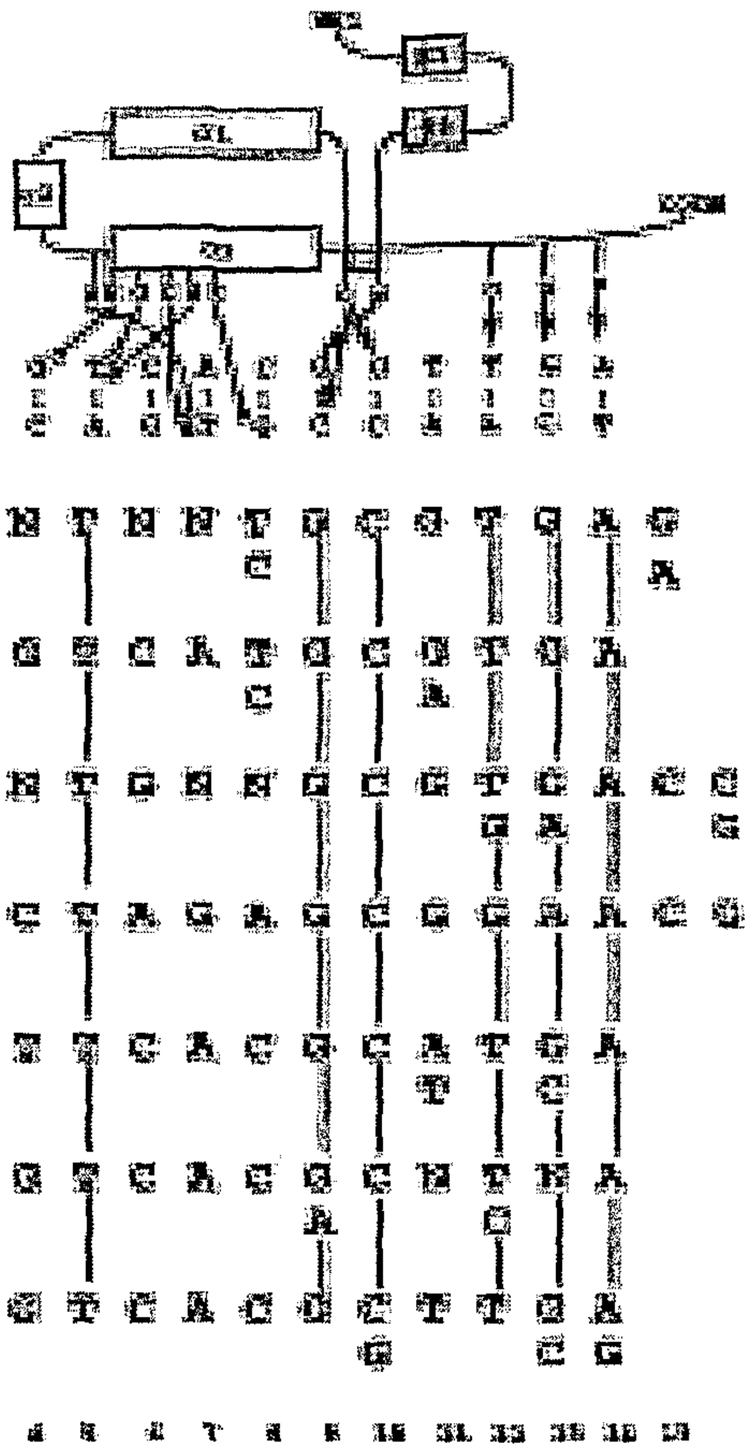
(54) Titre : CIBLAGE DE PAX2 EN VUE D'INDUIRE LE TRAITEMENT DU CANCER ET L'IMMUNITE TUMORALE INDUITE PAR DEFB1

(54) Title: INHIBITION OF PAX2 BY DEFB1 INDUCTION AS A THERAPY FOR CANCER



(57) Abrégé/Abstract:
Provided is a method of treating cancer in a subject by inhibiting expression of PAX2. An example of a cancer treated by the present method is prostate cancer. Similarly, provided is a method of treating cancer in a subject by increasing expression of

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(57) Abrégé(suite)/Abstract(continued):
DEFBI in the subject. In the method wherein the expression of DEFBI is increased, it can be increased by blocking the binding of PAX2 to the DEFBI promoter. Blocking the binding of PAX2 to the DEFBI promoter can be by administration of an oligonucleotide containing the PAX2 DNA binding site of DEFBI. This oligonucleotide can be complementary to the sequence of PAX2 that binds to the DEFBI promoter. Alternatively, the oligonucleotide can interact with the PAX2 in a way that inhibits binding to DEFBI.

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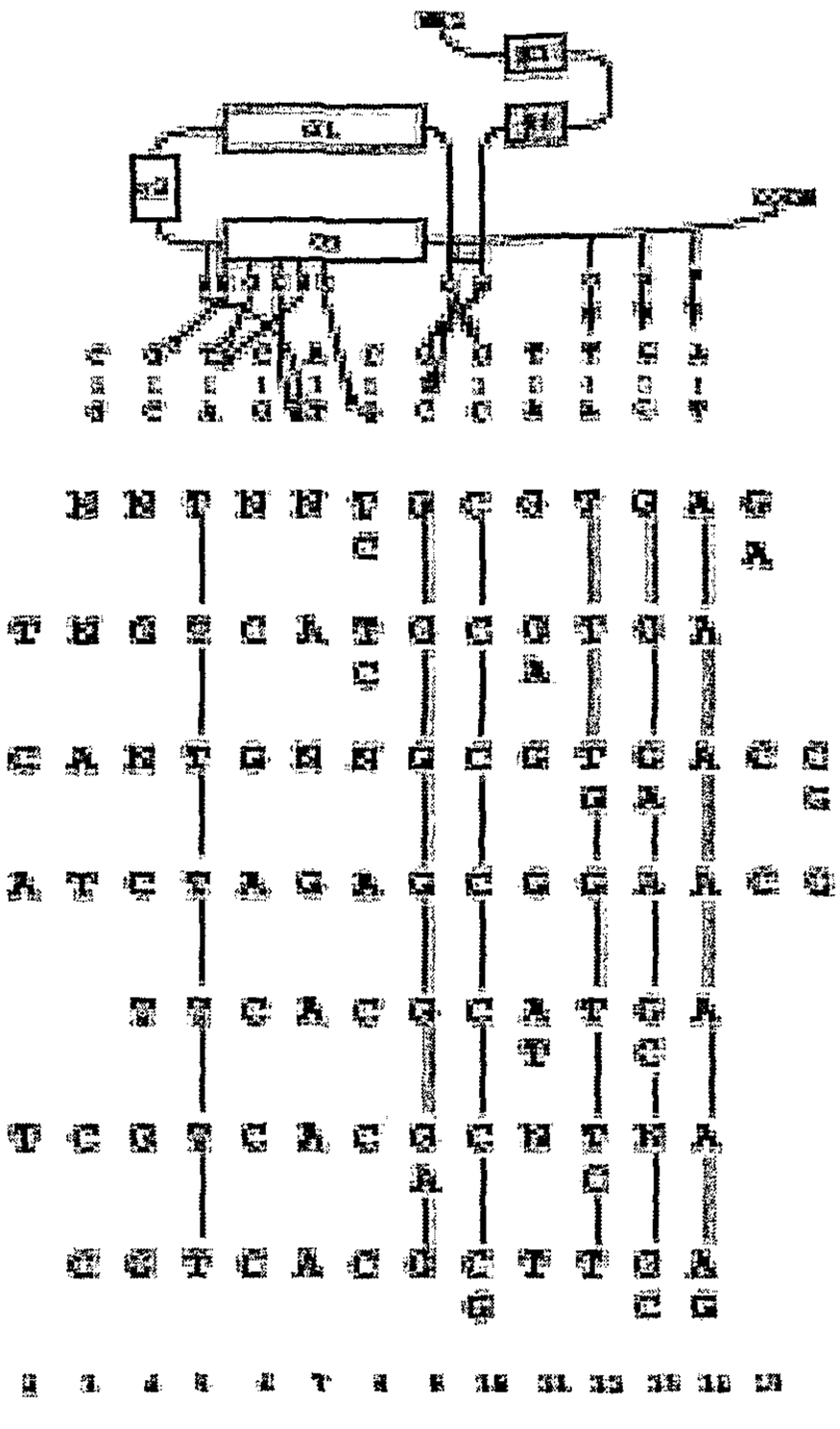
(54) Title: INHIBITION OF PAX2 BY DEFB1 INDUCTION AS A THERAPY FOR CANCER



(57) Abstract: Provided is a method of treating cancer in a subject by inhibiting expression of PAX2. An example of a cancer treated by the present method is prostate cancer. Similarly, provided is a method of treating cancer in a subject by increasing expression of DEFB1 in the subject. In the method wherein the expression of DEFB1 is increased, it can be increased by blocking the binding of PAX2 to the DEFB1 promoter. Blocking the binding of PAX2 to the DEFB1 promoter can be by administration of an oligonucleotide containing the PAX2 DNA binding site of DEFB1. This oligonucleotide can be complementary to the sequence of PAX2 that binds to the DEFB1 promoter. Alternatively, the oligonucleotide can interact with the PAX2 in a way that inhibits binding to DEFB1.

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TARGETING PAX2 FOR THE INDUCTION OF DEFB1-MEDIATED TUMOR IMMUNITY AND CANCER THERAPY

Brief Description of Invention

Current anticancer chemotherapies that are based on alkylating agents, anti-metabolites and natural products are heterogeneous in their mechanism of action. Consequently, most of them also act against normal cells resulting in severe side effects and toxicity to the patient. Disclosed herein is a method for the treatment of advanced prostate cancer using human beta defensin-1 (DEFB1), which is a naturally component of the innate immune system, to induce prostate cancer tumor immunity. This is accomplished through endogenously added DEFB1, ectopically expressed DEFB1 or de novo expression of DEFB1 by inhibiting the transcriptional repressor PAX2 by a variety of mechanisms or agents. Inhibiting PAX2 expression by siRNA therapy turns on DEFB1 expression and generates DEFB1-mediated cell death in prostate cancer. With this, the technology described here is used for the design of small molecules to specifically block PAX2 expression. Alternatively provided are molecules containing the CCTTG (SEQ ID NO:1) recognition sequence (in either forward of reverse orientation) that to bind to the DNA-binding domain of PAX2 preventing its binding to the DEFB1 promoter through competitive inhibition. This permits DEFB1 expression, triggering both an innate and adaptive immune response, and resulting in the killing of prostate cancer cells and the suppression of prostate tumor formation. In conclusion, these modulators of innate tumor immunity, PAX2 and DEFB1, and the molecular therapies based on them provide for the treatment of prostate cancer with little toxicity to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings illustrate one or more embodiments of the invention and, together with the written description, serve to explain the principles of the invention. Wherever possible, the same reference numbers are used throughout the drawings to refer to the same or like elements of an embodiment.

Figure 1. QRT-PCR analysis of DEFB1 Expression. In order to verify induction of DEFB1 expression, QRT-PCR was performed. *A*, DEFB1 relative expression levels were compared in clinical samples from 6 patients that underwent radical prostatectomies. *B*,

DEFB1 relative expression levels were compared in benign and malignant prostatic clinical samples, hPrEC cells and in prostate cancer cell lines before and after DEFB1 induction. *C*, DEFB1 relative expression levels were analyzed in benign tissue, malignant tissue and PIN in a single tissue section. *D*, DEFB1 expression in benign tissue, malignant tissue and PIN in one patient was compared to the average DEFB1 expression level found in benign tissue.

Figure 2. Microscopic analysis of DEFB1 induced changes in membrane integrity and cell morphology. Cell morphology of DU145, PC3 and LNCaP was analyzed by phase contrast microscopy after 48 hours of DEFB1 induction. Membrane ruffling is indicated by black arrows and apoptotic bodies are indicated white arrows.

Figure 3. Analysis of DEFB1 Cytotoxicity in Prostate Cancer Cells. The prostate cell lines DU145, PC3 and LNCaP were treated with PonA to induce DEFB1 expression for 1-3 days after which MTT assay was performed to determine cell viability. Results represent mean ± s.d., n=9.

Figure 4. Induction of cell death in DU145 and PC3 cells by DEFB1. DEFB1 expression was induced in prostate cancer cell lines DU145 (A) and PC3 (B) and then subjected to annexin V/FITC/propidium iodide staining and flow cytometric analysis. Cells positive for propidium iodide and annexin V were considered apoptotic. Times of induction are shown under each panel. Numbers next to the boxes for each time point represent the percentages of propidium iodide $(PI)^-$ annexin V^+ cells (lower right quadrant), and PI^+ annexin V^+ cells (upper right quadrant). The data are from a single experiment that is representative of three separate experiments.

Figure 5. Pan-caspase analysis following DEFB1 induction. DU145 and PC3 cells were stained with FAM-VAD-FMK-labeled fluoromethyl ketone to detect caspase activity. Cells were visible under DIC for each condition. Confocal microscopic analysis revealed no caspase staining in control DU145 (B), PC3 cells (F) and LNCaP (J). Cells treated with PonA for 24 hours to induce DEFB1 revealed caspase activity in DU145 (D) and PC3 (H). No caspase activity was detected in LNCaP (L).

Figure 6. Silencing of PAX2 Protein Expression Following PAX2 siRNA Treatment. **(a)** Western blot analysis of PC3 and DU145 cells transfected with PAX2 siRNA duplex at day zero (lane 1), day two (lane 2), and day four (lane 3). **(b)** Western blot analysis of PC3 and DU145 cells transfected with PAX2 siRNA duplex at day zero (lane 1), day two (lane 2), day four (lane 3) and day 6 (lane 4). PAX2 protein was undetectable as early as after four days of treatment (lane 3) in DU145 cells and after six days of treatment in PC3. Blots were stripped and re-probed for β-actin as an internal control.

Figure 7. Analysis of Prostate Cancer Cells Growth after Treatment with Pax 2 siRNA. Phase contrast microscopic analysis of DU145, PC3 and LNCaP at 6 days in the presence of normal growth media. Treatment with negative control siRNA had no effect on the cells. However, there was a significant reduction in cell number in all three lines following treatment with PAX2 siRNA.

Figure 8. Analysis of Cell Death Following siRNA Silencing of PAX2. Prostate cancer cell lines PC3, DU145, and LNCaP were treated with 0.5 μg of a pool of four PAX2 siRNA's or four non-specific control siRNA's for 2, 4 or 6 days after which MTT assay was done to determine cell viability. Results represent mean ± s.d., n=9.

Figure 9. Analysis of Caspase Activity. DU145, PC3 and LNCaP cells were stained with carboxyfluorescein-labeled fluoromethyl ketone to detected caspase activity following treatment with PAX2 siRNA. Confocal microscopic analysis of untreated and treated cells show cells were visible with DIC. Analysis under fluorescence revealed no caspase staining in control DU145 (B), PC3 cells (F) and LNCaP cells (J). However, cell treated with PAX2 siRNA induced caspase activity in DU145 (D), PC3 (H) and LNCaP (L).

Figure 10. Analysis of Apoptotic Factors Following PAX2 siRNA Treatment. Changes in expression of pro-apoptotic factors were compared in untreated control cells and in cells treated for six days with PAX2 siRNA. *A*, BAX expression levels increased in DU145, PC3 and LNCaP. *B*, BID expression increased in DU145 and LNCaP, but change in PC3. *C*, BAD expression levels increased in all three cell lines.

Figure 11. Model of PAX2 Binding to DNA Recognition Sequence. The PAX2 transcriptional repressor binds to a CCTTG (SEQ ID NO:1) recognition site immediately adjacent to the DEFB1 TATA box preventing transcription and DEFB1 protein expression. Inhibition of PAX2 protein expression allows normal DEFB1 expression.

Figure 12. Illustration of the DEFB1 Reporter Construct. The DEFB1 promoter consisting of the first 160 bases upstream of the mRNA start site was PCR amplified from DU145 cell and ligated into the pGL3 luciferase reporter plasmid.

Figure 13. Inhibition of PAX2 Results in DEFB1 Expression. DU145, PC3, LNCaP and HPrEC were treated for 48 hours with PAX2 siRNA. QRT-PCR analysis before treatment showed no DEFB1 expression in DU145, PC3 and LNCaP. However, DEFB1 expression was restored following treatment in all lines. There was no change in DEFB1 expression following siRNA treatment of PAX2-null HprEC.

Figure 14. Inhibition of PAX2 Results in Increased DEFB1 Promoter Activity. PC3 promoter/pGL3 and DU145 promoter/pGL3 construct were generated and were transfected into PC3 and DU145 cells, respectively. Promoter activity was compared before and after PAX2 inhibition by siRNA treatment. DEFB1 promoter activity increased 2.65-fold in DU145 and 3.78 fold in PC3 following treatment.

Figure 15. DEFB1 Causes Loss of Membrane Integrity. Membrane integrity of PC3 and DU145 cells was analyzed by confocal laser microscopy following the induction of DEFB1 expression for 48 hours. Green staining was indicative of the localization of AO, and red staining represents EtBr. Yellow staining represents the co-localization of both AO and EtBr in the nucleus.

Figure 16. PAX2 Inhibition Results in Loss of Membrane Integrity. Cells were treated for 48 hours with PAX2 siRNA and membrane integrity was analyzed by confocal laser microscopy.

Figure 17. ChIP Analysis of PAX2 binding to DEFB1 Promoter. ChIP analysis was performed on DU145 and PC3 cells. Following immunoprecipitation with an anti-PAX2

antibody, PCR was performed to detect the DEFB1 promoter region containing the GTTCC PAX2 recognition site. This demonstrates that the PAX2 transcriptional repressor is bound to the DEFB1 promoter in prostate cancer cell lines.

Figure 18. Predicted Structure of the PrdPD and PrdHD with DNA. The coordinates of the structures of the PrdPD bound to DNA (Xu et al., 1995 and the PrdHD bound to DNA (Wilson et al., 1995) were used to construct a model of the two domains as they bound to a PH0 site. The individual binding sites are abutted next to each other with a specific orientation as indicated. The PAI binding site is in red, the HD binding site is in blue, and the corresponding PAI domain is in turquoise, and HD is in yellow. The RED domain is oriented based on the PrdPD crystal structure.

Figure 19. Comparison of Consensus Sequences of Different Paired Domains. At the top of the Figure is drawn a schematic representation of protein±DNA contacts described in the crystallographic analysis of the Prd-paired-domain±DNA complex [9]. Empty boxes indicate a-helices, shaded boxes indicates b-sheets and a thick line indicate a b-turn. Contacting amino acids are shown by single-letter code. Only direct amino acid±base contacts are shown. Empty circles indicate major groove contacts while red arrows indicate minor groove contacts. This scheme is aligned to all known consensus sequences for paired-domain proteins (top strands only are shown). Vertical lines between consensus sequences indicate conserved base-pairs. Numbering of the positions is shown at the bottom of the Figure and it is the same as that used in [9].

DETAILED DESCRIPTION OF THE INVENTION

As shown herein, PAX2 inhibits expression of DEFB1, and DEFB1 is shown to have tumor cell killing activity. Thus, provided is a method of treating cancer in a subject by inhibiting expression of PAX2. An example of a cancer treated by the present method is prostate cancer. The present methods are particularly effective for treatment of late stage prostate cancer.

In the cancer treatment methods disclosed, the method of inhibiting expression of PAX 2 can be by administration of a nucleic acid encoding a siRNA for PAX 2. Dharmachon is a commercial source for such siRNAs.

The siRNA for use in the methods can be selected from the group consisting of:

AUAGACUCGACUUGACUUCUU (SEQ ID NO:2)

AUCUUCAUCACGUUUCCUCUU (SEQ ID NO:4)

GUAUUCAGCAAUCUUGUCCUU (SEQ ID NO:6)

GAUUUGAUGUGCUCUGAUGUU (SEQ ID NO:8)

The following table illustrates the above antisense sequences and their corresponding sense sequences.

	Sense (5'-3')	Antisense (5'-3')
Sequence A	5'- GAAGUCAAGUCGAGUCUAUUU-3'	5'–AUAGACUCGACUUGACUUCUU–3'
Sequence B	5'-GAGGAAACGUGAUGAAGAUUU-3'	5'-AUCUUCAUCACGUUUCCUCUU-3'
Sequence C	5'-GGACAAGAUUGCUGAAUACUU-3'	5'-GUAUUCAGCAAUCUUGUCCUU-3'
Sequence D	5'-CAUCAGAGCA-CAUCAAAUCUU-3'	5'-GAUUUGAUGUGCUCUGAUGUU-3'

Further examples of molecules that inhibit PAX2 include:

#1 ACCCGACTATGTTCGCCTGG (SEQ ID NO:XXX),

#2 AAGCTCTGGATCGAGTCTTTG (SEQ ID NO:XXX),

and #4 ATGTGTCAGGCACACAGACG (SEQ ID NO:XXX). #4 was shown to inhibit PAX2 (Davies et al., Hum. Mol. Gen Jan. 15, 13 (2); 235).

Another paper (Muratovska et al., Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival Oncogene (2003) 22, 7989–7997) discloses the following siRNAs: GUCGAGUCUAUCUGCAUCCTT (SEQ ID NO:xxx) and GGAUGCAGAUAGACUCGACTT (SEQ ID NO:XXX).

To down-regulate Pax2 expression, Fonsato et al. transfected tumor-derived endothelial cells with an anti-sense PAX2 vector. See Fonsato V. et al (Expression of Pax2 in human renal tumor-derived endothelial cells sustains apoptosis resistance and angiogenesis, Am J Pathol. 2006 Feb;168(2):706-1), incorporated herein by referene for its description of this molecule. Similarly, Hueber et al. teach that PAX2 antisense cDNA and PAX2-small interfering RNA (100 nM) reduce endogenous PAX2 protein. See Hueber et al. PAX2 inactivation enhances cisplatin-induced apoptosis in renal carcinoma cells, Kidney Int. 2006 Apr;69(7):1139-45 incorporated herein for its teaching of PAX2 antisense and PAX2 siRNA.

Additional inhibitors of PAX2 expression or the binding of PAX2 to the DEFB1 promoter are provided to increase DEFB1 expression in the presently disclosed methods.

For example, small molecules and antibodies are designed based on the present studies to interfere with or inhibit the binding of PAX2 to the DEFB1 promoter.

As shown herein, PAX2 inhibits expression of DEFB1, and DEFB1 is shown to have tumor cell killing activity. Thus, a method of treating cancer in a subject by administering DEFB1 is also provided. An example of a cancer treated by the present method is prostate cancer.

Similarly, provided is a method of treating cancer in a subject by increasing expression of DEFB1 in the subject. The present methods of administering or increasing the expression of DEFB1 are particularly effective for treatment of late stage prostate cancer.

In one embodiment of the methods of the invention for treating cancer by administering DEFB1 or increasing DEFB1 expression (e.g., by inhibiting expression or binding of PAX2), the subject is a subject diagnosed with prostate cancer. In a further embodiment of the methods of the invention for treating cancer by administering DEFB1 or increasing DEFB1 expression (e.g., by inhibiting expression or binding of PAX2), the subject is a subject diagnosed with advanced (late stage) prostate cancer.

In the method wherein the expression of DEFB1 is increased, it can be increased by blocking the binding of PAX2 to the DEFB1 promoter. The blocking of binding of PAX2 to the DEFB1 promoter can be by administration of an oligonucleotide containing the PAX2 DNA binding site of DEFB1. This oligonucleotide can be complementary to the sequence of PAX2 that binds to the DEFB1 promoter. Alternatively, the oligonucleotide can interact with the PAX2 in a way that inhibits binding to DEFB1. This interaction can be based on three-dimensional structure rather than primary nucleotide sequence.

PAX proteins are a family of transcription factors conserved during evolution and able to bind specific DNA sequences through a domains called a "paired domain" and a "homeodomain". The paired domain (PD) is a consensus sequence shared by certain PAX proteins (e.g., PAX2 and PAX6). The PD directs DNA binding of amino acids located in the α3-helix forming a DNA-Protein complex. For PAX2, the amino acids in the HD recognize and interact specifically with a **CCTTG** DNA core sequence. Therefore, the critical region for PAX2 binding to DEFB1 would be AAGTTCAC**CCTTG**ACTGTG. Oligonucleotides up to and exceeding 64 bases in length, which include this sequence or its complement are expected to be inhibitors.

The DNA-binding specificity of the PAX-8 paired domain was investigated. Site selection experiments indicate that PAX-8 binds to a consensus sequence similar to those bound by PAX-2 and PAX-5. When consensus sequences of various paired domains are observed in light of recent structural studies describing paired-domain-DNA interaction [Xu, Rould, Jun, Desplan and Pabo (1995) Cell 80, 639-650], it appears that base-pairs contacted in the minor groove are conserved, while most of the base-pairs contacted in the major groove are not. Therefore a network of specific minor groove contacts is a common characteristic of paired-domain-DNA interactions. The functional importance of such a network can be successfully tested by analyzing the effect of consensus-based mutations on the PAX2 binding site of the DEFB1 promoter.

The PAX2 DNA binding site of DEFB1 can comprise SEQ ID NO:1 (CCTTG).

The oligonucleotide comprising to the PAX2 DNA binding site of DEFB1 is selected from the group consisting of

X_1 CCTTG (SEQ ID NO:1)X_2, wherein X_1 is from 1 to 35 contiguous flanking nucleotides of DEFB1 and X_2 is from 1 to 35 nucleotides. The nucleotides can be contiguous nucleotides that normally flank the PAX2 DNA binding site of DEFB1. Alternatively, they can be unrelated to DEFB1, and selected routinely to avoid interference with the recognition sequence.

For example, the inhibitory oligonucleotides can be selected from the group consisting of:

CTCCCTTCAGTTCCGTCGAC (SEQ ID NO:9)

CTCCCTTCACCTTGGTCGAC (SEQ ID NO:10)

ACTGTGGCACCTCCCTTCAGTTCCGTCGACGAGGTTGTGC (SEQ ID NO:12)

ACTGTGGCACCTCCCTTCACCTTGGTCGACGAGGTTGTGC (SEQ ID NO:13)

The disclosed compositions can be used to treat any disease where uncontrolled cellular proliferation occurs such as cancers. A non-limiting list of different types of cancers is as follows: lymphomas (Hodgkins and non-Hodgkins), leukemias, carcinomas, carcinomas of solid tissues, squamous cell carcinomas, adenocarcinomas, sarcomas, gliomas, high grade gliomas, blastomas, neuroblastomas, plasmacytomas, histiocytomas, melanomas, adenomas, hypoxic tumors, myelomas, AIDS-related lymphomas or sarcomas, metastatic cancers, or cancers in general.

A representative but non-limiting list of cancers that the disclosed compositions can be used to treat is the following: lymphoma, B cell lymphoma, T cell lymphoma, mycosis

fungoides, Hodgkin's Disease, myeloid leukemia, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, squamous cell carcinoma of head and neck, kidney cancer, lung cancers such as small cell lung cancer and non-small cell lung cancer, neuroblastoma/glioblastoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, colon cancer, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon and rectal cancers, prostatic cancer, or pancreatic cancer. Compounds disclosed herein may also be used for the treatment of precancer conditions such as cervical and anal dysplasias, other dysplasias, severe dysplasias, hyperplasias, atypical hyperplasias, and neoplasias. Further, a number of diseases stemming from chronic inflammation, e.g., prostatitis and Benign Prostatic Hypertrophy (BPH), as well as various cancers of the prostate, can be impacted by the present methods and compounds.

DEFB1's gene locus (8p23.3) is a hotspot for deletions and has been linked to patients with poorer prognosis. Thus, DEFB1 (and perhaps PAX2) can be used as a biomarker, e.g., in a screening for the early detection of prostate cancer. Furthermore, data presented here indicate that its loss may occur as early as PIN (or even before), and may be a major contributing factor to the onset of prostate cancer.

Nucleic Acid Homology/Identity/Similarity

It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In

general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated

by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

Hybridization/selective hybridization

The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as an oligonucleotide inhibitor, a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide derivatives in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization may involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the Tm (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the Tm. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The conditions can be used as described above to achieve stringency, or as is known in the art. (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed.,

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. Methods Enzymol. 1987:154:367, 1987 which is herein incorporated by reference for material at least related to hybridization of nucleic acids). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

Another way to define selective hybridization is by looking at the amount (percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting nucleic acid. Typically, the non-limiting nucleic acid is in for example, 10 or 100 or 1000 fold excess. This type of assay can be performed at under conditions where both the limiting and non-limiting nucleic acid are for example, 10 fold or 100 fold or 1000 fold below their k_d, or where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where one or both nucleic acid molecules are above their k_d.

Another way to define selective hybridization is by looking at the percentage of nucleic acid that gets enzymatically manipulated under conditions where hybridization is required to promote the desired enzymatic manipulation, e.g., for primers. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions which promote the enzymatic manipulation, for example if the enzymatic manipulation is DNA extension, then selective hybridization conditions would be when at least about 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred conditions also include those suggested by the manufacturer or indicated in the art as being appropriate for the enzyme performing the manipulation.

Just as with homology, it is understood that there are a variety of methods herein disclosed for determining the level of hybridization between two nucleic acid molecules. It is understood that these methods and conditions may provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required parameters in any one of these methods it is considered disclosed herein.

It is understood that those of skill in the art understand that if a composition or method meets any one of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

Nucleotides and related molecules

A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

A nucleotide analog is a nucleotide which contains some type of modification to any of the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl (.psi.), hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and

2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, Antisense Research and

Applications, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxyribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10}, alkyl or C_2 to C_{10} alkenyl and alkynyl. 2' sugar modifications also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_nON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

Other modifications at the 2' position include but are not limited to: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, $SO_2 CH_3$, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137;

5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

It is understood that nucleotide analogs need only contain a single modification, but may also contain multiple modifications within one of the moieties or between different moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic

internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones;formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage (aminoethylglycine) (PNA). United States patents 5,539,082; 5,714,331;and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by reference. (See also Nielsen et al., Science, 1991, 254, 1497-1500).

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,

86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36,

3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937. Numerous United States patents teach the preparation of such conjugates and include, but are not limited to U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

Sequences

There are a variety of sequences related to the DEFB1 gene and to the PAX2 transcriptional factor, respectively, having the following GenBank Accession Numbers: U50930 and NM_003989.1. These sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

The one particular sequence set forth in SEQ ID NO: xxx and having GenBank accession number U50930 is used herein as an example to exemplify a source for the disclosed DEFB1 nucleic acids. The one particular sequence set forth in SEQ ID NO:xxx and having GenBank accession number NM_003989 (see Appendix A).1 is used herein as an example, to exemplify a source for the disclosed PAX2 nucleic acids. Other examples of PAX2 sequences, based on alternative splicing are also found in GenBank. These are

variants a-e, shown in Appendices B-F. It is understood that the description related to this sequence is applicable to any sequence related to unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. siRNA molecules, competitive inhibitors of DEFB1 promoter-PAX2, and primers and/or probes can be designed for any DEFB1 or PAX2 sequence given the information disclosed herein and known in the art.

Nucleic acid synthesis

The nucleic acids, such as, the oligonucleotides to be used as inhibitors can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* **53**:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* **5**:3-7 (1994).

Primers and probes

Disclosed are compositions including primers and probes, which are capable of interacting with the DEFB1 gene as disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner. Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA extension, DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific

manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using non-enzymatic techniques, where for example, the nucleotides or oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner.

The size of the primers or probes for interaction with the DEFB1 or PAX2 gene in certain embodiments can be any size that supports the desired enzymatic manipulation of the primer, such as DNA amplification or the simple hybridization of the probe or primer. A typical primer or probe would be at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3500, or 4000 nucleotides long.

In other embodiments a primer or probe can be less than or equal to 6, 7, 8, 9, 10, 11, 12 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3500, or 4000 nucleotides long.

The primers for the DEFB1 or PAX2 gene typically will be used to produce an amplified DNA product that contains the region of the DEFB1 gene to which PAX2 binds. In general, typically the size of the product will be such that the size can be accurately determined to within 3, or 2 or 1 nucleotides.

In certain embodiments this product is at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650,

700, 750, 800, 850, 900, 950, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3500, or 4000 nucleotides long.

In other embodiments the product is less than or equal to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3500, or 4000 nucleotides long.

Functional Nucleic Acids

RNAi

It is also understood that the disclosed nucleic acids can be used for RNAi or RNA interference. It is thought that RNAi involves a two-step mechanism for RNA interference (RNAi): an initiation step and an effector step. For example, in the first step, input double-stranded (ds) RNA (siRNA) is processed into small fragments, such as 21–23-nucleotide 'guide sequences'. RNA amplification occurs in whole animals. Typically then, the guide RNAs can be incorporated into a protein RNA complex which is capable of degrading RNA, the nuclease complex, which has been called the RNA-induced silencing complex (RISC). This RISC complex acts in the second effector step to destroy mRNAs that are recognized by the guide RNAs through base-pairing interactions. RNAi involves the introduction by any means of double stranded RNA into the cell which triggers events that cause the degradation of a target RNA. RNAi is a form of post-transcriptional gene silencing. In addition to the siRNAs disclosed herein, disclosed are RNA hairpins that can act in RNAi. For description of making and using RNAi molecules see, e.g., Hammond et al., Nature Rev Gen 2: 110-119 (2001); Sharp, Genes Dev 15: 485-490 (2001), Waterhouse et al., Proc. Natl. Acad. Sci. USA 95(23): 13959-13964 (1998) all of which are incorporated herein by reference in their entireties and at least form material related to delivery and making of RNAi molecules.

RNAi has been shown to work in many types of cells, including mammalian cells. For work in mammalian cells it is preferred that the RNA molecules which will be used as targeting sequences within the RISC complex are shorter. For example, less than or equal to 50 or 40 or 30 or 29, 28, 27, 26, 25, 24, 23, ,22, 21, 20, 19, 18, 17, 16 , 15, 14, 13 , 12,

11, or 10 nucleotides in length. These RNA molecules can also have overhangs on the 3' or 5' ends relative to the target RNA which is to be cleaved. These overhangs can be at least or less than or equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 nucleotides long. RNAi works in mammalian stem cells, such as mouse ES cells. Examples of siRNAs can be found in Table 4.

Functional nucleic acids are nucleic acid molecules that have a specific function, such as binding a target molecule or catalyzing a specific reaction. Functional nucleic acid molecules can be divided into the following categories, which are not meant to be limiting. For example, functional nucleic acids include antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional nucleic acid molecules can act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a de novo activity independent of any other molecules.

Functional nucleic acid molecules can interact with any macromolecule, such as DNA, RNA, polypeptides, or carbohydrate chains. Thus, functional nucleic acids can interact with mRNA or the genomic DNA of PAX2. Often functional nucleic acids are designed to interact with other nucleic acids based on sequence homology between the target molecule and the functional nucleic acid molecule. In other situations, the specific recognition between the functional nucleic acid molecule and the target molecule is not based on sequence homology between the functional nucleic acid molecule and the target molecule, but rather is based on the formation of tertiary structure that allows specific recognition to take place.

Antisense molecules are designed to interact with a target nucleic acid molecule through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule is designed to promote the destruction of the target molecule through, for example, RNAseH mediated RNA-DNA hybrid degradation. Alternatively the antisense molecule is designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. Numerous methods for optimization of antisense efficiency by finding the most accessible regions of the target molecule exist. Exemplary methods would be in vitro selection experiments and DNA modification studies using DMS and DEPC. It is preferred that

antisense molecules bind the target molecule with a dissociation constant (k_d)less than or equal to 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. A representative sample of methods and techniques which aid in the design and use of antisense molecules can be found in the following non-limiting list of United States patents: 5,135,917, 5,294,533, 5,627,158, 5,641,754, 5,691,317, 5,780,607, 5,786,138, 5,849,903, 5,856,103, 5,919,772, 5,955,590, 5,990,088, 5,994,320, 5,998,602, 6,005,095, 6,007,995, 6,013,522, 6,017,898, 6,018,042, 6,025,198, 6,033,910, 6,040,296, 6,046,004, 6,046,319, and 6,057,437.

Aptamers are molecules that interact with a target molecule, preferably in a specific way. Typically aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets. Aptamers can bind small molecules, such as ATP (United States patent 5,631,146) and theophiline (United States patent 5,580,737), as well as large molecules, such as reverse transcriptase (United States patent 5,786,462) and thrombin (United States patent 5,543,293). Aptamers can bind very tightly with k_ds from the target molecule of less than 10^{-12} M. It is preferred that the aptamers bind the target molecule with a k_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. Aptamers can bind the target molecule with a very high degree of specificity. For example, aptamers have been isolated that have greater than a 10000 fold difference in binding affinities between the target molecule and another molecule that differ at only a single position on the molecule (United States patent 5,543,293). It is preferred that the aptamer have a k_d with the target molecule at least 10, 100, 1000, 10,000, or 100,000 fold lower than the k_d with a background binding molecule. Representative examples of how to make and use aptamers to bind a variety of different target molecules can be found in the following non-limiting list of United States patents: 5,476,766, 5,503,978, 5,631,146, 5,731,424 , 5,780,228, 5,792,613, 5,795,721, 5,846,713, 5,858,660 , 5,861,254, 5,864,026, 5,869,641, 5,958,691, 6,001,988, 6,011,020, 6,013,443, 6,020,130, 6,028,186, 6,030,776, and 6,051,698.

Ribozymes are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecularly or intermolecularly. Ribozymes are thus catalytic nucleic acid. It is preferred that the ribozymes catalyze intermolecular reactions. There are a number of different types of ribozymes that catalyze nuclease or nucleic acid polymerase type reactions which are based on ribozymes found in natural systems, such as hammerhead ribozymes, (for example, but not limited to the following United States patents: 5,334,711, 5,436,330, 5,616,466, 5,633,133, 5,646,020, 5,652,094, 5,712,384, 5,770,715, 5,856,463,

5,861,288, 5,891,683, 5,891,684, 5,985,621, 5,989,908, 5,998,193, 5,998,203, WO 9858058 by Ludwig and Sproat, WO 9858057 by Ludwig and Sproat, and WO 9718312 by Ludwig and Sproat) hairpin ribozymes (for example, but not limited to the following United States patents: 5,631,115, 5,646,031, 5,683,902, 5,712,384, 5,856,188, 5,866,701, 5,869,339, and 6,022,962), and tetrahymena ribozymes (for example, but not limited to the following United States patents: 5,595,873 and 5,652,107). There are also a number of ribozymes that are not found in natural systems, but which have been engineered to catalyze specific reactions de novo (for example, but not limited to the following United States patents: 5,580,967, 5,688,670, 5,807,718, and 5,910,408). Preferred ribozymes cleave RNA or DNA substrates, and more preferably cleave RNA substrates. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. This recognition is often based mostly on canonical or non-canonical base pair interactions. This property makes ribozymes particularly good candidates for target specific cleavage of nucleic acids because recognition of the target substrate is based on the target substrates sequence. Representative examples of how to make and use ribozymes to catalyze a variety of different reactions can be found in the following non-limiting list of United States patents: 5,646,042, 5,693,535, 5,731,295, 5,811,300, 5,837,855, 5,869,253, 5,877,021, 5,877,022, 5,972,699, 5,972,704, 5,989,906, and 6,017,756.

Triplex forming functional nucleic acid molecules are molecules that can interact with either double-stranded or single-stranded nucleic acid. When triplex molecules interact with a target region, a structure called a triplex is formed, in which there three strands of DNA are forming a complex dependant on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules are preferred because they can bind target regions with high affinity and specificity. It is preferred that the triplex forming molecules bind the target molecule with a k_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. Representative examples of how to make and use triplex forming molecules to bind a variety of different target molecules can be found in the following non-limiting list of United States patents: 5,176,996, 5,645,985, 5,650,316, 5,683,874, 5,693,773, 5,834,185, 5,869,246, 5,874,566, and 5,962,426.

External guide sequences (EGSs) are molecules that bind a target nucleic acid molecule forming a complex, and this complex is recognized by RNase P, which cleaves the target molecule. EGSs can be designed to specifically target a RNA molecule of choice.

RNAse P aids in processing transfer RNA (tRNA) within a cell. Bacterial RNAse P can be recruited to cleave virtually any RNA sequence by using an EGS that causes the target RNA:EGS complex to mimic the natural tRNA substrate. (WO 92/03566 by Yale, and Forster and Altman, Science 238:407-409 (1990)).

Similarly, eukaryotic EGS/RNAse P-directed cleavage of RNA can be utilized to cleave desired targets within eukaryotic cells. (Yuan et al., Proc. Natl. Acad. Sci. USA 89:8006-8010 (1992); WO 93/22434 by Yale; WO 95/24489 by Yale; Yuan and Altman, EMBO J 14:159-168 (1995), and Carrara et al., Proc. Natl. Acad. Sci. (USA) 92:2627-2631 (1995)). Representative examples of how to make and use EGS molecules to facilitate cleavage of a variety of different target molecules be found in the following non-limiting list of United States patents: 5,168,053, 5,624,824, 5,683,873, 5,728,521, 5,869,248, and 5,877,162.

Delivery of the compositions to cells

There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991)Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

Nucleic acid based delivery systems

Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

As used herein, plasmid or viral vectors are agents that transport the disclosed nucleic acids, such as DEFB1 coding sequences, PAX2 siRNAs or other antisense molecules into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. Viral vectors are, for example, Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone. Also preferred are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not as useful in non-proliferating cells. Adenovirus vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or 10.

Viral vectors can have higher transaction (ability to introduce genes) abilities than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

Retroviral Vectors

A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985), which is

incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference.

A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

Adenoviral Vectors

The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, in vivo delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993); Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin. Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem. 267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research 73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

A viral vector can be one based on an adenovirus which has had the E1 gene removed and these virions are generated in a cell line such as the human 293 cell line. In another preferred embodiment both the E1 and E3 genes are removed from the adenovirus genome.

Adeno-associated viral vectors

Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus is a preferred vector because it can infect many cell types and is

nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type AAV is known to stably insert into chromosome 19. Vectors which contain this site specific integration property are preferred. An especially preferred embodiment of this type of vector is the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

In another type of AAV virus, the AAV contains a pair of inverted terminal repeats (ITRs) which flank at least one cassette containing a promoter which directs cell-specific expression operably linked to a heterologous gene. Heterologous in this context refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

Typically the AAV and B19 coding regions have been deleted, resulting in a safe, noncytotoxic vector. The AAV ITRs, or modifications thereof, confer infectivity and site-specific integration, but not cytotoxicity, and the promoter directs cell-specific expression. United states Patent No. 6,261,834 is herein incorporated by reference for material related to the AAV vector.

1. The disclosed vectors thus provide DNA molecules which are capable of integration into a mammalian chromosome without substantial toxicity.

2. The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

Large payload viral vectors

Molecular genetic experiments with large human herpes viruses have provided a means whereby large heterologous DNA fragments can be cloned, propagated and established in cells permissive for infection with herpes viruses (Sun et al., Nature genetics 8: 33-41, 1994; Cotter and Robertson, Curr Opin Mol Ther 5: 633-644, 1999). These large DNA viruses (herpes simplex virus (HSV) and Epstein-Barr virus (EBV), have the potential to deliver fragments of human heterologous DNA > 150 kb to specific cells. EBV recombinants can maintain large pieces of DNA in the infected B-cells as episomal DNA. Individual clones carried human genomic inserts up to 330 kb appeared genetically stable The maintenance of these episomes requires a specific EBV nuclear protein, EBNA1,

constitutively expressed during infection with EBV. Additionally, these vectors can be used for transfection, where large amounts of protein can be generated transiently in vitro. Herpesvirus amplicon systems are also being used to package pieces of DNA > 220 kb and to infect cells that can stably maintain DNA as episomes.

Other useful systems include, for example, replicating and host-restricted non-replicating vaccinia virus vectors.

Non-nucleic acid based systems

The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

Thus, the compositions can comprise, in addition to the disclosed vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, and DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the disclosed nucleic acid or vector can be delivered *in vivo* by electroporation, the

technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other specific cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellular, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral integration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic

acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

In vivo/ex vivo

As described herein, the compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

Expression systems

The nucleic acids that are delivered to cells typically contain expression controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

Viral Promoters and Enhancers

Preferred promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTR.

It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

Proteins

Protein variants

Variants of the DEFB1 protein are provided. Derivatives of the DEFB1 protein function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include

amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

TABLE 1:Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	AlaA
allosoleucine	AIle
arginine	ArgR
asparagine	AsnN
aspartic acid	AspD
cysteine	CysC
glutamic acid	GluE

Amino Acid	Abbreviations
glutamine	GlnK
glycine	GlyG
histidine	HisH
isolelucine	IleI
leucine	LeuL
lysine	LysK
phenylalanine	PheF
proline	ProP
pyroglutamic acidp	Glu
serine	SerS
threonine	ThrT
tyrosine	TyrY
tryptophan	TrpW
valine	ValV

TABLE 2: Amino Acid Substitutions

Original Residue Exemplary Conservative Substitutions, others are known in the art.
Alaser
Arglys, gln
Asngln; his
Aspglu
Cysser
Glnasn, lys
Gluasp
Glypro
Hisasn;gln
Ileleu; val
Leuile; val
Lysarg; gln;
MetLeu; ile
Phemet; leu; tyr
Serthr
Thrser
Trptyr
Tyrtrp; phe
Valile; leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic

residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:xxx sets forth a particular sequence of DEFB1 and SEQ ID NO:xxx sets forth a particular sequence of PAX2. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA,

and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. For example, one of the many nucleic acid sequences that can encode the DEFB1 protein sequence set forth in SEQ ID NO:xxx is set forth in SEQ ID NO:xxx. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular DEFB1 from which that protein arises is also known and herein disclosed and described.

It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent then the amino acids shown in Table 1 and Table 2. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec. Biol. 77:43-73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetic Enginerring Reviews 13:197-216 (1995),

Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994) all of which are herein incorporated by reference at least for material related to amino acid analogs).

Molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, --CH_2--CH_2 --, --CH=CH-- (cis and trans), --$COCH_2$ --, --$CH(OH)CH_2$--, and --CHH_2SO—(These and others can be found in Spatola, A. F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, Trends Pharm Sci (1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. Life Sci 38:1243-1249 (1986) (--CH H_2--S); Hann J. Chem. Soc Perkin Trans. I 307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. J. Med. Chem. 23:1392-1398 (1980) (--$COCH_2$--); Jennings-White et al. Tetrahedron Lett 23:2533 (1982) (--$COCH_2$--); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982) (--$CH(OH)CH_2$--); Holladay et al. Tetrahedron. Lett 24:4401-4404 (1983) (--$C(OH)CH_2$--); and Hruby Life Sci 31:189-199 (1982) (--CH_2--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH_2NH--. It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

Amino acid analogs and analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others.

D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference).

Pharmaceutical carriers/Delivery of pharmaceutical products

As described above, the compositions can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use

of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

Pharmaceutically Acceptable Carriers

The compositions, including DEFB1, DEFB1-encoding nucleic acids, oligonucleotide inhibitors of PAX2 binding, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose

solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable..

Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

Therapeutic Uses

Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, guidance in selecting appropriate doses for antibodies can be found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical daily dosage of the oligonucleotide used alone might range from about 1 μg/kg to up to 100 mg/kg of body

weight or more per day, depending on the factors mentioned above. In a more specific example, 5 to 7 mg/kg/day can be used. This is appropriate for i.v. administration and higher dosages, up to about 150mg/m^2/day s.c. are appropriate. This can be administered daily for a week in from 1 to 24 courses. See for example A Phase I Pharmacokinetic and Biological Correlative Study of Oblimersen Sodium (Genasense, G3139), an Antisense Oligonucleotide to the Bcl-2 mRNA, and of Docetaxel in Patients with Hormone-Refractory Prostate Cancer, Clinical Cancer Research, Vol. 10, 5048–5057, August 1, 2004, incorporated herein for it's teaching of dosages for oligonucleotides.

Following administration of a disclosed composition for treating cancer, the efficacy of the therapeutic composition can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, DEFB1, DEFB1-encoding nucleic acid, inhibitor of PAX2, disclosed herein is efficacious in treating cancer in a subject by observing that the composition reduces tumor load or prevents a further increase in tumor load. Methods of assessing tumor load are known in the art

The compositions that inhibit the interactions between PAX2 and the DEFB1 promoter can be administered prophylactically to patients or subjects who are at risk for cancer.

Other molecules that interact with PAX2 to inhibit its interaction with the DEFB1 promoter can be delivered in ways similar to those described for the pharmaceutical products.

The disclosed compositions and methods can also be used for example as tools to isolate and test new drug candidates for a variety of related diseases. Thus, a method of identifying inhibitors of the binding of PAX2 to the DEFB1 promoter is provided. The method can comprise contacting a system that expresses DEFB1 with a putative inhibitor in the presence and/or absence of PAX2 to determine whether there is an inhibitory effect on this interaction.

Compositions identified by screening with disclosed compositions / combinatorial chemistry

Combinatorial chemistry

The disclosed compositions can be used as targets for any combinatorial technique to identify molecules or macromolecular molecules that interact with the disclosed

compositions in a desired way. Also disclosed are the compositions that are identified through combinatorial techniques or screening techniques in which the compositions disclosed as the PAX2 sequence or portions thereof (e.g., PAX2 DNA–binding domain), are used as the target in a combinatorial or screening protocol.

It is understood that when using the disclosed compositions in combinatorial techniques or screening methods, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as, DEFB1 or PAX2, are also disclosed. Thus, the products produced using the combinatorial or screening approaches that involve the disclosed compositions are also considered herein disclosed.

It is understood that the disclosed methods for identifying molecules that inhibit the interactions between, for example, DEFB1 promoter and PAX2 can be performed using high through put means. For example, putative inhibitors can be identified using Fluorescence Resonance Energy Transfer (FRET) to quickly identify interactions. The underlying theory of the techniques is that when two molecules are close in space, ie, interacting at a level beyond background, a signal is produced or a signal can be quenched. Then, a variety of experiments can be performed, including, for example, adding in a putative inhibitor. If the inhibitor competes with the interaction between the two signaling molecules, the signals will be removed from each other in space, and this will cause a decrease or an increase in the signal, depending on the type of signal used. This decrease or increasing signal can be correlated to the presence or absence of the putative inhibitor. Any signaling means can be used. For example, disclosed are methods of identifying an inhibitor of the interaction between any two of the disclosed molecules comprising, contacting a first molecule and a second molecule together in the presence of a putative inhibitor, wherein the first molecule or second molecule comprises a fluorescence donor, wherein the first or second molecule, typically the molecule not comprising the donor, comprises a fluorescence acceptor; and measuring Fluorescence Resonance Energy Transfer (FRET), in the presence of the putative inhibitor and the in absence of the putative inhibitor, wherein a decrease in FRET in the presence of the putative inhibitor as compared to FRET measurement in its absence indicates the putative inhibitor inhibits binding between the two molecules. This type of method can be performed with a cell system as well.

Combinatorial chemistry includes but is not limited to all methods for isolating small molecules or macromolecules that are capable of binding either a small molecule or another macromolecule, typically in an iterative process. Proteins, oligonucleotides, and sugars are examples of macromolecules. For example, oligonucleotide molecules with a given function, catalytic or ligand-binding, can be isolated from a complex mixture of random oligonucleotides in what has been referred to as "in vitro genetics" (Szostak, TIBS 19:89, 1992). One synthesizes a large pool of molecules bearing random and defined sequences and subjects that complex mixture, for example, approximately 10^{15} individual sequences in 100 μg of a 100 nucleotide RNA, to some selection and enrichment process. Through repeated cycles of affinity chromatography and PCR amplification of the molecules bound to the ligand on the column, Ellington and Szostak (1990) estimated that 1 in 10^{10} RNA molecules folded in such a way as to bind a small molecule dyes. DNA molecules with such ligand-binding behavior have been isolated as well (Ellington and Szostak, 1992; Bock et al, 1992). Techniques aimed at similar goals exist for small organic molecules, proteins, antibodies and other macromolecules known to those of skill in the art. Screening sets of molecules for a desired activity whether based on small organic libraries, oligonucleotides, or antibodies is broadly referred to as combinatorial chemistry.

Combinatorial techniques are particularly suited for defining binding interactions between molecules and for isolating molecules that have a specific binding activity, often called aptamers when the macromolecules are nucleic acids.

There are a number of methods for isolating proteins which either have de novo activity or a modified activity. For example, phage display libraries have been used to isolate numerous peptides that interact with a specific target. (See for example, United States Patent No. 6,031,071; 5,824,520; 5,596,079; and 5,565,332 which are herein incorporated by reference at least for their material related to phage display and methods relate to combinatorial chemistry)

A preferred method for isolating proteins that have a given function is described by Roberts and Szostak (Roberts R.W. and Szostak J.W. Proc. Natl. Acad. Sci. USA, 94(23)12997-302 (1997). This combinatorial chemistry method couples the functional power of proteins and the genetic power of nucleic acids. An RNA molecule is generated in which a puromycin molecule is covalently attached to the 3'-end of the RNA molecule. An *in vitro* translation of this modified RNA molecule causes the correct protein, encoded by the RNA to be translated. In addition, because of the attachment of the puromycin, a

peptdyl acceptor which cannot be extended, the growing peptide chain is attached to the puromycin which is attached to the RNA. Thus, the protein molecule is attached to the genetic material that encodes it. Normal *in vitro* selection procedures can now be done to isolate functional peptides. Once the selection procedure for peptide function is complete traditional nucleic acid manipulation procedures are performed to amplify the nucleic acid that codes for the selected functional peptides. After amplification of the genetic material, new RNA is transcribed with puromycin at the 3'-end, new peptide is translated and another functional round of selection is performed. Thus, protein selection can be performed in an iterative manner just like nucleic acid selection techniques. The peptide which is translated is controlled by the sequence of the RNA attached to the puromycin. This sequence can be anything from a random sequence engineered for optimum translation (i.e. no stop codons etc.) or it can be a degenerate sequence of a known RNA molecule to look for improved or altered function of a known peptide. The conditions for nucleic acid amplification and in vitro translation are well known to those of ordinary skill in the art and are preferably performed as in Roberts and Szostak (Roberts R.W. and Szostak J.W. Proc. Natl. Acad. Sci. USA, 94(23)12997-302 (1997)).

Another preferred method for combinatorial methods designed to isolate peptides is described in Cohen et al. (Cohen B.A.,et al., Proc. Natl. Acad. Sci. USA 95(24):14272-7 (1998)). This method utilizes and modifies two-hybrid technology. Yeast two-hybrid systems are useful for the detection and analysis of protein:protein interactions. The two-hybrid system, initially described in the yeast *Saccharomyces cerevisiae*, is a powerful molecular genetic technique for identifying new regulatory molecules, specific to the protein of interest (Fields and Song, *Nature* 340:245-6 (1989)). Cohen et al., modified this technology so that novel interactions between synthetic or engineered peptide sequences could be identified which bind a molecule of choice. The benefit of this type of technology is that the selection is done in an intracellular environment. The method utilizes a library of peptide molecules that attached to an acidic activation domain.

Using methodology well known to those of skill in the art, in combination with various combinatorial libraries, one can isolate and characterize those small molecules or macromolecules, which bind to or interact with the desired target. The relative binding affinity of these compounds can be compared and optimum compounds identified using competitive binding studies, which are well known to those of skill in the art.

Techniques for making combinatorial libraries and screening combinatorial libraries to isolate molecules which bind a desired target are well known to those of skill in the art. Representative techniques and methods can be found in but are not limited to United States patents 5,084,824, 5,288,514, 5,449,754, 5,506,337, 5,539,083, 5,545,568, 5,556,762, 5,565,324, 5,565,332, 5,573,905, 5,618,825, 5,619,680, 5,627,210, 5,646,285, 5,663,046, 5,670,326, 5,677,195, 5,683,899, 5,688,696, 5,688,997, 5,698,685, 5,712,146, 5,721,099, 5,723,598, 5,741,713, 5,792,431, 5,807,683, 5,807,754, 5,821,130, 5,831,014, 5,834,195, 5,834,318, 5,834,588, 5,840,500, 5,847,150, 5,856,107, 5,856,496, 5,859,190, 5,864,010, 5,874,443, 5,877,214, 5,880,972, 5,886,126, 5,886,127, 5,891,737, 5,916,899, 5,919,955, 5,925,527, 5,939,268, 5,942,387, 5,945,070, 5,948,696, 5,958,702, 5,958,792, 5,962,337, 5,965,719, 5,972,719, 5,976,894, 5,980,704, 5,985,356, 5,999,086, 6,001,579, 6,004,617, 6,008,321, 6,017,768, 6,025,371, 6,030,917, 6,040,193, 6,045,671, 6,045,755, 6,060,596, and 6,061,636.

Combinatorial libraries can be made from a wide array of molecules using a number of different synthetic techniques. For example, libraries containing fused 2,4-pyrimidinediones (United States patent 6,025,371) dihydrobenzopyrans (United States Patent 6,017,768and 5,821,130), amide alcohols (United States Patent 5,976,894), hydroxy-amino acid amides (United States Patent 5,972,719) carbohydrates (United States patent 5,965,719), 1,4-benzodiazepin-2,5-diones (United States patent 5,962,337), cyclics (United States patent 5,958,792), biaryl amino acid amides (United States patent 5,948,696), thiophenes (United States patent 5,942,387), tricyclic Tetrahydroquinolines (United States patent 5,925,527), benzofurans (United States patent 5,919,955), isoquinolines (United States patent 5,916,899), hydantoin and thiohydantoin (United States patent 5,859,190), indoles (United States patent 5,856,496), imidazol-pyrido-indole and imidazol-pyrido-benzothiophenes (United States patent 5,856,107) substituted 2-methylene-2, 3-dihydrothiazoles (United States patent 5,847,150), quinolines (United States patent 5,840,500), PNA (United States patent 5,831,014), containing tags (United States patent 5,721,099), polyketides (United States patent 5,712,146), morpholino-subunits (United States patent 5,698,685 and 5,506,337), sulfamides (United States patent 5,618,825), and benzodiazepines (United States patent 5,288,514).

Screening molecules similar to the disclosed siRNA molecules for inhibition of PAX2 suppression of DEFB1 expresson is a method of isolating desired compounds.

Molecules isolated which can either be competitive inhibitors or non-competitive inhibitors.

In another embodiment the inhibitors are non-competitive inhibitors. One type of non-competitive inhibitor will cause allosteric rearrangements.

As used herein combinatorial methods and libraries included traditional screening methods and libraries as well as methods and libraries used in iterative processes.

Computer assisted drug design

The disclosed compositions can be used as targets for any molecular modeling technique to identify either the structure of the disclosed compositions or to identify potential or actual molecules, such as small molecules, which interact in a desired way with the disclosed compositions. The nucleic acids, peptides, and related molecules disclosed herein can be used as targets in any molecular modeling program or approach.

It is understood that when using the disclosed compositions in modeling techniques, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation of the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as, SEQ ID NO:1, are also disclosed. Thus, the products produced using the molecular modeling approaches that involve the disclosed compositions, such as, SEQ ID NO:1, are also considered herein disclosed.

Thus, one way to isolate molecules that bind a molecule of choice is through rational design. This is achieved through structural information and computer modeling. Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analyses or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity. Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

Examples of molecular modeling systems are the CHARMm and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, et al., 1988 *Acta Pharmaceutica Fennica* 97, 159-166; Ripka, *New Scientist* 54-57 (June 16, 1988); McKinaly and Rossmann, 1989 *Annu. Rev. Pharmacol. Toxiciol.* 29, 111-122; Perry and Davies, QSAR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236, 125-140 and 141-162; and, with respect to a model enzyme for nucleic acid components, Askew, et al., 1989 *J. Am. Chem. Soc.* 111, 1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, CA., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of molecules specifically interacting with specific regions of DNA or RNA, once that region is identified.

Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which alter substrate binding or enzymatic activity.

Computer readable mediums

It is understood that the disclosed nucleic acids and proteins can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and video disks, or other computer readable mediums. Also disclosed are the binary code

representations of the disclosed sequences. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved.

Disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth herein. Also disclosed are computer readable mediums comprising the sequences and information regarding the sequences set forth.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in C or is at ambient temperature, and pressure is at or near atmospheric.

Example I

Human Beta Defensin-1 is Cytotoxic to Late-Stage Prostate Cancer and Plays a Role in Prostate Cancer Tumor Immunity

Abstract

DEFB1 was cloned into an inducible expression system to examine what effect it had on normal prostate epithelial cells, as well as androgen receptor positive (AR^+) and androgen receptor negative (AR^-) prostate cancer cell lines. Induction of DEFB1 expression resulted in a decrease in cellular growth in AR^- cells DU145 and PC3, but had no effect on

the growth of the AR^+ prostate cancer cells LNCaP. DEFB1 also caused rapid induction of caspase-mediated apoptosis. Data presented here are the first to provide evidence of its role in innate tumor immunity and indicate that its loss contributes to tumor progression in prostate cancer.

Materials and Methods

Cell Lines

The cell lines DU145 were cultured in DMEM medium, PC3 were grown in F12 medium, and LNCaP were grown in RPMI medium (Life Technologies, Inc., Grand Island, NY). Growth media for all three lines was supplemented with 10% (v/v) fetal bovine serum (Life Technologies). The hPrEC cells were cultured in prostate epithelium basal media (Cambrex Bio Science, Inc., Walkersville, MD). All cell lines were maintained at 37°C and 5% CO_2.

Tissue Samples and Laser Capture Microdissection

Prostate tissues obtained from consented patients that underwent radical prostatectomy were acquired through the Hollings Cancer Center tumor bank in accordance with an Institutional Review Board-approved protocol. This included guidelines for the processing, sectioning, histological characterization, RNA purification and PCR amplification of samples. Following pathologic examination of frozen tissue sections, laser capture microdissection (LCM) was performed to ensure that the tissue samples assayed consisted of pure populations of benign prostate cells. For each tissue section analyzed, LCM was performed at three different regions containing benign tissue and the cells collected were then pooled.

Cloning of DEFB1 Gene

DEFB1 cDNA was generated from RNA by reverse transcription-PCR. The PCR primers were designed to contain *ClaI* and *KpnI* restriction sites. DEFB1 PCR products were restriction digested with *ClaI* and *KpnI* and ligated into a TA cloning vector. The TA/DEFB1 vector was then transfected into E. coli by heat shock and individual clones were selected and expanded. Plasmids were isolated by Cell Culture DNA Midiprep (Qiagen, Valencia, CA) and sequence integrity verified by automated sequencing. The DEFB1 gene fragment was then ligated into the pTRE2 digested with *ClaI* and *KpnI*, which

served as an intermediate vector for orientation purposes. Then the pTRE2/DEFB1 construct was digested with *ApaI* and *KpnI* to excise the DEFB1 insert, which was ligated into pIND vector of the Ecdysone Inducible Expression System (Invitrogen, Carlsbad, CA) also double digested with *ApaI* and *KpnI*. The construct was again transfected into E. coli and individual clones were selected and expanded. Plasmids were isolated and sequence integrity of pIND/DEFB1 was again verified by automated sequencing.

Transfection

Cells (1 x 10^6) were seeded onto 100-mm Petri dishes and grown overnight. Then the cells were co-transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with 1μg of pVgRXR plasmid, which expresses the heterodimeric ecdysone receptor, and 1μg of the pIND/DEFB1 vector construct or empty pIND control vector in Opti-MEM media (Life Technologies, Inc., Grand Island, NY).

RNA Isolation and Quantitative RT-PCR

In order to verify DEFB1 protein expression in the cells transfected with DEFB1 construct, RNA was collected after a 24 hour induction period with Ponasterone A (Pon A). Briefly, total RNA was isolated using the SV Total RNA Isolation System (Promega, Madison, WI) from approximately 1 × 10^6 cells harvested by trypsinizing. Here, cells were lysed and total RNA was isolated by centrifugation through spin columns. For cells collected by LCM, total RNA was isolated using the PicoPure RNA Isolation Kit (Arcturus Biosciences, Mt. View, CA) following the manufacturer's protocol. Total RNA (0.5 μg per reaction) from both sources was reverse transcribed into cDNA utilizing random primers (Promega). AMV Reverse Transcriptase II enzyme (500 units per reaction; Promega) was used for first strand synthesis and Tfl DNA Polymerase for second strand synthesis (500 units per reaction; Promega) as per the manufacturer's protocol. In each case, 50 pg of cDNA was used per ensuing PCR reaction. Two-step QRT-PCR was performed on cDNA generated using the MultiScribe Reverse Transcripatase from the TaqMan Reverse Transcription System and the SYBR Green PCR Master Mix (Applied Biosystems).

The primer pair for DEFB1 (Table 3) was generated from the published DEFB1 sequence (GenBank Accession No. U50930)[10]. Forty cycles of PCR were performed under standard conditions using an annealing temperature of 56°C. In addition, β-actin (Table 3) was amplified as a housekeeping gene to normalize the initial content of total cDNA.

DEFB1 expression was calculated as the relative expression ratio between DEFB1 and β-actin and was compared in cells lines induced and uninduced for DEFB1 expression, as well as LCM benign prostatic tissue. As a negative control, QRT-PCR reactions without cDNA template were also performed. All reactions were run three times in triplicate.

MTT Cell Viability Assay

To examine the effects of DEFB1 on cell growth, metabolic 3-[4,5-dimethylthiazol-2yl]-2,5 diphenyl tetrazolium bromide (MTT) assays were performed. PC3, DU145 and LNCaP cells co-transfected with pVgRXR plasmid and pIND/DEFB1 construct or empty pIND vector were seeded onto a 96-well plate at 1-5 x10^3 cells per well. Twenty-four hours after seeding, fresh growth medium was added containing 10 μM Ponasterone A daily to induce DEFB1 expression for 24-, 48- and 72 hours after which the MTT assay was performed according to the manufacturer's instructions (Promega). Reactions were performed three times in triplicate.

Flow Cytometry

PC3 and DU145 cells co-transfected with the DEFB1 expression system were grown in 60-mm dishes and induced for 12, 24, and 48 hours with 10 μM Ponasterone A. Following each incubation period, the medium was collected from the plates (to retain any detached cells) and combined with PBS used to wash the plates. The remaining attached cells were harvested by trypsinization and combined with the detached cells and PBS. The cells were then pelleted at 4°C (500 x g) for 5 min, washed twice in PBS, and resuspended in 100ul of 1x Annexin binding buffer (0.1 M Hepes/NaOH at pH 7.4, 1.4 M NaCl, 25 mM $CaCl_2$) containing 5 μl of Annexin V-FITC and 5 μl of PI. The cells were incubated at RT for 15 min in the dark, then diluted with 400 μl of 1x Annexin binding buffer and analyzed by FACscan (Becton Dickinson, San Jose, CA). All reactions were performed three times.

Microscopic Analysis

Cell morphology was analyzed by phase contrast microscopy. DU145, PC3 and LNCaP cells containing no vector, empty plasmid or DEFB1 plasmid were seeded onto 6 well culture plates (BD Falcon, USA). The following day plasmid-containing cells were induced for a period of 48h with media containing 10 μM Ponasterone A, while control cells received fresh media. The cells were then viewed under an inverted Zeiss IM 35

microscope (Carl Zeiss, Germany). Phase contrast pictures of a field of cells were obtained using the SPOT Insight Mosaic 4.2 camera (Diagnostic Instruments, USA). Cells were examined by phase contrast microscopy under 32X magnification and digital images were stored as uncompressed TIFF files and exported into Photoshop CS software (Adobe Systems, San Jose, CA) for image processing and hard copy presentation.

Caspase Detection

Detection of caspase activity in the prostate cancer cell lines was performed using APO LOGIX^{TM} Carboxyfluorescin Caspase detection kit (Cell Technology, Mountain View, CA). Active caspases were detected through the use of a FAM-VAD-FMK inhibitor that irreversibly binds to active caspases. Briefly, DU145 and PC3 cells (1.5-3 X10^5) containing the DEFB1 expression system were plated in 35 mm glass bottom microwell dishes (Matek, Ashland, MA) and treated for 24 hours with media only or with media containing PonA as previously described. Next, 10 µl of a 30X working dilution of carboxyfluorescein labeled peptide fluoromethyl ketone (FAM-VAD-FMK) was added to 300µl of media and added to each 35 mm dish. Cells were then incubated for 1 hour at 37°C under 5% CO_2. Then, the medium was aspirated and the cells were washed twice with 2 ml of a 1X Working dilution Wash Buffer. Cells were viewed under differential interference contrast (DIC) or under laser excitation at 488nm. The fluorescent signal was analyzed using a confocal microscope (Zeiss LSM 5 Pascal) and a 63X DIC oil lens with a Vario 2 RGB Laser Scanning Module.

Statistical Analysis

Statistical differences were evaluated using the Student's t-test for unpaired values. P values were determined by a two-sided calculation, and a P value of less than 0.05 was considered statistically significant.

Results

DEFB1 Expression in Prostate Tissue and Cell Lines

DEFB1 expression levels were measured by QRT-PCR in benign and malignant prostatic tissue, hPrEC prostate epithelial cells and DU145, PC3 and LNCaP prostate cancer

cells. DEFB1 expression was detected in all of the benign clinical samples. The average amount of DEFB1 relative expression was 0.0073. In addition, DEFB1 relative expression in hPrEC cells was 0.0089. There was no statistical difference in DEFB1 expression detected in the benign prostatic tissue samples and hPrEC (Figure 1A). Analysis of the relative DEFB1 expression levels in the prostate cancer cell lines revealed significantly lower levels in DU145, PC3 and LNCaP. As a further point of reference, relative DEFB1 expression was measured in the adjacent malignant section of prostatic tissue from patient #1215. There were no significant differences in the level of DEFB1 expression observed in the three prostate cancer lines compared to malignant prostatic tissue from patient #1215 (Figure 1B). In addition, expression levels in all four samples were close to the no template negative controls which confirmed little to no endogenous DEFB1 expression (data not shown). QRT-PCR was also performed on the prostate cancer cell lines transfected with the DEFB1 expression system. Following a 24 hour induction period, relative expression levels were 0.01360 in DU145, 0.01503 in PC3 and 0.138 in LNCaP. Amplification products were verified by gel electrophoresis.

QRT-PCR was performed on LCM tissues regions containing benign, PIN and cancer. DEFB1 relative expression was 0.0146 in the benign region compared to 0.0009 in the malignant region (Figure 1C.). This represents a 94% decrease which again demonstrates a significant down-regulation of expression. Furthermore, analysis of PIN revealed that DEFB1 expression level was 0.044 which was a 70% decrease. Comparing expression in patient #1457 to the average expression level found in benign regions of six other patients (Figure 1 A.) revealed a ratio of 1.997 representing almost twice as much expression (Figure 1D.). However, the expression ratio was 0.0595 in PIN and was 0.125 in malignant tissue compared to average expression levels in benign tissue.

DEFB1 Causes Cell Membrane Permeability and Ruffling

Induction of DEFB1 in the prostate cancer cell lines resulted in a significant reduction in cell number in DU145 and PC3, but had no effect on cell proliferation in LNCaP (Figure 2). As a negative control, cell proliferation was monitored in all three lines containing empty plasmid. There were no observable changes in cell morphology in DU145, PC3 or LNCaP cells following the addition of PonA. In addition, DEFB1 induction resulted in morphological changes in both DU145 and PC3. Here cells appeared

more rounded and exhibited membrane ruffling indicative of cell death. Apoptotic bodies were also present in both lines.

Expression of DEFB1 Results in Decreased Cell Viability

The MTT assay showed a reduction in cell viability by DEFB1 in PC3 and DU145 cells, but no significant effect on LNCaP cells (Figure 3). After 24 hours, relative cell viability was 72% in DU145 and 56% in PC3. Analysis 48 hours after induction revealed 49% cell viability in DU145 and 37% cell viability in PC3. After 72 hours of DEFB1 expression resulted in 44% and 29% relative cell viability in DU145 and PC3 cells, respectively.

DEFB1 Causes Rapid Caspase-mediated Apoptosis in Late-stage Prostate Cancer Cells

In order to determine whether the effects of DEFB1 on PC3 and DU145 were cytostatic or cytotoxic, FACS analysis was performed. Under normal growth conditions, more than 90% of PC3 and DU145 cultures were viable and non-apoptotic (lower left quadrant) and did not stain with annexin V or PI (Figure 4). After inducing DEFB1 expression in PC3 cells, the number of apoptotic cells (lower and upper right quadrants) totaled 10% at 12 hours, 20% at 24 hours, and 44% at 48 hours. For DU145 cells, the number of apoptotic cells totaled 12% after 12 hours, 34% at 24 hours, and 59% after 48 hours of induction. There was no increase in apoptosis observed in cells containing empty plasmid following induction with PonA (data not shown).

Caspase activity was determined by confocal laser microscopic analysis (Figure 5). DU145 and PC3 cell were induced for DEFB1 expression and activity was monitored based on the binding of green fluoresing FAM-VAD-FMK to caspases in cells actively undergoing apoptosis. Analysis of cells under DIC showed the presence of viable control DU145 (A), PC3 (E) and LNCaP (I) cells at 0 hours. Excitation by the confocal laser at 488 nm produced no detectable green staining which indicates no caspase activity in DU145 (B), PC3 (F) or LNCaP (J). Following induction for 24 hours, DU145 (C), PC3 (G) and LNCaP (K) cells were again visible under DIC. Confocal analysis under fluorescence revealed green staining in DU145 (D) and PC3 (H) cell indicating caspase activity. However, there was no green staining in LNCaP (L), indicating no induction of apoptosis by DEFB1.

siRNA Silencing of PAX2

In order to achieve efficient gene silencing, a pool of four complementary short interfering ribonucleotides (siRNAs) targeting human PAX2 mRNA (Accession No. NM_003989.1), were synthesized (Dharmacon Research, Lafayette, CO, USA). A second pool of four siRNAs were used as an internal control to test for the specificity of PAX2 siRNAs. Two of the sequences synthesized target the GL2 luciferase mRNA (Accession No. X65324), and two were non-sequence-specific (Table 4). For annealing of siRNAs, 35 M of single strands were incubated in annealing buffer (100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2 mM magnesium acetate) for 1 min at 90°C followed by 1 h incubation at 37°C.

Western Analysis

Briefly, cells were harvested by trypsinization and washed twice with PBS. Lysis buffer was prepared according to the manufacturer's instructions (Sigma), and was then added to the cells. Following a 15 minute incubation period at 4°C on an orbital shaker, cell lysate were then collected and centrifuged for 10 minutes at 12000xg to pellet cellular debris. The protein-containing supernatant were then collected and quantitated. Next, 25 µg protein extract was loaded onto an 8-16% gradient SDS-PAGE (Novex). Following electrophoresis, proteins were transferred to PVDF membranes, and then blocked with 5% nonfat dry milk in TTBS (0.05% Tween 20 and 100mM Tris-Cl) for 1 hour. Blots were then probed with rabbit anti-Pax2 primary antibody (Zymed, San Francisco, CA) at a 1:2000 dilution. After washing, the membranes were incubated with anti-rabbit antibody conjugated to horseradish peroxidase (HRP) (dilution 1:5000; Sigma), and signal detection was visualized using chemilluminescence reagents (Pierce) on an Alpha Innotech Fluorchem 8900. As a control, blots were stripped and reprobed with mouse anti-β-actin primary antibody (1:5000; Sigma-Aldrich) and HRP- conjugated anti-mouse secondary antibody (1:5000; Sigma-Aldrich) and signal detection was again visualized.

Phase Contrast Microscopy

The effect of PAX2 knock-down on cell growth was analyzed by phase contrast microscopy. Here, 1-2 x10^4 cells were seeded onto 6 well culture plates (BD Falcon, USA). The following day cells were treated with media only, negative control non-specific siRNA or PAX2 siRNA and allowed to incubate for six days. The cells were then viewed under an inverted Zeiss IM 35 microscope (Carl Zeiss, Germany) at 32x magnification. Phase contrast pictures of a field of cells were obtained using the SPOT Insight Mosaic 4.2 camera (Diagnostic Instruments, USA).

MTT Cytotoxicity Assay

DU145, PC3 and LNCaP cells (1x10^5) were transfected with 0.5µg of the PAX2 siRNA pool or control siRNA pool using Codebreaker transfection reagent according to the manufacturer's protocol (Promega). Next, cell suspensions were diluted and seeded onto a 96-well plate at 1-5 x10^3 cells per well and allowed to grow for 2-, 4- or 6 days. After culture, cell viability was determined by measuring the conversion of 3-[4,5-dimethylthiazol-2yl]-2,5 diphenyl tetrazolium bromide, MTT (Promega), to a colored formazan product. Absorbance was read at 540 nm on a scanning multiwell spectrophotometer.

Pan-Caspase Detection

Detection of caspase activity in the prostate cancer cell lines was performed using APO LOGIX^{TM} Carboxyfluorescin Caspase detection kit (Cell Technology, Mountain View, CA). Active caspases were detected through the use of a FAM-VAD-FMK inhibitor that irreversibly binds to active caspases. Briefly, cells (1-2 X10^4) onto 35 mm glass bottom microwell dishes (Matek, Ashland, MA) and treated with media only or PAX2 siRNA as previously described. Next, 10 µl of a 30X working dilution of carboxyfluorescein labeled peptide fluoromethyl ketone (FAM-VAD-FMK) was added to 300µl of media and added to each 35 mm dish. Cells were then incubated for 1 hour at 37°C under 5% CO_2. Then, the medium was aspirated and the cells were washed twice with 2 ml of a 1X Working dilution Wash Buffer. Cells were viewed under differential interference contrast (DIC) or under laser excitation at 488nm. The fluorescent signal was

analyzed using a confocal microscope (Zeiss LSM 5 Pascal) and a 63X DIC oil lens with a Vario 2 RGB Laser Scanning Module.

Quantitative Real-time RT-PCR

Quantitative real-time RT-PCR was performed in order to verify gene expression after PAX2 siRNA treatment in PC3, DU145 and LNCaP cell lines. Total RNA was isolated using the SV Total RNA Isolation System (Promega). Briefly, approximately 1 × 10^6 cells were harvested by trypsinizing, and rinsed in PBS. Cells were then lysed and total RNA was isolated by centrifugation through spin columns. Total RNA (0.5 µg per reaction) was reverse transcribed into cDNA utilizing Oligo (dT) 15 primer (Promega) and AMV Reverse Transcriptase II enzyme (500 units per reaction; Promega) for first strand synthesis and Tfl DNA Polymerase for for second strand synthesis (500 units per reaction; Promega) as per the manufacturers' protocol, with identical control samples treated without RT enzyme. Typically, 50 pg of each cDNA was used per ensuing PCR reactionTwo-step QRT-PCR was performed on cDNA generated using the MultiScribe Reverse Transcripatase from the TaqMan Reverse Transcription System and the SYBR Green PCR Master Mix (PE Biosystems). The primer pairs for BAX, BID and BAD were generated from the published sequences (Table3). Reactions were performed in MicroAmp Optical 96-well Reaction Plate (PE Biosystems). Forty cycles of PCR were performed under standard conditions using an annealing temperature of 60°C. Quantification was determined by the cycle number where exponential amplification began (threshold value) and averaged from the values obtained from the triplicate repeats. There was an inverse relationship between message level and threshold value. In addition, GAPDH was used as a housekeeping gene to normalize the initial content of total cDNA. Gene expression was calculated as the relative expression ratio between the pro-apoptotic genes and GAPDH. All reactions were carried out in triplicate.

Results

siRNA Inhibition of PAX2 Protein

In order to confirm that the siRNA effective targeted the PAX2 mRNA, Western Analysis was performed to monitor PAX2 protein expression levels over a six day treatment period. Cells were given a single round of transfection with the pool of PAX2 siRNA. The

results confirmed specific targeting of PAX2 mRNA by showing knock-down of PAX2 protein by day four in DU145 (Figure 6a) and by day six in PC3 (Figure 6b).

Knock-down of PAX2 inhibit Prostate Cancer Cell Growth

Cells were analyzed following a six day treatment period with media only, negative control non-specific siRNA or PAX2 siRNA (Figure 7). DU145 (a), PC3 (d) and LNCaP (g) cells all reached at least 90% confluency in the culture dishes containing media only. Treatment of DU145 (b), PC3 (e) and LNCaP (h) with negative control non-specific siRNA had no effect on cell growth, and cells again reached confluency after six days. However, treatment with PAX2 siRNA resulted in a significant decrease in cell number. DU145 cells were approximately 15% confluent (c) and PC3 cells were only 10% confluent (f). LNCaP cell were 5% confluent following siRNA treatment.

Cytotoxicity Assays

Cell viability was measured after two-, four-, and six-day exposure times, and is expressed as a ratio of the 570-630 nm absorbance of treated cells divided by that of the untreated control cells (Figure 8). Relative cell viability following 2 days of treatment was 77% in LNCaP, 82% in DU145 and 78 % in PC3. After four days, relative cell viability was 46% in LNCaP, 53% in DU145 and 63% in PC3. After six days of treatment, relative cell viability decreased to 31% in LNCaP, 37% in PC3, and was 53% in DU145. As negative controls, cell viability was measured in after a six day treatment period with negative control non-specific siRNA or transfection reagent alone. For both conditions, there was no statistically significant change in cell viability compared to normal growth media (data not shown).

Pan-Caspase Detection

Caspase activity was detected by confocal laser microscopic analysis. DU145, PC3 and LNCaP cells were treated with PAX2 siRNA and activity was monitored based on the binding of FAM-labeled peptide to caspases in cells actively undergoing apoptosis which will fluoresce green. Analysis of cells with media only under DIC shows the presence of viable DU145 (A), PC3 (E) and LNCaP (I) cells at 0 hours (Figure 9). Excitation by the confocal laser at 488 nm produced no detectable green staining which indicates no caspase activity in untreated DU145 (B), PC3 (F) or LNCaP (J). Following four days of treatment

with PAX2 siRNA, DU145 (C), PC3 (G) and LNCaP (K) cells were again visible under DIC. Under fluorescence, the treated DU145 (D), PC3 (H) and LNCaP (L) cells presented green staining indicating caspase activity.

Effect of Pax2 Inhibition on Pro-apoptotic Factors

DU145, PC3 and LNCaP cells were treated with siRNA against PAX2 for six days and expression of pro-apoptotic genes dependent and independent of p53 transcription regulation were measured to monitor cell death pathways. For BAX, there was a 1.81-fold increase in LNCaP, a 2.73-fold increase in DU145, and a 1.87-fold increase in PC3 (Figure 10a). Expression levels of BID increased by 1.38-fold in LNCaP and 1.77-fold in DU145 (Figure 10b). However, BID expression levels decreased by 1.44-fold in PC3 following treatment (Figure 10c). Analysis of BAD revealed a 2.0-fold increase in expression in LNCaP, a 1.38-fold increase in DU145, and a 1.58-fold increase in PC3.

Conclusion

Despite significant advances in cancer therapy there is still little progress in the treatment of advanced disease. Successful drug treatment of prostate cancer requires the use of therapeutics with specific effects on target cells while maintaining minimal clinical effects on the host. The goal of cancer therapy is to trigger tumor-selective cell death. Therefore, understanding the mechanisms in such death is critical in determining the efficacy of a specific treatment.

The dependency of prostate cancer cell survival on PAX2 expression is shown here. In order to distinguish between death observed in the p53-expressing cell line LNCaP, the p53-mutated line DU145, and the p53-null line PC3 downstream events that follow p53 activation as a result of PAX2 knock-down were examined. Caspase activity was detected in all three lines indicative of the initiation of programmed cell death. With this, changes in the expression of pro-apoptotic genes were examined. Here, BAX expression was upregulated in all three cell lines independent of p53 status. The expression of pro-apoptotic factor BAD was increased in all three lines following PAX2 inhibition. Following treatment with PAX2 siRNA, BID expression was increased in LNCaP and DU145, but actually decreased in PC3. This indicates that cell death observed in prostate cancer is influenced by but is not dependent on p53 expression. The initiation of apoptosis

in prostate cancer cells through different cell death pathways irrespective of p53 status indicates that PAX2 inhibits other tumor suppressors

Example III

Inhibition of PAX2 Oncogene Results in DEFB1-Mediated Death of Prostate Cancer Cells

Abstract

The identification of tumor-specific molecules that serve as targets for the development of new cancer drugs is considered to be a major goal in cancer research. Example I demonstrated that there is a high frequency of DEFB1 expression loss in prostate cancer, and that induction of DEFB1 expression results in rapid apoptosis in androgen receptor negative-stage prostate cancer. These data show that DEFB1 plays a role in prostate tumor suppression. In addition, given that it is a naturally occurring component of the immune system of normal prostate epithelium, DEFB1 is expected to be a viable therapeutic agent with little to no side effects. Example II demonstrated that inhibition of PAX2 expression results in prostate cancer cell death independent of p53. These data indicate that there is an addition pro-apoptotic factor or tumor suppressor that is inhibited by PAX2. In addition, the data show that the oncogenic factor PAX2, which is over-expressed in prostate cancer, is a transcriptional repressor of DEFB1. The purpose of this study is to determine if DEFB1 loss of expression is due to aberrant expression of the PAX2 oncogene, and whether inhibiting PAX2 results in DEFB1-mediated cell death.

The data show that loss of DEFB1 expression occurs at the transcriptional level. Furthermore, computational analysis of the DEFB1 promoter revealed the presence of a ***GTTCC*** DNA binding site for the PAX2 transcriptional repressor next to the DEFB1 TATA box (Figure 1). The results presented here show that PAX2 and DEFB1 exhibit several attributes of suitable cancer targets, including a role in the suppression of cell death. Therefore, DEFB1 plays a role in tumor immunity and its expression is modulated through therapeutic down-regulation of the PAX2 oncogene.

Materials and Methods

RNA Isolation and Quantitative RT-PCR

In order to verify changes in DEFB1 expression levels RNA was collected after 4 days of PAX2 siRNA treatment. Briefly, total RNA was isolated using the SV Total RNA Isolation System (Promega, Madison, WI) from approximately 1×10^6 cells harvested by trypsinizing. Here, cells were lysed and total RNA was isolated by centrifugation through spin columns. Total RNA (0.5 µg per reaction) from both sources was reverse transcribed into cDNA utilizing random primers (Promega). AMV Reverse Transcriptase II enzyme (500 units per reaction; Promega) was used for first strand synthesis and Tfl DNA Polymerase for second strand synthesis (500 units per reaction; Promega) as per the manufacturer's protocol. In each case, 50 pg of cDNA was used per ensuing PCR reaction. Two-step QRT-PCR was performed on cDNA generated using the MultiScribe Reverse Transcripatase from the TaqMan Reverse Transcription System and the SYBR Green PCR Master Mix (Applied Biosystems).

The primer pair for DEFB1 was generated from the published DEFB1 sequence (Accession No. U50930). Forty cycles of PCR were performed under standard conditions using an annealing temperature of 56°C. In addition, GAPDH was amplified as a housekeeping gene to normalize the initial content of total cDNA. DEFB1 expression was calculated as the relative expression ratio between DEFB1 and GAPDH and was compared in cells lines before and after siRNA knock-down of PAX2 expression. All reactions were run three times in triplicate.

Generation of the DEFB1 Reporter Construct

The pGL3 luciferase reporter plasmid was used to monitor DEFB1 reporter activity. Here, a region 160 bases upstream of the DEFB1 transcription initiation site and included the DEFB1 TATA box. The region also included the GTTCC sequence which is necessary for PAX2 binding. The PCR primers were designed to contain *Kpn1* and *Nhe1* restriction sites. The DEFB1 promoter PCR products were restriction digested *Kpn I* and *NheI* and ligated into a similary restriction digested pGL3 plasmid (Figure 2). The constructs were transfected into E. coli and individual clones were selected and expanded. Plasmids were isolated and sequence integrity of the DEFB1/pGL3 construct was verified by automated sequencing.

Luciferase Reporter Assay

Here, 1μg of the DEFB1 reporter construct or the control pGL3 plasmid was transfected into $1x10^6$ DU145 cells. Next, $0.5x10^3$ cells were seeded onto each well of a 96-well plate and allowed to grow overnight. Then fresh medium was added containing PAX2 siRNA or media only and the cells were incubated for 48 hours. Luciferase was detected by the BrightGlo kit accourding to the manufacturer's protocol (Promega) and the plates were read on a Veritas automated 96-well luminometer. Promoter activity was expressed as relative luminescence.

Analysis of Membrane Permeability

Acridine orange (AO)/ethidium bromide (EtBr) dual staining was performed to identify changes in cell membrane integrity, as well as apoptotic cells by staining the condensed chromatin. AO stains viable cells as well as early apoptotic cells, whereas EtBr stains late stage apoptotic cells that have lost membrane permeability. Briefly, cells were seeded into 2 chamber culture slides (BD Falcon, USA). Cells transfected with empty pIND plasmid/pvgRXR or pIND DEFB1/pvgRXR were induced for 24 or 48 h with media containing 10 μM Ponasterone A. Control cells were provided fresh media at 24 and 48h. In order to determine the effect of PAX2 inhibition on membrane integrity, separate culture slides containing DU145, PC3 and LNCaP were treated with PAX2 siRNA and incubated for 4 days. Following this, cells were washed once with PBS and stained with 2 ml of a mixture (1:1) of AO (Sigma, USA) and EtBr (Promega, USA) (5ug/ml) solution for 5 min. Following staining, the cells were again washed with PBS. Fluorescence was viewed by a Zeiss LSM 5 Pascal Vario 2 Laser Scanning Confocal Microscope (Carl Zeiss Jena, Germany). The excitation color wheel contain BS505-530 (green) and LP560 (red) filter blocks which allowed for the separation of emitted green light from AO into the green channel and red light from EtBr into the red channel. The laser power output and gain control settings within each individual experiment were identical between control and DEFB1 induced cells. The excitation was provided by a Kr/Ar mixed gas laser at wavelengths of 543nm for AO and 488 nm for EtBr. Slides were analyzed under 40X magnification and digital images were stored as uncompressed TIFF files and exported into Photoshop CS software (Adobe Systems, San Jose, CA) for image processing and hard copy presentation.

ChIP Analysis of PAX2

Chromatin immunoprecipitation (ChIP) allows the identification of binding sites for DNA-binding proteins based upon *in vivo* occupancy of a promoter by a transcription factor and enrichment of transcription factor bound chromatin by immunoprecipitation (66). A modification of the protocol described by the Farnham laboratory ((67, 68) was used; also on line at http://mcardle.oncology.wisc.edu/farnham/). The DU145 and PC3 cell lines over-expresses the PAX2 protein, but does not express DEFB1. Cells were incubated with PBS containing 1.0% formaldehyde for 10 minutes to crosslink proteins to DNA. Samples were then sonicated to yield DNA with an average length of 600 bp. Sonicated chromatin precleared with Protein A Dynabeads was incubated with PAX2-specific antibody or "no antibody" control [isotype-matched control antibodies]. Washed immunoprecipitates were then collected. After reversal of the crosslinks, DNA was analyzed by PCR using promoter-specific primers to determine whether DEFB1 is represented in the PAX2-immunoprecipitated samples. Primers were designed to amplify the 160 bp region immediately upstream of the DEFB1 mRNA start site which contained the DEFB1 TATA box and the functional ***GTTCC*** PAX2 recognition site. For these studies, positive controls included PCR of an aliquot of the input chromatin (prior to immunoprecipitation, but crosslinks reversed). All steps were performed in the presence of protease inhibitors.

Results

siRNA Inhibition of PAX2 Increases DEFB1 Expression

QRT-PCR analysis of DEFB1 expression before siRNA treatment revealed relative expression levels of 0.00097 in DU145, 0.00001 in PC3, and .00004 LNCaP (Figure 13). Following siRNA knock-down of PAX2, relative expression was .03294 (338-fold increase) in DU145, .00020 (22.2-fold increase) in PC3 and 0.00019 (4.92-fold increase) in LNCaP. As a negative control, the human prostate epithelial cell line (hPrEC) which is PAX2 null, revealed expression levels at 0.00687 before treatment and 0.00661 following siRNA treatment confirming no statistical change in DEFB1 expression.

DEFB1 Causes Cell Membrane Permeability

Membrane integrity was monitored by confocal analysis (Figure 14). Here, intact cells stain green due to AO which is membrane permeable. In addition, cells with compromised plasma membranes would stain red by EtBr which is membrane impermeable.

Here, uninduced DU145 (A) and PC3 (D) cells stained positively with AO and emitted green color, but did not stain with EtBr. However, DEFB1 induction in both DU145 (B) and PC3 (E) resulted in the accumulation of EtBr in the cytoplasm at 24 hours indicated by the red staining. By 48 hours, DU145 (C) and PC3 (F) possessed condensed nuclei and appeared yellow, which was due to the presence of both green and red staining resulting from the accumulation of AO and EtBr, respectively.

Inhibition of PAX2 Results in Membrane Permeability

Cells were treated with PAX2 siRNA for 4 days and membrane integrity was monitored again by confocal analysis (Figure 15). Here, both DU145 (B) and PC3 (E) possessed condensed nuclei and appeared yellow. However, LNCaP cells' cytoplasm and nuclei remained green following siRNA treament. Also red staining at the cell periphery indicates the maintenance of cell membrane integrity. These findings indicate that the inhibition of PAX2 results in specifically DEFB1-mediated cell death in DU1145 and PC3, but not LNCaP cells. Death observed in LNCaP (refer to Chapter II) is due to the transactivation of the existing wild-type p53 in LNCap following PAX2 inhibition.

siRNA Inhibition of PAX2 Increases DEFB1 Promoter Activity

Analysis of DEFB1 promoter activity in DU145 cells containing the DEFB1/pGL3 construct revealed a 2.65 fold increase in relative light units following 48 hours of treatment compared to untreated cells (Figure 16). In PC3 cells, there was a 3.78-fold increase in relative light units compared to untreated cells.

PAX2 Binds to the DEFB1 Promoter

ChIP analysis was performed on DU145 and PC3 cells to determine if the PAX2 transcriptional repressor is bound to the DEFB1 promoter (Figure 17). Lane 1 contains a 100 bp molecular weight marker. Lane 2 is a positive control representing 160 bp region of the DEFB1 promoter amplified from DU145 before cross-linking and immunoprecipitation. Lane 3 is a negative control representing PCR performed without DNA. Lane 4 and 5 are negative controls representing PCR from immunoprecipitations performed with IgG from cross-linked DU145 and PC3, respectively. PCR amplification of 25pg of DNA (lane 6 and 8) and 50pg of DNA (lane 7 and 9) immunoprecitipated with anti-PAX2 antibody after crosslinking show 160 bp promoter fragment in DU145 and PC3, respectively.

Conclusion

The present novel data are the first to disclose the role of DEFB1 in prostate cancer tumor immunity. The data also show that the oncogenic factor PAX2 suppresses DEFB1 expression. One of the hallmarks of defensin cytotoxicity is the disruption of membrane integrity. The present results show that ectopic expression of DEFB1 in prostate cancer cells results in a loss of membrane potential due to compromised cell membranes. The same phenomenon is observed after inhibiting PAX2 protein expression. ChIP analysis was also performed and confirmed that PAX2 is bound to the DEFB1 promoter resulting in the repression of DEFB1 expression. Therefore, suppression of PAX2 expression or function, results in the re-establishment of DEFB1 expression and subsequently DEFB1-mediated cell death. Also, the present data establish the utility of DEFB1 as a directed therapy for prostate cancer treatment through innate immunity.

Example IV

Expression of DEFB1 Results in Tumor Shrinkage

The anti-tumoral ability of DEFB1 is evaluated by injecting tumor cells that overexpress DEFB1 into nude mice. DEFB1 is cloned into pBI-EGFP vector, which has a bidirectional tetracycline responsible promoter. Tet-Off Cell lines are generated by transfecting pTet-Off into DU145, PC3 and LNCaP cells and selecting with G418. The pBI-EGFP-DEFB1 plasmid is co-transfected with pTK-Hyg into the Tet-off cell lines and selected with hygromycin. Only single-cell suspensions with a viability of >90% are used. Each animal receives approximately 500,000 cells administered subcutaneously into the right flank of female nude mice. There are two groups, a control group injected with vector only clones and a group injected with the DEFB1 over-expressing clones. 35 mice are in each group as determined by a statistician. Animals are weighed twice weekly, tumor growth monitored by calipers and tumor volumes determined using the following formula: volume = 0.5 x (width)2 x length. All animals are sacrificed by CO_2 overdose when tumor size reaches 2 mm^3 or 6 months following implantation; tumors are excised, weighed and stored in neutral buffered formalin for pathological examination. Differences in tumor growth between the groups are descriptively characterized through summary statistics and

graphical displays. Statistical significance is evaluated with either the t-test or non-parametric equivalent.

Example V

Expression of PAX2 siRNA Results in Up-Regulation of DEFB1 Expression and Tumor Shrinkage In Vivo

Hairpin PAX2 siRNA template oligonucleotides utilized in the in vitro studies are utilized to examine the effect of the up-regulation of DEFB1 expression in vivo. The sense and antisense strand (see Table 4) are annealed and cloned into pSilencer 2.1 U6 hygro siRNA expression vector (Ambion) under the control of the human U6 RNA pol III promoter. The cloned plasmid is sequenced, verified and transfected into PC3, Du145, and LNCap cell lines. Scrambled shRNA is cloned and used as a negative control in this study. Hygromycin resistant colonies are selected, cells are introduced into the mice subcutaneously and tumor growth is monitored as described above.

Example VI

Small Molecule Inhibitors of PAX2 Binding Results in Up-Regulation of DEFB1 Expression and Tumor Shrinkage In Vivo

The DNA recognition sequence for PAX2 binding resides in the DEFB1 promoter between nucleotides -75 and -71 [+1 refers to the transcriptional start site]. Short oligonucleotides complementary to the PAX2 DNA–binding domain are provided. Examples of such oligonucleotides include the 20-mer and 40-mer oligonucleotides containing the GTTCC recognition sequence provided below. These lengths were randomly selected, and other lengths are expected to be effective as blockers of binding. As a negative control, oligonicleotides with a scrambled sequence (CTCTG) were designed to verify specificity. The oligonucleotides are transfected into the prostate cancer cells and the HPrEC cells with lipofectamine reagent or Codebreaker transfection reagent (Promega, Inc). In order to confirm DNA-protein interactions, double stranded oligonucleotides will be labeled with [32P] dCTP and electrophoretic mobility shift assays are performed. In

addition, DEFB1 expression is monitored by QRT-PCR and Western analysis following treatment with oligonucleotides. Finally, cell death is detected by MTT assay and flow cytometry as previously described.

Recognition Sequence #1: CTCCCTTCA**GTTCC**GTCGAC (SEQ ID NO:9)

Recognition Sequence #2: CTCCCTTCA**CCTTG**GTCGAC (SEQ ID NO:10)

Scramble Sequence #1: CTCCCTTCA**CTCTG**GTCGAC (SEQ ID NO:11)

Recognition Sequence #3:

ACTGTGGCACCTCCCTTCA**GTTCC**GTCGACGAGGTTGTGC (SEQ ID NO:12)

Recognition Sequence #4:

ACTGTGGCACCTCCCTTCA**CCTTG**GTCGACGAGGTTGTGC (SEQ ID NO:13)

Scramble Sequence #2:

ACTGTGGCACCTCCCTTCA**CTCTG**GTCGACGAGGTTGTGC (SEQ ID NO:14)

Further examples of oligonucleotides of the invention include:

Recognition Sequence #1: 5'-AGAAGTTCAC**CCTTG**ACTGT-3' (SEQ ID No:x)

Recognition Sequence #2: 5'-AGAAGTTCAC**GTTCC**ACTGT-3' (SEQ ID No:x)

Scramble Sequence #1: 5'-AGAAGTTCAC**GCTCT**ACTGT-3' (SEQ ID No:x)

Recognition Sequence #3:

5'-TTAGCGATTAGAAGTTCAC**CCTTG**ACTGTGGCACCTCCC-3' (SEQ ID No:x)

Recognition Sequence #4:

5'-GTTAGCGATTAGAAGTTCAC**GTTCC**ACTGTGGCACCTCCC-3' (SEQ ID No:x)

Scramble Sequence #2:

5'-GTTAGCGATTAGAAGTTCAC**GCTCT**ACTGTGGCACCTCCC-3' (SEQ ID No:x)

This set of alternative inhibitory oligonucleotides represents the recognition sequence (along with the CCTTG core sequence) for the PAX2 binding domain and homeobox. These include actual sequences from the DEFB1 promoter.

The PAX2 gene is required for the growth and survival of various cancer cells including prostate. In addition, the inhibition of PAX2 expression results in cell death mediated by the innate immunity component DEFB1. Suppression of DEFB1 expression and activity is accomplished by binding of the PAX2 protein to a GTTCC recognition site in the DEFB1 promoter. Therefore, this pathway provides a viable therapeutic target for the treatment of prostate cancer. In this method, the sequences bind to the PAX2 DNA binding site and block PAX2 binding to the DEFB1 promoter thus allowing DEFB1 expression and

activity. The oligonucleotide sequences and experiment described above are examples of and demonstrate a model for the design of additional PAX2 inhibitor drugs.

Given that the GTTCC sequence exists in interleukin-3, interleukin-4, the insulin receptor and others, PAX2 regulates their expression and activity as well. Therefore the PAX2 inhibitors disclosed herein have utility in a number of other diseases including those directed related to inflammation including prostatitis and benign prostatic hypertrophy (BPH).

Example VII

Loss of DEFB1 Expression Results in Increased Tumorigenesis

Generation of Loss of Function Mice

The Cre/loxP system has been useful in elucidating the molecular mechanisms underlying prostate carcinogenesis. Here a DEFB1 Cre conditional KO is used for inducible disruption within the prostate. The DEFB1 Cre conditional KO involves the generation of a targeting vector containing loxP sites flanking DEFB1 coding exons, targeted ES cells with this vector and the generation of germline chimeric mice from these targeted ES cells. Heterozygotes are mated to prostate-specific Cre transgenics and heterozygous intercross is used to generate prostate-specific DEFB1 KO mice. Four genotoxic chemical compounds have been found to induce prostate carcinomas in rodents: N-methyl-N-nitrosourea (MNU), N-nitrosobis 2-oxopropyl. amine (BOP), 3,2X-dimethyl-4-amino-biphenyl (MAB) and 2-amino-1-methyl-6-phenylimidazow 4,5-bxpyridine (PhIP). DEFB1-transgenic mice are treated with these carcinogenic compounds via intra-gastric administration or i.v. injection for prostate adenoma and adenocarcinoma induction studies. Prostate samples are studied for differences in tumor growth and changes gene expression though histological, immunohistological, mRNA and protein analyses.

Generation of GOF mice

For PAX2 inducible GOF mice, PAX2 GOF (bi-transgenic) and wild-type (mono-transgenic) littermates are administered doxycycline (Dox) from 5 weeks of age to induce prostate-specific PAX2 expression. Briefly, PROBASIN-rtTA mono-transgenic mice (prostate cell-specific expression of tet-dependent rtTA inducer) are crossed to our PAX2 transgenic responder lines. For induction, bi-transgenic mice are fed Dox via the drinking water (500 mg/L freshly prepared twice a week). Initial experiments verify low background

levels, good inducibility and cell-type specific expression of PAX2 and the EGFP reporter using transgenic founder line in bi-transgenic mice. Regarding experimental group sizes, 5-7 age- and sex-matched individuals in each group (wild-type and GOF) allow for statistical significance. For all animals in this study, prostate tissues are collected initially at weekly intervals for analysis and comparison, to determine carcinogenic time parameters.

PCR Genotyping, RT-PCR and qPCR

PROBASIN-rtTA transgenic mice are genotyped using the following PCR primers and conditions: PROBASIN5 (forward) 5'-ACTGCCCATTGCCCAAACAC-3'; RTTA3 (reverse) 5'-AAAATCTTGCCAGCTTTCCCC-3'; 95°C denaturation for 5 min, followed by 30cycles of 95°C for 30 sec, 57°C for 30 sec, 72°C for 30 sec, followed by a 5 min extension at 72°C, yielding a 600 bp product. PAX2 inducible transgenic mice are genotyped using the following PCR primers and conditions: PAX2For 5'-GTCGGTTACGGAGCGGACCGGAG-3'; Rev5'IRES 5'-TAACATATAGACAAACGCACACCG-3'; 95°C denaturation for 5 min, followed by 34cycles of 95°C for 30 sec, 63°C for 30 sec, 72°C for 30 sec, followed by a 5 min extension at 72°C, yielding a 460 bp product. Immortomouse hemizygotes are be genotyped using the following PCR primers and conditions: Immol1, 5'-GCGCTTGTGTC GCCATTGTATTC-3'; Immol2, 5'-GTCACACCACAGAAGTAAGGTTCC-3'; 94°C 30 sec, 58°C 1 min, 72°C 1min 30 sec, 30 cycles to yield a ~1kb transgene band. For genotyping PAX2 knockout mice, the following PCR primers and conditions are used: PAX2 For 5'-GTCGGTTACGGAGCGGACCGGAG-3'; PAX2Rev 5'-CACAGAGCATTGGCGATCTCGATGC-3'; 94°C 1 min, 65°C 1 min, 72°C 30 sec, 36 cycles to yield a 280 bp band.

DEFB1 Peptide Animal Studies

Six-week-old male athymic (nude) mice purchased from Charles River Laboratories are injected sub-cutaneously over the scapula with 10^6 viable PC3 cells. One week after injection, the animals are randomly allocated to one of three groups –group I: control; group II: intraperitoneal injections of DEFB1, 100 μg/day, 5 days a week, for weeks 2-14; group III: intraperitoneal injections of DEFB1, 100 mg/day, 5 days a week, for weeks 8-14. Animals are maintained in sterile housing, four animals to a cage, and observed on a daily

basis. At 10-day intervals, the tumors are measured by using calipers, and the volumes of the tumors are calculated by using $V = (L \times W2)/2$.

Appendix A

LOCUS HUMPAX201 7331 bp DNA linear PRI 28-JAN-2002
DEFINITION Homo sapiens paired-box protein (PAX2) gene, promoter and exon 1.
ACCESSION L09748 AF433639
VERSION L09748.2 GI:18141563
KEYWORDS .
SEGMENT 1 of 3
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 7096 to 7291)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Chromosomal localization of seven PAX genes and cloning of a novel
family member, PAX-9
JOURNAL Nat. Genet. 3 (4), 292-298 (1993)
PUBMED 7981748
REFERENCE 2 (bases 1 to 7331)
AUTHORS Pfeffer,P.L., Payer,B., Reim,G., di Magliano,M.P. and Busslinger,M.
TITLE The activation and maintenance of Pax2 expression at the
mid-hindbrain boundary is controlled by separate enhancers
JOURNAL Development 129 (2), 307-318 (2002)
PUBMED 11807024
REFERENCE 3 (bases 7096 to 7291)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Direct Submission
JOURNAL Submitted (12-JUN-1993) Research Institute of Molecular Pathology,
Dr. Bohr-Gasse 7, Vienna, Austria
REFERENCE 4 (bases 1 to 7331)
AUTHORS Pfeffer,P.L. and Busslinger,M.
TITLE Direct Submission
JOURNAL Submitted (14-JAN-2002) Research Institute of Molecular Pathology,
Dr. Bohr-Gasse 7, Vienna, Austria
REMARK Sequence update by submitter
COMMENT On Jan 14, 2002 this sequence version replaced gi:292378.
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Appendix B

LOCUS NM_003987 4276 bp mRNA linear PRI 24-SEP-2005
DEFINITION Homo sapiens paired box gene 2 (PAX2), transcript variant a, mRNA.
ACCESSION NM_003987
VERSION NM_003987.2 GI:34878698
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 1 to 4276)
AUTHORS Yoshimura,K., Yoshida,S., Yamaji,Y., Komori,A., Yoshida,A.,
Hatae,K., Kubota,T. and Ishibashi,T.
TITLE De novo insG619 mutation in PAX2 gene in a Japanese patient with
papillorenal syndrome
JOURNAL Am. J. Ophthalmol. 139 (4), 733-735 (2005)
PUBMED 15808183
REMARK GeneRIF: Molecular genetic analysis of the PAX2 gene in combination
with renal ultrasonography can help in making an earlier diagnosis
of the disease.
REFERENCE 2 (bases 1 to 4276)
AUTHORS Mazal,P.R., Stichenwirth,M., Koller,A., Blach,S., Haitel,A. and
Susani,M.
TITLE Expression of aquaporins and PAX-2 compared to CD10 and cytokeratin
7 in renal neoplasms: a tissue microarray study
JOURNAL Mod. Pathol. 18 (4), 535-540 (2005)
PUBMED 15502805
REMARK GeneRIF: PAX-2 is a reliable marker for clear cell renal cell
carcinomas of lower grades but not for higher grades.
REFERENCE 3 (bases 1 to 4276)
AUTHORS Higashide,T., Wada,T., Sakurai,M., Yokoyama,H. and Sugiyama,K.
TITLE Macular abnormalities and optic disk anomaly associated with a new
PAX2 missense mutation
JOURNAL Am. J. Ophthalmol. 139 (1), 203-205 (2005)
PUBMED 15652857
REMARK GeneRIF: A new PAX2 missense mutation, R71T, may cause macular
abnormalities in addition to anomalies of the optic disk and the
kidney.
REFERENCE 4 (bases 1 to 4276)
AUTHORS Buttiglieri,S., Deregibus,M.C., Bravo,S., Cassoni,P., Chiarle,R.,
Bussolati,B. and Camussi,G.
TITLE Role of Pax2 in apoptosis resistance and proinvasive phenotype of
Kaposi's sarcoma cells

JOURNAL J. Biol. Chem. 279 (6), 4136-4143 (2004)
PUBMED 14627715
REMARK GeneRIF: expression of Pax2 by Kaposi's sarcoma cells correlated with an enhanced resistance against apoptotic signals and with the proinvasive phenotype
REFERENCE 5 (bases 1 to 4276)
AUTHORS Brophy,P.D., Lang,K.M. and Dressler,G.R.
TITLE The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor
JOURNAL J. Biol. Chem. 278 (52), 52401-52405 (2003)
PUBMED 14561758
REMARK GeneRIF: Pax2 protein regulates expression of secreted frizzled related protein 2
REFERENCE 6 (bases 1 to 4276)
AUTHORS Schimmenti,L.A., Manligas,G.S. and Sieving,P.A.
TITLE Optic nerve dysplasia and renal insufficiency in a family with a novel PAX2 mutation, Arg115X: further ophthalmologic delineation of the renal-coloboma syndrome
JOURNAL Ophthalmic Genet. 24 (4), 191-202 (2003)
PUBMED 14566649
REMARK GeneRIF: PAX2 mutation is associated with Optic nerve dysplasia and renal insufficiency of the renal-coloboma syndrome
REFERENCE 7 (bases 1 to 4276)
AUTHORS Muratovska,A., Zhou,C., He,S., Goodyer,P. and Eccles,M.R.
TITLE Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival
JOURNAL Oncogene 22 (39), 7989-7997 (2003)
PUBMED 12970747
REMARK GeneRIF: The PAX2 gene was frequently expressed in a panel of 406 common primary tumor tissues and endogenous PAX gene expression is often required for the growth and survival of cancer cells
REFERENCE 8 (bases 1 to 4276)
AUTHORS Gough,S.M., McDonald,M., Chen,X.N., Korenberg,J.R., Neri,A., Kahn,T., Eccles,M.R. and Morris,C.M.
TITLE Refined physical map of the human PAX2/HOX11/NFKB2 cancer gene region at 10q24 and relocalization of the HPV6AI1 viral integration site to 14q13.3-q21.1
JOURNAL BMC Genomics 4 (1), 9 (2003)
PUBMED 12697057
REFERENCE 9 (bases 1 to 4276)
AUTHORS Hoffmeister,A., Ropolo,A., Vasseur,S., Mallo,G.V., Bodeker,H., Ritz-Laser,B., Dressler,G.R., Vaccaro,M.I., Dagorn,J.C., Moreno,S. and Iovanna,J.L.
TITLE The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter
JOURNAL J. Biol. Chem. 277 (25), 22314-22319 (2002)
PUBMED 11940591

REMARK GeneRIF: The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter.
REFERENCE 10 (bases 1 to 4276)
AUTHORS Cai,Y., Lechner,M.S., Nihalani,D., Prindle,M.J., Holzman,L.B. and Dressler,G.R.
TITLE Phosphorylation of Pax2 by the c-Jun N-terminal kinase and enhanced Pax2-dependent transcription activation
JOURNAL J. Biol. Chem. 277 (2), 1217-1222 (2002)
PUBMED 11700324
REFERENCE 11 (bases 1 to 4276)
AUTHORS Becker,K., Beales,P.L., Calver,D.M., Matthijs,G. and Mohammed,S.N.
TITLE Okihiro syndrome and acro-renal-ocular syndrome: clinical overlap, expansion of the phenotype, and absence of PAX2 mutations in two new families
JOURNAL J. Med. Genet. 39 (1), 68-71 (2002)
PUBMED 11826030
REMARK GeneRIF: The absence of PAX2 mutations has been identified in two families with histories of clinical overlap of Okihiro and acro-renal-ocular syndromes.
REFERENCE 12 (bases 1 to 4276)
AUTHORS Eccles,M.R., He,S., Legge,M., Kumar,R., Fox,J., Zhou,C., French,M. and Tsai,R.W.
TITLE PAX genes in development and disease: the role of PAX2 in urogenital tract development
JOURNAL Int. J. Dev. Biol. 46 (4), 535-544 (2002)
PUBMED 12141441
REMARK Review article
GeneRIF: PAX2 has a role in urogenital tract development and disease [review]
REFERENCE 13 (bases 1 to 4276)
AUTHORS Chung,G.W., Edwards,A.O., Schimmenti,L.A., Manligas,G.S., Zhang,Y.H. and Ritter,R. III.
TITLE Renal-coloboma syndrome: report of a novel PAX2 gene mutation
JOURNAL Am. J. Ophthalmol. 132 (6), 910-914 (2001)
PUBMED 11730657
REMARK GeneRIF: The causal relationship between PAX2 gene mutations and renal-coloboma syndrome is further supported
REFERENCE 14 (bases 1 to 4276)
AUTHORS Nishimoto,K., Iijima,K., Shirakawa,T., Kitagawa,K., Satomura,K., Nakamura,H. and Yoshikawa,N.
TITLE PAX2 gene mutation in a family with isolated renal hypoplasia
JOURNAL J. Am. Soc. Nephrol. 12 (8), 1769-1772 (2001)
PUBMED 11461952
REFERENCE 15 (bases 1 to 4276)
AUTHORS Ritz-Laser,B., Estreicher,A., Gauthier,B. and Philippe,J.
TITLE The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene

promoter
JOURNAL J. Biol. Chem. 275 (42), 32708-32715 (2000)
PUBMED 10938089
REFERENCE 16 (bases 1 to 4276)
AUTHORS Lechner,M.S., Levitan,I. and Dressler,G.R.
TITLE PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin
JOURNAL Nucleic Acids Res. 28 (14), 2741-2751 (2000)
PUBMED 10908331
REFERENCE 17 (bases 1 to 4276)
AUTHORS Tavassoli,K., Ruger,W. and Horst,J.
TITLE Alternative splicing in PAX2 generates a new reading frame and an extended conserved coding region at the carboxy terminus
JOURNAL Hum. Genet. 101 (3), 371-375 (1997)
PUBMED 9439670
REFERENCE 18 (bases 1 to 4276)
AUTHORS Dahl,E., Koseki,H. and Balling,R.
TITLE Pax genes and organogenesis
JOURNAL Bioessays 19 (9), 755-765 (1997)
PUBMED 9297966
REMARK Review article
REFERENCE 19 (bases 1 to 4276)
AUTHORS Schimmenti,L.A., Cunliffe,H.E., McNoe,L.A., Ward,T.A., French,M.C., Shim,H.H., Zhang,Y.H., Proesmans,W., Leys,A., Byerly,K.A., Braddock,S.R., Masuno,M., Imaizumi,K., Devriendt,K. and Eccles,M.R.
TITLE Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations
JOURNAL Am. J. Hum. Genet. 60 (4), 869-878 (1997)
PUBMED 9106533
REFERENCE 20 (bases 1 to 4276)
AUTHORS Narahara,K., Baker,E., Ito,S., Yokoyama,Y., Yu,S., Hewitt,D., Sutherland,G.R., Eccles,M.R. and Richards,R.I.
TITLE Localisation of a 10q breakpoint within the PAX2 gene in a patient with a de novo t(10;13) translocation and optic nerve coloboma-renal disease
JOURNAL J. Med. Genet. 34 (3), 213-216 (1997)
PUBMED 9132492
REFERENCE 21 (bases 1 to 4276)
AUTHORS Dehbi,M., Ghahremani,M., Lechner,M., Dressler,G. and Pelletier,J.
TITLE The paired-box transcription factor, PAX2, positively modulates expression of the Wilms' tumor suppressor gene (WT1)
JOURNAL Oncogene 13 (3), 447-453 (1996)
PUBMED 8760285
REFERENCE 22 (bases 1 to 4276)
AUTHORS Sanyanusin,P., Norrish,J.H., Ward,T.A., Nebel,A., McNoe,L.A. and Eccles,M.R.
TITLE Genomic structure of the human PAX2 gene
JOURNAL Genomics 35 (1), 258-261 (1996)
PUBMED 8661132

REFERENCE 23 (bases 1 to 4276)
AUTHORS Sanyanusin,P., Schimmenti,L.A., McNoe,L.A., Ward,T.A., Pierpont,M.E., Sullivan,M.J., Dobyns,W.B. and Eccles,M.R.
TITLE Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux
JOURNAL Nat. Genet. 9 (4), 358-364 (1995)
PUBMED 7795640
REFERENCE 24 (bases 1 to 4276)
AUTHORS Ward,T.A., Nebel,A., Reeve,A.E. and Eccles,M.R.
TITLE Alternative messenger RNA forms and open reading frames within an additional conserved region of the human PAX-2 gene
JOURNAL Cell Growth Differ. 5 (9), 1015-1021 (1994)
PUBMED 7819127
REFERENCE 25 (bases 1 to 4276)
AUTHORS Noll,M.
TITLE Evolution and role of Pax genes
JOURNAL Curr. Opin. Genet. Dev. 3 (4), 595-605 (1993)
PUBMED 8241771
REMARK Review article
REFERENCE 26 (bases 1 to 4276)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Chromosomal localization of seven PAX genes and cloning of a novel family member, PAX-9
JOURNAL Nat. Genet. 3 (4), 292-298 (1993)
PUBMED 7981748
REFERENCE 27 (bases 1 to 4276)
AUTHORS Pilz,A.J., Povey,S., Gruss,P. and Abbott,C.M.
TITLE Mapping of the human homologs of the murine paired-box-containing genes
JOURNAL Mamm. Genome 4 (2), 78-82 (1993)
PUBMED 8431641
REFERENCE 28 (bases 1 to 4276)
AUTHORS Eccles,M.R., Wallis,L.J., Fidler,A.E., Spurr,N.K., Goodfellow,P.J. and Reeve,A.E.
TITLE Expression of the PAX2 gene in human fetal kidney and Wilms' tumor
JOURNAL Cell Growth Differ. 3 (5), 279-289 (1992)
PUBMED 1378753
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U45255.1 and BM671839.1.
On Sep 22, 2003 this sequence version replaced gi:4557822.

Summary: PAX2 encodes paired box gene 2, one of many human homologues of the Drosophila melanogaster gene prd. The central feature of this transcription factor gene family is the conserved DNA-binding paired box domain. PAX2 is believed to be a target of transcriptional supression by the tumor supressor gene WT1. Mutations within PAX2 have been shown to result in optic nerve colobomas and renal hypoplasia. Alternative splicing of this gene results in multiple transcript variants.

Transcript Variant: This variant (a) uses an alternate in-frame splice site in the 3' coding region, compared to variant e, resulting in a shorter protein (isoform a) that has a shorter, distinct C-terminus compared to isoform e.
COMPLETENESS: complete on the 3' end.

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go_process: GTP biosynthesis [goid 0006183] [evidence IEA];
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Appendix C

LOCUS NM_000278 4207 bp mRNA linear PRI 24-SEP-2005
DEFINITION Homo sapiens paired box gene 2 (PAX2), transcript variant b, mRNA.
ACCESSION NM_000278
VERSION NM_000278.2 GI:34878700
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 1 to 4207)
AUTHORS Yoshimura,K., Yoshida,S., Yamaji,Y., Komori,A., Yoshida,A.,
Hatae,K., Kubota,T. and Ishibashi,T.
TITLE De novo insG619 mutation in PAX2 gene in a Japanese patient with
papillorenal syndrome
JOURNAL Am. J. Ophthalmol. 139 (4), 733-735 (2005)
PUBMED 15808183
REMARK GeneRIF: Molecular genetic analysis of the PAX2 gene in combination
with renal ultrasonography can help in making an earlier diagnosis
of the disease.
REFERENCE 2 (bases 1 to 4207)
AUTHORS Mazal,P.R., Stichenwirth,M., Koller,A., Blach,S., Haitel,A. and
Susani,M.
TITLE Expression of aquaporins and PAX-2 compared to CD10 and cytokeratin
7 in renal neoplasms: a tissue microarray study
JOURNAL Mod. Pathol. 18 (4), 535-540 (2005)
PUBMED 15502805
REMARK GeneRIF: PAX-2 is a reliable marker for clear cell renal cell
carcinomas of lower grades but not for higher grades.
REFERENCE 3 (bases 1 to 4207)
AUTHORS Higashide,T., Wada,T., Sakurai,M., Yokoyama,H. and Sugiyama,K.
TITLE Macular abnormalities and optic disk anomaly associated with a new
PAX2 missense mutation
JOURNAL Am. J. Ophthalmol. 139 (1), 203-205 (2005)
PUBMED 15652857
REMARK GeneRIF: A new PAX2 missense mutation, R71T, may cause macular
abnormalities in addition to anomalies of the optic disk and the
kidney.
REFERENCE 4 (bases 1 to 4207)
AUTHORS Buttiglieri,S., Deregibus,M.C., Bravo,S., Cassoni,P., Chiarle,R.,
Bussolati,B. and Camussi,G.
TITLE Role of Pax2 in apoptosis resistance and proinvasive phenotype of
Kaposi's sarcoma cells
JOURNAL J. Biol. Chem. 279 (6), 4136-4143 (2004)
PUBMED 14627715
REMARK GeneRIF: expression of Pax2 by Kaposi's sarcoma cells correlated

with an enhanced resistance against apoptotic signals and with the proinvasive phenotype
REFERENCE 5 (bases 1 to 4207)
AUTHORS Brophy,P.D., Lang,K.M. and Dressler,G.R.
TITLE The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor
JOURNAL J. Biol. Chem. 278 (52), 52401-52405 (2003)
PUBMED 14561758
REMARK GeneRIF: Pax2 protein regulates expression of secreted frizzled related protein 2
REFERENCE 6 (bases 1 to 4207)
AUTHORS Schimmenti,L.A., Manligas,G.S. and Sieving,P.A.
TITLE Optic nerve dysplasia and renal insufficiency in a family with a novel PAX2 mutation, Arg115X: further ophthalmologic delineation of the renal-coloboma syndrome
JOURNAL Ophthalmic Genet. 24 (4), 191-202 (2003)
PUBMED 14566649
REMARK GeneRIF: PAX2 mutation is associated with Optic nerve dysplasia and renal insufficiency of the renal-coloboma syndrome
REFERENCE 7 (bases 1 to 4207)
AUTHORS Muratovska,A., Zhou,C., He,S., Goodyer,P. and Eccles,M.R.
TITLE Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival
JOURNAL Oncogene 22 (39), 7989-7997 (2003)
PUBMED 12970747
REMARK GeneRIF: The PAX2 gene was frequently expressed in a panel of 406 common primary tumor tissues and endogenous PAX gene expression is often required for the growth and survival of cancer cells
REFERENCE 8 (bases 1 to 4207)
AUTHORS Gough,S.M., McDonald,M., Chen,X.N., Korenberg,J.R., Neri,A., Kahn,T., Eccles,M.R. and Morris,C.M.
TITLE Refined physical map of the human PAX2/HOX11/NFKB2 cancer gene region at 10q24 and relocalization of the HPV6AI1 viral integration site to 14q13.3-q21.1
JOURNAL BMC Genomics 4 (1), 9 (2003)
PUBMED 12697057
REFERENCE 9 (bases 1 to 4207)
AUTHORS Hoffmeister,A., Ropolo,A., Vasseur,S., Mallo,G.V., Bodeker,H., Ritz-Laser,B., Dressler,G.R., Vaccaro,M.I., Dagorn,J.C., Moreno,S. and Iovanna,J.L.
TITLE The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter
JOURNAL J. Biol. Chem. 277 (25), 22314-22319 (2002)
PUBMED 11940591
REMARK GeneRIF: The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription

factors on the glucagon gene promoter.
REFERENCE 10 (bases 1 to 4207)
AUTHORS Cai,Y., Lechner,M.S., Nihalani,D., Prindle,M.J., Holzman,L.B. and Dressler,G.R.
TITLE Phosphorylation of Pax2 by the c-Jun N-terminal kinase and enhanced Pax2-dependent transcription activation
JOURNAL J. Biol. Chem. 277 (2), 1217-1222 (2002)
PUBMED 11700324
REFERENCE 11 (bases 1 to 4207)
AUTHORS Becker,K., Beales,P.L., Calver,D.M., Matthijs,G. and Mohammed,S.N.
TITLE Okihiro syndrome and acro-renal-ocular syndrome: clinical overlap, expansion of the phenotype, and absence of PAX2 mutations in two new families
JOURNAL J. Med. Genet. 39 (1), 68-71 (2002)
PUBMED 11826030
REMARK GeneRIF: The absence of PAX2 mutations has been identified in two families with histories of clinical overlap of Okihiro and acro-renal-ocular syndromes.
REFERENCE 12 (bases 1 to 4207)
AUTHORS Eccles,M.R., He,S., Legge,M., Kumar,R., Fox,J., Zhou,C., French,M. and Tsai,R.W.
TITLE PAX genes in development and disease: the role of PAX2 in urogenital tract development
JOURNAL Int. J. Dev. Biol. 46 (4), 535-544 (2002)
PUBMED 12141441
REMARK Review article
GeneRIF: PAX2 has a role in urogenital tract development and disease [review]
REFERENCE 13 (bases 1 to 4207)
AUTHORS Chung,G.W., Edwards,A.O., Schimmenti,L.A., Manligas,G.S., Zhang,Y.H. and Ritter,R. III.
TITLE Renal-coloboma syndrome: report of a novel PAX2 gene mutation
JOURNAL Am. J. Ophthalmol. 132 (6), 910-914 (2001)
PUBMED 11730657
REMARK GeneRIF: The causal relationship between PAX2 gene mutations and renal-coloboma syndrome is further supported
REFERENCE 14 (bases 1 to 4207)
AUTHORS Nishimoto,K., Iijima,K., Shirakawa,T., Kitagawa,K., Satomura,K., Nakamura,H. and Yoshikawa,N.
TITLE PAX2 gene mutation in a family with isolated renal hypoplasia
JOURNAL J. Am. Soc. Nephrol. 12 (8), 1769-1772 (2001)
PUBMED 11461952
REFERENCE 15 (bases 1 to 4207)
AUTHORS Ritz-Laser,B., Estreicher,A., Gauthier,B. and Philippe,J.
TITLE The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter
JOURNAL J. Biol. Chem. 275 (42), 32708-32715 (2000)
PUBMED 10938089

REFERENCE 16 (bases 1 to 4207)
AUTHORS Lechner,M.S., Levitan,I. and Dressler,G.R.
TITLE PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin
JOURNAL Nucleic Acids Res. 28 (14), 2741-2751 (2000)
PUBMED 10908331
REFERENCE 17 (bases 1 to 4207)
AUTHORS Tavassoli,K., Ruger,W. and Horst,J.
TITLE Alternative splicing in PAX2 generates a new reading frame and an extended conserved coding region at the carboxy terminus
JOURNAL Hum. Genet. 101 (3), 371-375 (1997)
PUBMED 9439670
REFERENCE 18 (bases 1 to 4207)
AUTHORS Dahl,E., Koseki,H. and Balling,R.
TITLE Pax genes and organogenesis
JOURNAL Bioessays 19 (9), 755-765 (1997)
PUBMED 9297966
REMARK Review article
REFERENCE 19 (bases 1 to 4207)
AUTHORS Schimmenti,L.A., Cunliffe,H.E., McNoe,L.A., Ward,T.A., French,M.C., Shim,H.H., Zhang,Y.H., Proesmans,W., Leys,A., Byerly,K.A., Braddock,S.R., Masuno,M., Imaizumi,K., Devriendt,K. and Eccles,M.R.
TITLE Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations
JOURNAL Am. J. Hum. Genet. 60 (4), 869-878 (1997)
PUBMED 9106533
REFERENCE 20 (bases 1 to 4207)
AUTHORS Narahara,K., Baker,E., Ito,S., Yokoyama,Y., Yu,S., Hewitt,D., Sutherland,G.R., Eccles,M.R. and Richards,R.I.
TITLE Localisation of a 10q breakpoint within the PAX2 gene in a patient with a de novo t(10;13) translocation and optic nerve coloboma-renal disease
JOURNAL J. Med. Genet. 34 (3), 213-216 (1997)
PUBMED 9132492
REFERENCE 21 (bases 1 to 4207)
AUTHORS Dehbi,M., Ghahremani,M., Lechner,M., Dressler,G. and Pelletier,J.
TITLE The paired-box transcription factor, PAX2, positively modulates expression of the Wilms' tumor suppressor gene (WT1)
JOURNAL Oncogene 13 (3), 447-453 (1996)
PUBMED 8760285
REFERENCE 22 (bases 1 to 4207)
AUTHORS Sanyanusin,P., Norrish,J.H., Ward,T.A., Nebel,A., McNoe,L.A. and Eccles,M.R.
TITLE Genomic structure of the human PAX2 gene
JOURNAL Genomics 35 (1), 258-261 (1996)
PUBMED 8661132
REFERENCE 23 (bases 1 to 4207)
AUTHORS Sanyanusin,P., Schimmenti,L.A., McNoe,L.A., Ward,T.A., Pierpont,M.E., Sullivan,M.J., Dobyns,W.B. and Eccles,M.R.

TITLE Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux
JOURNAL Nat. Genet. 9 (4), 358-364 (1995)
PUBMED 7795640
REFERENCE 24 (bases 1 to 4207)
AUTHORS Ward,T.A., Nebel,A., Reeve,A.E. and Eccles,M.R.
TITLE Alternative messenger RNA forms and open reading frames within an additional conserved region of the human PAX-2 gene
JOURNAL Cell Growth Differ. 5 (9), 1015-1021 (1994)
PUBMED 7819127
REFERENCE 25 (bases 1 to 4207)
AUTHORS Noll,M.
TITLE Evolution and role of Pax genes
JOURNAL Curr. Opin. Genet. Dev. 3 (4), 595-605 (1993)
PUBMED 8241771
REMARK Review article
REFERENCE 26 (bases 1 to 4207)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Chromosomal localization of seven PAX genes and cloning of a novel family member, PAX-9
JOURNAL Nat. Genet. 3 (4), 292-298 (1993)
PUBMED 7981748
REFERENCE 27 (bases 1 to 4207)
AUTHORS Pilz,A.J., Povey,S., Gruss,P. and Abbott,C.M.
TITLE Mapping of the human homologs of the murine paired-box-containing genes
JOURNAL Mamm. Genome 4 (2), 78-82 (1993)
PUBMED 8431641
REFERENCE 28 (bases 1 to 4207)
AUTHORS Eccles,M.R., Wallis,L.J., Fidler,A.E., Spurr,N.K., Goodfellow,P.J. and Reeve,A.E.
TITLE Expression of the PAX2 gene in human fetal kidney and Wilms' tumor
JOURNAL Cell Growth Differ. 3 (5), 279-289 (1992)
PUBMED 1378753
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U45255.1 and BM671839.1.
On Sep 22, 2003 this sequence version replaced gi:4557820.

Summary: PAX2 encodes paired box gene 2, one of many human homologues of the Drosophila melanogaster gene prd. The central feature of this transcription factor gene family is the conserved DNA-binding paired box domain. PAX2 is believed to be a target of transcriptional supression by the tumor supressor gene WT1. Mutations within PAX2 have been shown to result in optic nerve colobomas and renal hypoplasia. Alternative splicing of this gene results in multiple transcript variants.

Transcript Variant: This variant (b) lacks an alternate in-frame exon and uses an alternate splice site in the 3' coding region,

compared to variant e. This results in a protein (isoform b) with a shorter, distinct C-terminus compared to isoform e.
COMPLETENESS: complete on the 3' end.

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go_process: transcription [goid 0006350] [evidence IEA];
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go_process: UTP biosynthesis [goid 0006228] [evidence IEA];
go_process: axonogenesis [goid 0007409] [evidence TAS] [pmid 9106533];
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Appendix D

LOCUS NM_003988 4290 bp mRNA linear PRI 24-SEP-2005
DEFINITION Homo sapiens paired box gene 2 (PAX2), transcript variant c, mRNA.
ACCESSION NM_003988
VERSION NM_003988.2 GI:34878708
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 1 to 4290)
AUTHORS Yoshimura,K., Yoshida,S., Yamaji,Y., Komori,A., Yoshida,A.,
Hatae,K., Kubota,T. and Ishibashi,T.
TITLE De novo insG619 mutation in PAX2 gene in a Japanese patient with
papillorenal syndrome
JOURNAL Am. J. Ophthalmol. 139 (4), 733-735 (2005)
PUBMED 15808183
REMARK GeneRIF: Molecular genetic analysis of the PAX2 gene in combination
with renal ultrasonography can help in making an earlier diagnosis
of the disease.
REFERENCE 2 (bases 1 to 4290)
AUTHORS Mazal,P.R., Stichenwirth,M., Koller,A., Blach,S., Haitel,A. and
Susani,M.
TITLE Expression of aquaporins and PAX-2 compared to CD10 and cytokeratin
7 in renal neoplasms: a tissue microarray study
JOURNAL Mod. Pathol. 18 (4), 535-540 (2005)
PUBMED 15502805
REMARK GeneRIF: PAX-2 is a reliable marker for clear cell renal cell
carcinomas of lower grades but not for higher grades.
REFERENCE 3 (bases 1 to 4290)
AUTHORS Higashide,T., Wada,T., Sakurai,M., Yokoyama,H. and Sugiyama,K.
TITLE Macular abnormalities and optic disk anomaly associated with a new
PAX2 missense mutation
JOURNAL Am. J. Ophthalmol. 139 (1), 203-205 (2005)
PUBMED 15652857
REMARK GeneRIF: A new PAX2 missense mutation, R71T, may cause macular
abnormalities in addition to anomalies of the optic disk and the
kidney.
REFERENCE 4 (bases 1 to 4290)
AUTHORS Buttiglieri,S., Deregibus,M.C., Bravo,S., Cassoni,P., Chiarle,R.,
Bussolati,B. and Camussi,G.
TITLE Role of Pax2 in apoptosis resistance and proinvasive phenotype of
Kaposi's sarcoma cells
JOURNAL J. Biol. Chem. 279 (6), 4136-4143 (2004)
PUBMED 14627715
REMARK GeneRIF: expression of Pax2 by Kaposi's sarcoma cells correlated

with an enhanced resistance against apoptotic signals and with the proinvasive phenotype

REFERENCE 5 (bases 1 to 4290)
AUTHORS Brophy,P.D., Lang,K.M. and Dressler,G.R.
TITLE The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor
JOURNAL J. Biol. Chem. 278 (52), 52401-52405 (2003)
PUBMED 14561758
REMARK GeneRIF: Pax2 protein regulates expression of secreted frizzled related protein 2

REFERENCE 6 (bases 1 to 4290)
AUTHORS Schimmenti,L.A., Manligas,G.S. and Sieving,P.A.
TITLE Optic nerve dysplasia and renal insufficiency in a family with a novel PAX2 mutation, Arg115X: further ophthalmologic delineation of the renal-coloboma syndrome
JOURNAL Ophthalmic Genet. 24 (4), 191-202 (2003)
PUBMED 14566649
REMARK GeneRIF: PAX2 mutation is associated with Optic nerve dysplasia and renal insufficiency of the renal-coloboma syndrome

REFERENCE 7 (bases 1 to 4290)
AUTHORS Muratovska,A., Zhou,C., He,S., Goodyer,P. and Eccles,M.R.
TITLE Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival
JOURNAL Oncogene 22 (39), 7989-7997 (2003)
PUBMED 12970747
REMARK GeneRIF: The PAX2 gene was frequently expressed in a panel of 406 common primary tumor tissues and endogenous PAX gene expression is often required for the growth and survival of cancer cells

REFERENCE 8 (bases 1 to 4290)
AUTHORS Gough,S.M., McDonald,M., Chen,X.N., Korenberg,J.R., Neri,A., Kahn,T., Eccles,M.R. and Morris,C.M.
TITLE Refined physical map of the human PAX2/HOX11/NFKB2 cancer gene region at 10q24 and relocalization of the HPV6AI1 viral integration site to 14q13.3-q21.1
JOURNAL BMC Genomics 4 (1), 9 (2003)
PUBMED 12697057

REFERENCE 9 (bases 1 to 4290)
AUTHORS Hoffmeister,A., Ropolo,A., Vasseur,S., Mallo,G.V., Bodeker,H., Ritz-Laser,B., Dressler,G.R., Vaccaro,M.I., Dagorn,J.C., Moreno,S. and Iovanna,J.L.
TITLE The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter
JOURNAL J. Biol. Chem. 277 (25), 22314-22319 (2002)
PUBMED 11940591
REMARK GeneRIF: The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription

factors on the glucagon gene promoter.
REFERENCE 10 (bases 1 to 4290)
AUTHORS Cai,Y., Lechner,M.S., Nihalani,D., Prindle,M.J., Holzman,L.B. and Dressler,G.R.
TITLE Phosphorylation of Pax2 by the c-Jun N-terminal kinase and enhanced Pax2-dependent transcription activation
JOURNAL J. Biol. Chem. 277 (2), 1217-1222 (2002)
PUBMED 11700324
REFERENCE 11 (bases 1 to 4290)
AUTHORS Becker,K., Beales,P.L., Calver,D.M., Matthijs,G. and Mohammed,S.N.
TITLE Okihiro syndrome and acro-renal-ocular syndrome: clinical overlap, expansion of the phenotype, and absence of PAX2 mutations in two new families
JOURNAL J. Med. Genet. 39 (1), 68-71 (2002)
PUBMED 11826030
REMARK GeneRIF: The absence of PAX2 mutations has been identified in two families with histories of clinical overlap of Okihiro and acro-renal-ocular syndromes.
REFERENCE 12 (bases 1 to 4290)
AUTHORS Eccles,M.R., He,S., Legge,M., Kumar,R., Fox,J., Zhou,C., French,M. and Tsai,R.W.
TITLE PAX genes in development and disease: the role of PAX2 in urogenital tract development
JOURNAL Int. J. Dev. Biol. 46 (4), 535-544 (2002)
PUBMED 12141441
REMARK Review article
GeneRIF: PAX2 has a role in urogenital tract development and disease [review]
REFERENCE 13 (bases 1 to 4290)
AUTHORS Chung,G.W., Edwards,A.O., Schimmenti,L.A., Manligas,G.S., Zhang,Y.H. and Ritter,R. III.
TITLE Renal-coloboma syndrome: report of a novel PAX2 gene mutation
JOURNAL Am. J. Ophthalmol. 132 (6), 910-914 (2001)
PUBMED 11730657
REMARK GeneRIF: The causal relationship between PAX2 gene mutations and renal-coloboma syndrome is further supported
REFERENCE 14 (bases 1 to 4290)
AUTHORS Nishimoto,K., Iijima,K., Shirakawa,T., Kitagawa,K., Satomura,K., Nakamura,H. and Yoshikawa,N.
TITLE PAX2 gene mutation in a family with isolated renal hypoplasia
JOURNAL J. Am. Soc. Nephrol. 12 (8), 1769-1772 (2001)
PUBMED 11461952
REFERENCE 15 (bases 1 to 4290)
AUTHORS Ritz-Laser,B., Estreicher,A., Gauthier,B. and Philippe,J.
TITLE The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter
JOURNAL J. Biol. Chem. 275 (42), 32708-32715 (2000)
PUBMED 10938089

REFERENCE 16 (bases 1 to 4290)
AUTHORS Lechner,M.S., Levitan,I. and Dressler,G.R.
TITLE PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin
JOURNAL Nucleic Acids Res. 28 (14), 2741-2751 (2000)
PUBMED 10908331
REFERENCE 17 (bases 1 to 4290)
AUTHORS Tavassoli,K., Ruger,W. and Horst,J.
TITLE Alternative splicing in PAX2 generates a new reading frame and an extended conserved coding region at the carboxy terminus
JOURNAL Hum. Genet. 101 (3), 371-375 (1997)
PUBMED 9439670
REFERENCE 18 (bases 1 to 4290)
AUTHORS Dahl,E., Koseki,H. and Balling,R.
TITLE Pax genes and organogenesis
JOURNAL Bioessays 19 (9), 755-765 (1997)
PUBMED 9297966
REMARK Review article
REFERENCE 19 (bases 1 to 4290)
AUTHORS Schimmenti,L.A., Cunliffe,H.E., McNoe,L.A., Ward,T.A., French,M.C., Shim,H.H., Zhang,Y.H., Proesmans,W., Leys,A., Byerly,K.A., Braddock,S.R., Masuno,M., Imaizumi,K., Devriendt,K. and Eccles,M.R.
TITLE Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations
JOURNAL Am. J. Hum. Genet. 60 (4), 869-878 (1997)
PUBMED 9106533
REFERENCE 20 (bases 1 to 4290)
AUTHORS Narahara,K., Baker,E., Ito,S., Yokoyama,Y., Yu,S., Hewitt,D., Sutherland,G.R., Eccles,M.R. and Richards,R.I.
TITLE Localisation of a 10q breakpoint within the PAX2 gene in a patient with a de novo t(10;13) translocation and optic nerve coloboma-renal disease
JOURNAL J. Med. Genet. 34 (3), 213-216 (1997)
PUBMED 9132492
REFERENCE 21 (bases 1 to 4290)
AUTHORS Dehbi,M., Ghahremani,M., Lechner,M., Dressler,G. and Pelletier,J.
TITLE The paired-box transcription factor, PAX2, positively modulates expression of the Wilms' tumor suppressor gene (WT1)
JOURNAL Oncogene 13 (3), 447-453 (1996)
PUBMED 8760285
REFERENCE 22 (bases 1 to 4290)
AUTHORS Sanyanusin,P., Norrish,J.H., Ward,T.A., Nebel,A., McNoe,L.A. and Eccles,M.R.
TITLE Genomic structure of the human PAX2 gene
JOURNAL Genomics 35 (1), 258-261 (1996)
PUBMED 8661132
REFERENCE 23 (bases 1 to 4290)
AUTHORS Sanyanusin,P., Schimmenti,L.A., McNoe,L.A., Ward,T.A., Pierpont,M.E., Sullivan,M.J., Dobyns,W.B. and Eccles,M.R.

TITLE Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux
JOURNAL Nat. Genet. 9 (4), 358-364 (1995)
PUBMED 7795640
REFERENCE 24 (bases 1 to 4290)
AUTHORS Ward,T.A., Nebel,A., Reeve,A.E. and Eccles,M.R.
TITLE Alternative messenger RNA forms and open reading frames within an additional conserved region of the human PAX-2 gene
JOURNAL Cell Growth Differ. 5 (9), 1015-1021 (1994)
PUBMED 7819127
REFERENCE 25 (bases 1 to 4290)
AUTHORS Noll,M.
TITLE Evolution and role of Pax genes
JOURNAL Curr. Opin. Genet. Dev. 3 (4), 595-605 (1993)
PUBMED 8241771
REMARK Review article
REFERENCE 26 (bases 1 to 4290)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Chromosomal localization of seven PAX genes and cloning of a novel family member, PAX-9
JOURNAL Nat. Genet. 3 (4), 292-298 (1993)
PUBMED 7981748
REFERENCE 27 (bases 1 to 4290)
AUTHORS Pilz,A.J., Povey,S., Gruss,P. and Abbott,C.M.
TITLE Mapping of the human homologs of the murine paired-box-containing genes
JOURNAL Mamm. Genome 4 (2), 78-82 (1993)
PUBMED 8431641
REFERENCE 28 (bases 1 to 4290)
AUTHORS Eccles,M.R., Wallis,L.J., Fidler,A.E., Spurr,N.K., Goodfellow,P.J. and Reeve,A.E.
TITLE Expression of the PAX2 gene in human fetal kidney and Wilms' tumor
JOURNAL Cell Growth Differ. 3 (5), 279-289 (1992)
PUBMED 1378753
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U45255.1 and BM671839.1.
On Sep 22, 2003 this sequence version replaced gi:4557824.

Summary: PAX2 encodes paired box gene 2, one of many human homologues of the Drosophila melanogaster gene prd. The central feature of this transcription factor gene family is the conserved DNA-binding paired box domain. PAX2 is believed to be a target of transcriptional supression by the tumor supressor gene WT1. Mutations within PAX2 have been shown to result in optic nerve colobomas and renal hypoplasia. Alternative splicing of this gene results in multiple transcript variants.

Transcript Variant: This variant (c) has multiple differences in the coding region, compared to variant e, one of which results in a

translational frameshift. The resulting protein (isoform c) has a distinct C-terminus and is shorter than isoform e.
COMPLETENESS: complete on the 3' end.

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go_process: transcription [goid 0006350] [evidence IEA];
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go_process: GTP biosynthesis [goid 0006183] [evidence IEA];
go_process: UTP biosynthesis [goid 0006228] [evidence IEA];
go_process: axonogenesis [goid 0007409] [evidence TAS] [pmid 9106533];
go_process: cell differentiation [goid 0030154] [evidence IEA];
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Appendix E

LOCUS NM_003989 4188 bp mRNA linear PRI 24-SEP-2005
DEFINITION Homo sapiens paired box gene 2 (PAX2), transcript variant d, mRNA.
ACCESSION NM_003989
VERSION NM_003989.2 GI:34878715
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 1 to 4188)
AUTHORS Yoshimura,K., Yoshida,S., Yamaji,Y., Komori,A., Yoshida,A.,
Hatae,K., Kubota,T. and Ishibashi,T.
TITLE De novo insG619 mutation in PAX2 gene in a Japanese patient with
papillorenal syndrome
JOURNAL Am. J. Ophthalmol. 139 (4), 733-735 (2005)
PUBMED 15808183
REMARK GeneRIF: Molecular genetic analysis of the PAX2 gene in combination
with renal ultrasonography can help in making an earlier diagnosis
of the disease.
REFERENCE 2 (bases 1 to 4188)
AUTHORS Mazal,P.R., Stichenwirth,M., Koller,A., Blach,S., Haitel,A. and
Susani,M.
TITLE Expression of aquaporins and PAX-2 compared to CD10 and cytokeratin
7 in renal neoplasms: a tissue microarray study
JOURNAL Mod. Pathol. 18 (4), 535-540 (2005)
PUBMED 15502805
REMARK GeneRIF: PAX-2 is a reliable marker for clear cell renal cell
carcinomas of lower grades but not for higher grades.
REFERENCE 3 (bases 1 to 4188)
AUTHORS Higashide,T., Wada,T., Sakurai,M., Yokoyama,H. and Sugiyama,K.
TITLE Macular abnormalities and optic disk anomaly associated with a new
PAX2 missense mutation
JOURNAL Am. J. Ophthalmol. 139 (1), 203-205 (2005)
PUBMED 15652857
REMARK GeneRIF: A new PAX2 missense mutation, R71T, may cause macular
abnormalities in addition to anomalies of the optic disk and the
kidney.
REFERENCE 4 (bases 1 to 4188)
AUTHORS Buttiglieri,S., Deregibus,M.C., Bravo,S., Cassoni,P., Chiarle,R.,
Bussolati,B. and Camussi,G.
TITLE Role of Pax2 in apoptosis resistance and proinvasive phenotype of
Kaposi's sarcoma cells
JOURNAL J. Biol. Chem. 279 (6), 4136-4143 (2004)
PUBMED 14627715
REMARK GeneRIF: expression of Pax2 by Kaposi's sarcoma cells correlated

with an enhanced resistance against apoptotic signals and with the proinvasive phenotype

REFERENCE 5 (bases 1 to 4188)
AUTHORS Brophy,P.D., Lang,K.M. and Dressler,G.R.
TITLE The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor
JOURNAL J. Biol. Chem. 278 (52), 52401-52405 (2003)
PUBMED 14561758
REMARK GeneRIF: Pax2 protein regulates expression of secreted frizzled related protein 2

REFERENCE 6 (bases 1 to 4188)
AUTHORS Schimmenti,L.A., Manligas,G.S. and Sieving,P.A.
TITLE Optic nerve dysplasia and renal insufficiency in a family with a novel PAX2 mutation, Arg115X: further ophthalmologic delineation of the renal-coloboma syndrome
JOURNAL Ophthalmic Genet. 24 (4), 191-202 (2003)
PUBMED 14566649
REMARK GeneRIF: PAX2 mutation is associated with Optic nerve dysplasia and renal insufficiency of the renal-coloboma syndrome

REFERENCE 7 (bases 1 to 4188)
AUTHORS Muratovska,A., Zhou,C., He,S., Goodyer,P. and Eccles,M.R.
TITLE Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival
JOURNAL Oncogene 22 (39), 7989-7997 (2003)
PUBMED 12970747
REMARK GeneRIF: The PAX2 gene was frequently expressed in a panel of 406 common primary tumor tissues and endogenous PAX gene expression is often required for the growth and survival of cancer cells

REFERENCE 8 (bases 1 to 4188)
AUTHORS Gough,S.M., McDonald,M., Chen,X.N., Korenberg,J.R., Neri,A., Kahn,T., Eccles,M.R. and Morris,C.M.
TITLE Refined physical map of the human PAX2/HOX11/NFKB2 cancer gene region at 10q24 and relocalization of the HPV6AI1 viral integration site to 14q13.3-q21.1
JOURNAL BMC Genomics 4 (1), 9 (2003)
PUBMED 12697057

REFERENCE 9 (bases 1 to 4188)
AUTHORS Hoffmeister,A., Ropolo,A., Vasseur,S., Mallo,G.V., Bodeker,H., Ritz-Laser,B., Dressler,G.R., Vaccaro,M.I., Dagorn,J.C., Moreno,S. and Iovanna,J.L.
TITLE The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter
JOURNAL J. Biol. Chem. 277 (25), 22314-22319 (2002)
PUBMED 11940591
REMARK GeneRIF: The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription

factors on the glucagon gene promoter.
REFERENCE 10 (bases 1 to 4188)
AUTHORS Cai,Y., Lechner,M.S., Nihalani,D., Prindle,M.J., Holzman,L.B. and Dressler,G.R.
TITLE Phosphorylation of Pax2 by the c-Jun N-terminal kinase and enhanced Pax2-dependent transcription activation
JOURNAL J. Biol. Chem. 277 (2), 1217-1222 (2002)
PUBMED 11700324
REFERENCE 11 (bases 1 to 4188)
AUTHORS Becker,K., Beales,P.L., Calver,D.M., Matthijs,G. and Mohammed,S.N.
TITLE Okihiro syndrome and acro-renal-ocular syndrome: clinical overlap, expansion of the phenotype, and absence of PAX2 mutations in two new families
JOURNAL J. Med. Genet. 39 (1), 68-71 (2002)
PUBMED 11826030
REMARK GeneRIF: The absence of PAX2 mutations has been identified in two families with histories of clinical overlap of Okihiro and acro-renal-ocular syndromes.
REFERENCE 12 (bases 1 to 4188)
AUTHORS Eccles,M.R., He,S., Legge,M., Kumar,R., Fox,J., Zhou,C., French,M. and Tsai,R.W.
TITLE PAX genes in development and disease: the role of PAX2 in urogenital tract development
JOURNAL Int. J. Dev. Biol. 46 (4), 535-544 (2002)
PUBMED 12141441
REMARK Review article
GeneRIF: PAX2 has a role in urogenital tract development and disease [review]
REFERENCE 13 (bases 1 to 4188)
AUTHORS Chung,G.W., Edwards,A.O., Schimmenti,L.A., Manligas,G.S., Zhang,Y.H. and Ritter,R. III.
TITLE Renal-coloboma syndrome: report of a novel PAX2 gene mutation
JOURNAL Am. J. Ophthalmol. 132 (6), 910-914 (2001)
PUBMED 11730657
REMARK GeneRIF: The causal relationship between PAX2 gene mutations and renal-coloboma syndrome is further supported
REFERENCE 14 (bases 1 to 4188)
AUTHORS Nishimoto,K., Iijima,K., Shirakawa,T., Kitagawa,K., Satomura,K., Nakamura,H. and Yoshikawa,N.
TITLE PAX2 gene mutation in a family with isolated renal hypoplasia
JOURNAL J. Am. Soc. Nephrol. 12 (8), 1769-1772 (2001)
PUBMED 11461952
REFERENCE 15 (bases 1 to 4188)
AUTHORS Ritz-Laser,B., Estreicher,A., Gauthier,B. and Philippe,J.
TITLE The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter
JOURNAL J. Biol. Chem. 275 (42), 32708-32715 (2000)
PUBMED 10938089

REFERENCE 16 (bases 1 to 4188)
AUTHORS Lechner,M.S., Levitan,I. and Dressler,G.R.
TITLE PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin
JOURNAL Nucleic Acids Res. 28 (14), 2741-2751 (2000)
PUBMED 10908331
REFERENCE 17 (bases 1 to 4188)
AUTHORS Tavassoli,K., Ruger,W. and Horst,J.
TITLE Alternative splicing in PAX2 generates a new reading frame and an extended conserved coding region at the carboxy terminus
JOURNAL Hum. Genet. 101 (3), 371-375 (1997)
PUBMED 9439670
REFERENCE 18 (bases 1 to 4188)
AUTHORS Dahl,E., Koseki,H. and Balling,R.
TITLE Pax genes and organogenesis
JOURNAL Bioessays 19 (9), 755-765 (1997)
PUBMED 9297966
REMARK Review article
REFERENCE 19 (bases 1 to 4188)
AUTHORS Schimmenti,L.A., Cunliffe,H.E., McNoe,L.A., Ward,T.A., French,M.C., Shim,H.H., Zhang,Y.H., Proesmans,W., Leys,A., Byerly,K.A., Braddock,S.R., Masuno,M., Imaizumi,K., Devriendt,K. and Eccles,M.R.
TITLE Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations
JOURNAL Am. J. Hum. Genet. 60 (4), 869-878 (1997)
PUBMED 9106533
REFERENCE 20 (bases 1 to 4188)
AUTHORS Narahara,K., Baker,E., Ito,S., Yokoyama,Y., Yu,S., Hewitt,D., Sutherland,G.R., Eccles,M.R. and Richards,R.I.
TITLE Localisation of a 10q breakpoint within the PAX2 gene in a patient with a de novo t(10;13) translocation and optic nerve coloboma-renal disease
JOURNAL J. Med. Genet. 34 (3), 213-216 (1997)
PUBMED 9132492
REFERENCE 21 (bases 1 to 4188)
AUTHORS Dehbi,M., Ghahremani,M., Lechner,M., Dressler,G. and Pelletier,J.
TITLE The paired-box transcription factor, PAX2, positively modulates expression of the Wilms' tumor suppressor gene (WT1)
JOURNAL Oncogene 13 (3), 447-453 (1996)
PUBMED 8760285
REFERENCE 22 (bases 1 to 4188)
AUTHORS Sanyanusin,P., Norrish,J.H., Ward,T.A., Nebel,A., McNoe,L.A. and Eccles,M.R.
TITLE Genomic structure of the human PAX2 gene
JOURNAL Genomics 35 (1), 258-261 (1996)
PUBMED 8661132
REFERENCE 23 (bases 1 to 4188)
AUTHORS Sanyanusin,P., Schimmenti,L.A., McNoe,L.A., Ward,T.A., Pierpont,M.E., Sullivan,M.J., Dobyns,W.B. and Eccles,M.R.

TITLE Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux
JOURNAL Nat. Genet. 9 (4), 358-364 (1995)
PUBMED 7795640
REFERENCE 24 (bases 1 to 4188)
AUTHORS Ward,T.A., Nebel,A., Reeve,A.E. and Eccles,M.R.
TITLE Alternative messenger RNA forms and open reading frames within an additional conserved region of the human PAX-2 gene
JOURNAL Cell Growth Differ. 5 (9), 1015-1021 (1994)
PUBMED 7819127
REFERENCE 25 (bases 1 to 4188)
AUTHORS Noll,M.
TITLE Evolution and role of Pax genes
JOURNAL Curr. Opin. Genet. Dev. 3 (4), 595-605 (1993)
PUBMED 8241771
REMARK Review article
REFERENCE 26 (bases 1 to 4188)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Chromosomal localization of seven PAX genes and cloning of a novel family member, PAX-9
JOURNAL Nat. Genet. 3 (4), 292-298 (1993)
PUBMED 7981748
REFERENCE 27 (bases 1 to 4188)
AUTHORS Pilz,A.J., Povey,S., Gruss,P. and Abbott,C.M.
TITLE Mapping of the human homologs of the murine paired-box-containing genes
JOURNAL Mamm. Genome 4 (2), 78-82 (1993)
PUBMED 8431641
REFERENCE 28 (bases 1 to 4188)
AUTHORS Eccles,M.R., Wallis,L.J., Fidler,A.E., Spurr,N.K., Goodfellow,P.J. and Reeve,A.E.
TITLE Expression of the PAX2 gene in human fetal kidney and Wilms' tumor
JOURNAL Cell Growth Differ. 3 (5), 279-289 (1992)
PUBMED 1378753
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U45255.1 and BM671839.1.
On Sep 22, 2003 this sequence version replaced gi:4557826.

Summary: PAX2 encodes paired box gene 2, one of many human homologues of the Drosophila melanogaster gene prd. The central feature of this transcription factor gene family is the conserved DNA-binding paired box domain. PAX2 is believed to be a target of transcriptional supression by the tumor supressor gene WT1. Mutations within PAX2 have been shown to result in optic nerve colobomas and renal hypoplasia. Alternative splicing of this gene results in multiple transcript variants.

Transcript Variant: This variant (d) lacks an alternate in-frame exon compared to variant e. This results in an isoform (isoform d)

that is shorter than isoform e.
COMPLETENESS: complete on the 3' end.

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go_process: transcription [goid 0006350] [evidence IEA];
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go_process: GTP biosynthesis [goid 0006183] [evidence IEA];
go_process: UTP biosynthesis [goid 0006228] [evidence IEA];
go_process: axonogenesis [goid 0007409] [evidence TAS] [pmid 9106533];
go_process: cell differentiation [goid 0030154] [evidence IEA];
go_process: visual perception [goid 0007601] [evidence TAS] [pmid 9106533];
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APPENDIX F

LOCUS NM_003990 4257 bp mRNA linear PRI 24-SEP-2005
DEFINITION Homo sapiens paired box gene 2 (PAX2), transcript variant e, mRNA.
ACCESSION NM_003990
VERSION NM_003990.2 GI:34878702
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 4257)
AUTHORS Yoshimura,K., Yoshida,S., Yamaji,Y., Komori,A., Yoshida,A., Hatae,K., Kubota,T. and Ishibashi,T.
TITLE De novo insG619 mutation in PAX2 gene in a Japanese patient with papillorenal syndrome
JOURNAL Am. J. Ophthalmol. 139 (4), 733-735 (2005)
PUBMED 15808183
REMARK GeneRIF: Molecular genetic analysis of the PAX2 gene in combination with renal ultrasonography can help in making an earlier diagnosis of the disease.
REFERENCE 2 (bases 1 to 4257)
AUTHORS Mazal,P.R., Stichenwirth,M., Koller,A., Blach,S., Haitel,A. and Susani,M.
TITLE Expression of aquaporins and PAX-2 compared to CD10 and cytokeratin 7 in renal neoplasms: a tissue microarray study
JOURNAL Mod. Pathol. 18 (4), 535-540 (2005)
PUBMED 15502805
REMARK GeneRIF: PAX-2 is a reliable marker for clear cell renal cell carcinomas of lower grades but not for higher grades.
REFERENCE 3 (bases 1 to 4257)
AUTHORS Higashide,T., Wada,T., Sakurai,M., Yokoyama,H. and Sugiyama,K.
TITLE Macular abnormalities and optic disk anomaly associated with a new PAX2 missense mutation
JOURNAL Am. J. Ophthalmol. 139 (1), 203-205 (2005)
PUBMED 15652857
REMARK GeneRIF: A new PAX2 missense mutation, R71T, may cause macular abnormalities in addition to anomalies of the optic disk and the kidney.
REFERENCE 4 (bases 1 to 4257)
AUTHORS Buttiglieri,S., Deregibus,M.C., Bravo,S., Cassoni,P., Chiarle,R., Bussolati,B. and Camussi,G.
TITLE Role of Pax2 in apoptosis resistance and proinvasive phenotype of Kaposi's sarcoma cells
JOURNAL J. Biol. Chem. 279 (6), 4136-4143 (2004)

PUBMED 14627715
REMARK GeneRIF: expression of Pax2 by Kaposi's sarcoma cells correlated with an enhanced resistance against apoptotic signals and with the proinvasive phenotype
REFERENCE 5 (bases 1 to 4257)
AUTHORS Brophy,P.D., Lang,K.M. and Dressler,G.R.
TITLE The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor
JOURNAL J. Biol. Chem. 278 (52), 52401-52405 (2003)
PUBMED 14561758
REMARK GeneRIF: Pax2 protein regulates expression of secreted frizzled related protein 2
REFERENCE 6 (bases 1 to 4257)
AUTHORS Schimmenti,L.A., Manligas,G.S. and Sieving,P.A.
TITLE Optic nerve dysplasia and renal insufficiency in a family with a novel PAX2 mutation, Arg115X: further ophthalmologic delineation of the renal-coloboma syndrome
JOURNAL Ophthalmic Genet. 24 (4), 191-202 (2003)
PUBMED 14566649
REMARK GeneRIF: PAX2 mutation is associated with Optic nerve dysplasia and renal insufficiency of the renal-coloboma syndrome
REFERENCE 7 (bases 1 to 4257)
AUTHORS Muratovska,A., Zhou,C., He,S., Goodyer,P. and Eccles,M.R.
TITLE Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival
JOURNAL Oncogene 22 (39), 7989-7997 (2003)
PUBMED 12970747
REMARK GeneRIF: The PAX2 gene was frequently expressed in a panel of 406 common primary tumor tissues and endogenous PAX gene expression is often required for the growth and survival of cancer cells
REFERENCE 8 (bases 1 to 4257)
AUTHORS Gough,S.M., McDonald,M., Chen,X.N., Korenberg,J.R., Neri,A., Kahn,T., Eccles,M.R. and Morris,C.M.
TITLE Refined physical map of the human PAX2/HOX11/NFKB2 cancer gene region at 10q24 and relocalization of the HPV6AI1 viral integration site to 14q13.3-q21.1
JOURNAL BMC Genomics 4 (1), 9 (2003)
PUBMED 12697057
REFERENCE 9 (bases 1 to 4257)
AUTHORS Hoffmeister,A., Ropolo,A., Vasseur,S., Mallo,G.V., Bodeker,H., Ritz-Laser,B., Dressler,G.R., Vaccaro,M.I., Dagorn,J.C., Moreno,S. and Iovanna,J.L.
TITLE The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter
JOURNAL J. Biol. Chem. 277 (25), 22314-22319 (2002)
PUBMED 11940591

REMARK GeneRIF: The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter.
REFERENCE 10 (bases 1 to 4257)
AUTHORS Cai,Y., Lechner,M.S., Nihalani,D., Prindle,M.J., Holzman,L.B. and Dressler,G.R.
TITLE Phosphorylation of Pax2 by the c-Jun N-terminal kinase and enhanced Pax2-dependent transcription activation
JOURNAL J. Biol. Chem. 277 (2), 1217-1222 (2002)
PUBMED 11700324
REFERENCE 11 (bases 1 to 4257)
AUTHORS Becker,K., Beales,P.L., Calver,D.M., Matthijs,G. and Mohammed,S.N.
TITLE Okihiro syndrome and acro-renal-ocular syndrome: clinical overlap, expansion of the phenotype, and absence of PAX2 mutations in two new families
JOURNAL J. Med. Genet. 39 (1), 68-71 (2002)
PUBMED 11826030
REMARK GeneRIF: The absence of PAX2 mutations has been identified in two families with histories of clinical overlap of Okihiro and acro-renal-ocular syndromes.
REFERENCE 12 (bases 1 to 4257)
AUTHORS Eccles,M.R., He,S., Legge,M., Kumar,R., Fox,J., Zhou,C., French,M. and Tsai,R.W.
TITLE PAX genes in development and disease: the role of PAX2 in urogenital tract development
JOURNAL Int. J. Dev. Biol. 46 (4), 535-544 (2002)
PUBMED 12141441
REMARK Review article
GeneRIF: PAX2 has a role in urogenital tract development and disease [review]
REFERENCE 13 (bases 1 to 4257)
AUTHORS Chung,G.W., Edwards,A.O., Schimmenti,L.A., Manligas,G.S., Zhang,Y.H. and Ritter,R. III.
TITLE Renal-coloboma syndrome: report of a novel PAX2 gene mutation
JOURNAL Am. J. Ophthalmol. 132 (6), 910-914 (2001)
PUBMED 11730657
REMARK GeneRIF: The causal relationship between PAX2 gene mutations and renal-coloboma syndrome is further supported
REFERENCE 14 (bases 1 to 4257)
AUTHORS Nishimoto,K., Iijima,K., Shirakawa,T., Kitagawa,K., Satomura,K., Nakamura,H. and Yoshikawa,N.
TITLE PAX2 gene mutation in a family with isolated renal hypoplasia
JOURNAL J. Am. Soc. Nephrol. 12 (8), 1769-1772 (2001)
PUBMED 11461952
REFERENCE 15 (bases 1 to 4257)
AUTHORS Ritz-Laser,B., Estreicher,A., Gauthier,B. and Philippe,J.
TITLE The paired homeodomain transcription factor Pax-2 is expressed in

the endocrine pancreas and transactivates the glucagon gene promoter
JOURNAL J. Biol. Chem. 275 (42), 32708-32715 (2000)
PUBMED 10938089
REFERENCE 16 (bases 1 to 4257)
AUTHORS Lechner,M.S., Levitan,I. and Dressler,G.R.
TITLE PTIP, a novel BRCT domain-containing protein interacts with Pax2 and is associated with active chromatin
JOURNAL Nucleic Acids Res. 28 (14), 2741-2751 (2000)
PUBMED 10908331
REFERENCE 17 (bases 1 to 4257)
AUTHORS Tavassoli,K., Ruger,W. and Horst,J.
TITLE Alternative splicing in PAX2 generates a new reading frame and an extended conserved coding region at the carboxy terminus
JOURNAL Hum. Genet. 101 (3), 371-375 (1997)
PUBMED 9439670
REFERENCE 18 (bases 1 to 4257)
AUTHORS Dahl,E., Koseki,H. and Balling,R.
TITLE Pax genes and organogenesis
JOURNAL Bioessays 19 (9), 755-765 (1997)
PUBMED 9297966
REMARK Review article
REFERENCE 19 (bases 1 to 4257)
AUTHORS Schimmenti,L.A., Cunliffe,H.E., McNoe,L.A., Ward,T.A., French,M.C., Shim,H.H., Zhang,Y.H., Proesmans,W., Leys,A., Byerly,K.A., Braddock,S.R., Masuno,M., Imaizumi,K., Devriendt,K. and Eccles,M.R.
TITLE Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations
JOURNAL Am. J. Hum. Genet. 60 (4), 869-878 (1997)
PUBMED 9106533
REFERENCE 20 (bases 1 to 4257)
AUTHORS Narahara,K., Baker,E., Ito,S., Yokoyama,Y., Yu,S., Hewitt,D., Sutherland,G.R., Eccles,M.R. and Richards,R.I.
TITLE Localisation of a 10q breakpoint within the PAX2 gene in a patient with a de novo t(10;13) translocation and optic nerve coloboma-renal disease
JOURNAL J. Med. Genet. 34 (3), 213-216 (1997)
PUBMED 9132492
REFERENCE 21 (bases 1 to 4257)
AUTHORS Dehbi,M., Ghahremani,M., Lechner,M., Dressler,G. and Pelletier,J.
TITLE The paired-box transcription factor, PAX2, positively modulates expression of the Wilms' tumor suppressor gene (WT1)
JOURNAL Oncogene 13 (3), 447-453 (1996)
PUBMED 8760285
REFERENCE 22 (bases 1 to 4257)
AUTHORS Sanyanusin,P., Norrish,J.H., Ward,T.A., Nebel,A., McNoe,L.A. and Eccles,M.R.
TITLE Genomic structure of the human PAX2 gene

JOURNAL Genomics 35 (1), 258-261 (1996)
PUBMED 8661132
REFERENCE 23 (bases 1 to 4257)
AUTHORS Sanyanusin,P., Schimmenti,L.A., McNoe,L.A., Ward,T.A., Pierpont,M.E., Sullivan,M.J., Dobyns,W.B. and Eccles,M.R.
TITLE Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux
JOURNAL Nat. Genet. 9 (4), 358-364 (1995)
PUBMED 7795640
REFERENCE 24 (bases 1 to 4257)
AUTHORS Ward,T.A., Nebel,A., Reeve,A.E. and Eccles,M.R.
TITLE Alternative messenger RNA forms and open reading frames within an additional conserved region of the human PAX-2 gene
JOURNAL Cell Growth Differ. 5 (9), 1015-1021 (1994)
PUBMED 7819127
REFERENCE 25 (bases 1 to 4257)
AUTHORS Noll,M.
TITLE Evolution and role of Pax genes
JOURNAL Curr. Opin. Genet. Dev. 3 (4), 595-605 (1993)
PUBMED 8241771
REMARK Review article
REFERENCE 26 (bases 1 to 4257)
AUTHORS Stapleton,P., Weith,A., Urbanek,P., Kozmik,Z. and Busslinger,M.
TITLE Chromosomal localization of seven PAX genes and cloning of a novel family member, PAX-9
JOURNAL Nat. Genet. 3 (4), 292-298 (1993)
PUBMED 7981748
REFERENCE 27 (bases 1 to 4257)
AUTHORS Pilz,A.J., Povey,S., Gruss,P. and Abbott,C.M.
TITLE Mapping of the human homologs of the murine paired-box-containing genes
JOURNAL Mamm. Genome 4 (2), 78-82 (1993)
PUBMED 8431641
REFERENCE 28 (bases 1 to 4257)
AUTHORS Eccles,M.R., Wallis,L.J., Fidler,A.E., Spurr,N.K., Goodfellow,P.J. and Reeve,A.E.
TITLE Expression of the PAX2 gene in human fetal kidney and Wilms' tumor
JOURNAL Cell Growth Differ. 3 (5), 279-289 (1992)
PUBMED 1378753
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U45255.1 and BM671839.1. On Sep 22, 2003 this sequence version replaced gi:4557828.

Summary: PAX2 encodes paired box gene 2, one of many human homologues of the Drosophila melanogaster gene prd. The central feature of this transcription factor gene family is the conserved DNA-binding paired box domain. PAX2 is believed to be a target of transcriptional supression by the tumor supressor gene WT1.

Mutations within PAX2 have been shown to result in optic nerve colobomas and renal hypoplasia. Alternative splicing of this gene results in multiple transcript variants.

Transcript Variant: This variant (e) encodes the longest isoform (e).
COMPLETENESS: complete on the 3' end.

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go_function: ATP binding [goid 0005524] [evidence IEA];
go_function: DNA binding [goid 0003677] [evidence IEA];
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go_function: nucleoside diphosphate kinase activity [goid 0004550] [evidence IEA];
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go_process: transcription [goid 0006350] [evidence IEA];
go_process: CTP biosynthesis [goid 0006241] [evidence IEA];
go_process: GTP biosynthesis [goid 0006183] [evidence IEA];
go_process: UTP biosynthesis [goid 0006228] [evidence IEA];
go_process: axonogenesis [goid 0007409] [evidence TAS] [pmid 9106533];
go_process: cell differentiation [goid 0030154] [evidence IEA];
go_process: visual perception [goid 0007601] [evidence TAS] [pmid 9106533];
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CA 02625891 2008-04-14

Table 3.

Sequences of QRT-PCR Primers.

	Sense (5'-3')	Antisense (5'-3')
β-actin	5'-CCTGGCACCCAGCACAAT-3'	5'-GCC GATCCACACGGAGTACT-3'
DEFB1	5'-GTTGCCTGCCAGTC GCCAT GAGAACTTCCTAC-3'	5'-TGGCCTTCCCTCTGTA ACAGGTGCCTTGAATT-3'

Table 4.

PAX2 siRNA Sequences. A pool of four siRNA was utilized to inhibit PAX2 protein expression.

	Sense (5'-3')	**Antisense (5'-3')**
Sequence A	5'- GAAGUCAAGUCGAGUCUAUUU-3' (SEQ ID NO:1)	5'–AUAGACUCGACUUGACUUCUU–3' (SEQ ID NO:2)
Sequence B	5'-GAGGAAACGUGAUGAAGAUUU-3' (SEQ ID NO:3)	5'-AUCUUCAUCACGUUUCCUCUU-3' (SEQ ID NO:4)
Sequence C	5'-GGACAAGAUUGCUGAAUACUU-3' (SEQ ID NO:5)	5'-GUAUUCAGCAAUCUUGUCCUU-3' (SEQ ID NO:6)
Sequence D	5'-CAUCAGAGCACAUCAAAUCUU-3' (SEQ ID NO:7)	5'-GAUUUGAUGUGCUCUGAUGUU-3' (SEQ ID NO:8)

Table 5.

Quantitative RT-PCR Primers. Nucleotide sequences of primers used to amplify PAX2 and GAPDH.

	Sense (5'-3')	**Antisense (5'-3')**
GAPDH	5'-CCACCCATGGCAAATTCCATGGCA-3'	5'-TCTAGACGGCAGGTCAGGTCAACC-3'
BAD	5'-CTCAGGCCTATGCAAAAAGAGGA-3'	5'-GCCCTCCCTCCAAAGGAGAC-3'
BID	5'-AACCTACGCACCTACGTGAGGAG-3'	5'-CGTTCAGTCCATCCCATTTCTG-3'
BAX	5'-GACACCTGAGCTGACCTTGG-3'	5'-GAGGAAGTCCAGTGTCCAGC-3'

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. A method of treating cancer in a subject by inhibiting expression of PAX2.

2. The method of claim 1, wherein the method of inhibiting expression of PAX 2 is by administration of a nucleic acid encoding an siRNA for PAX 2.

3. The method of claim 2, wherein the siRNA is selected from the group consisting of:

AUAGACUCGACUUGACUUCUU (SEQ ID NO:2)

AUCUUCAUCACGUUUCCUCUU (SEQ ID NO:4)

GUAUUCAGCAAUCUUGUCCUU (SEQ ID NO:6)

GAUUUGAUGUGCUCUGAUGUU (SEQ ID NO:8)

4. The method of claim 2, wherein the cancer is prostate cancer.

5. A method of treating cancer in a subject by administering DEFB1.

6. A method of treating cancer in a subject by increasing expression of DEFB1 in the subject.

7. The method of claim 6, wherein the cancer is prostate cancer.

8. The method of claim 6, wherein the expression of DEFB1 is increased by blocking the binding of PAX2 to the DEFB1 promoter.

9. The method of claim 8, wherein the blocking of binding of PAX2 to the DEFB1 promoter is by administration of an oligonucleotide complementary to the PAX2 DNA binding site of DEFB1.

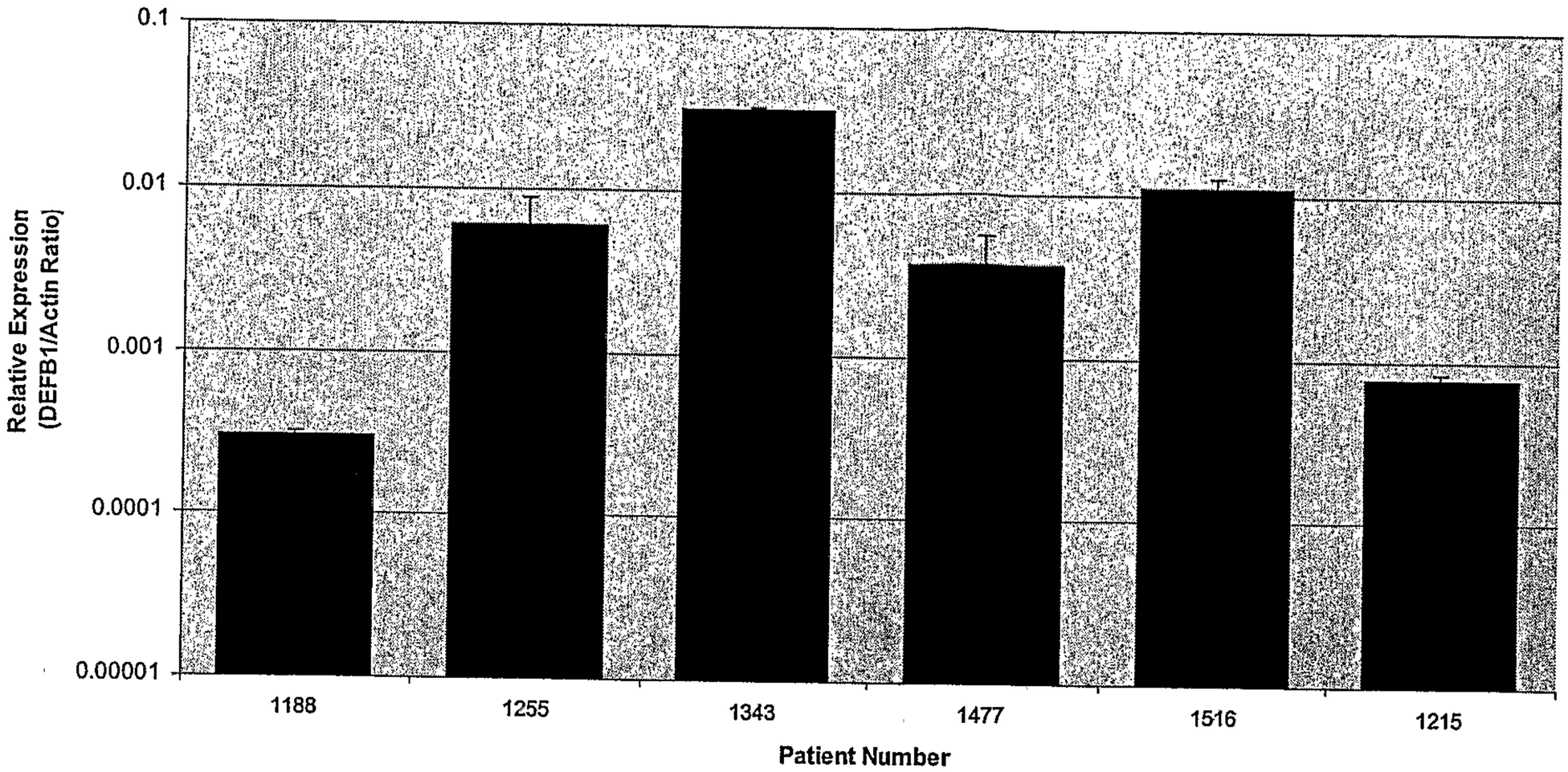
10. The method of claim 9, wherein the PAX2 DNA binding site of DEFB1 comprises SEQ ID NO:1 in forward or reverse orientation.

11. The method of claim 9, wherein the oligonucleotide complementary to the PAX2 DNA binding site of DEFB1 is selected from the group consisting of

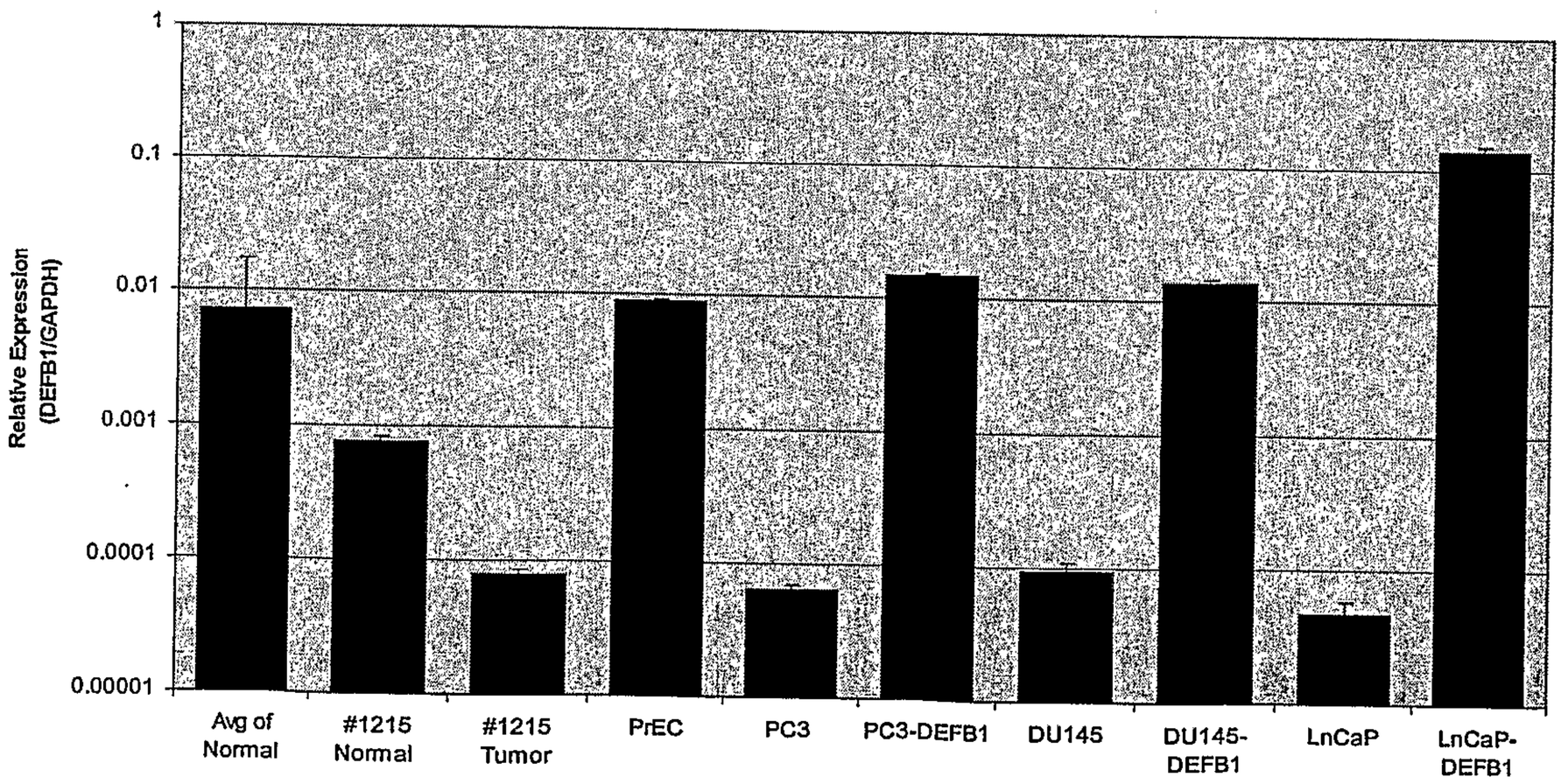
$X_1GGAACX_2$, wherein X_1 and X_2 are 0 to 30 nucleotides complementary to nucleotides contiguous to SEQ ID NO:1 in the DEFB1 coding sequence.

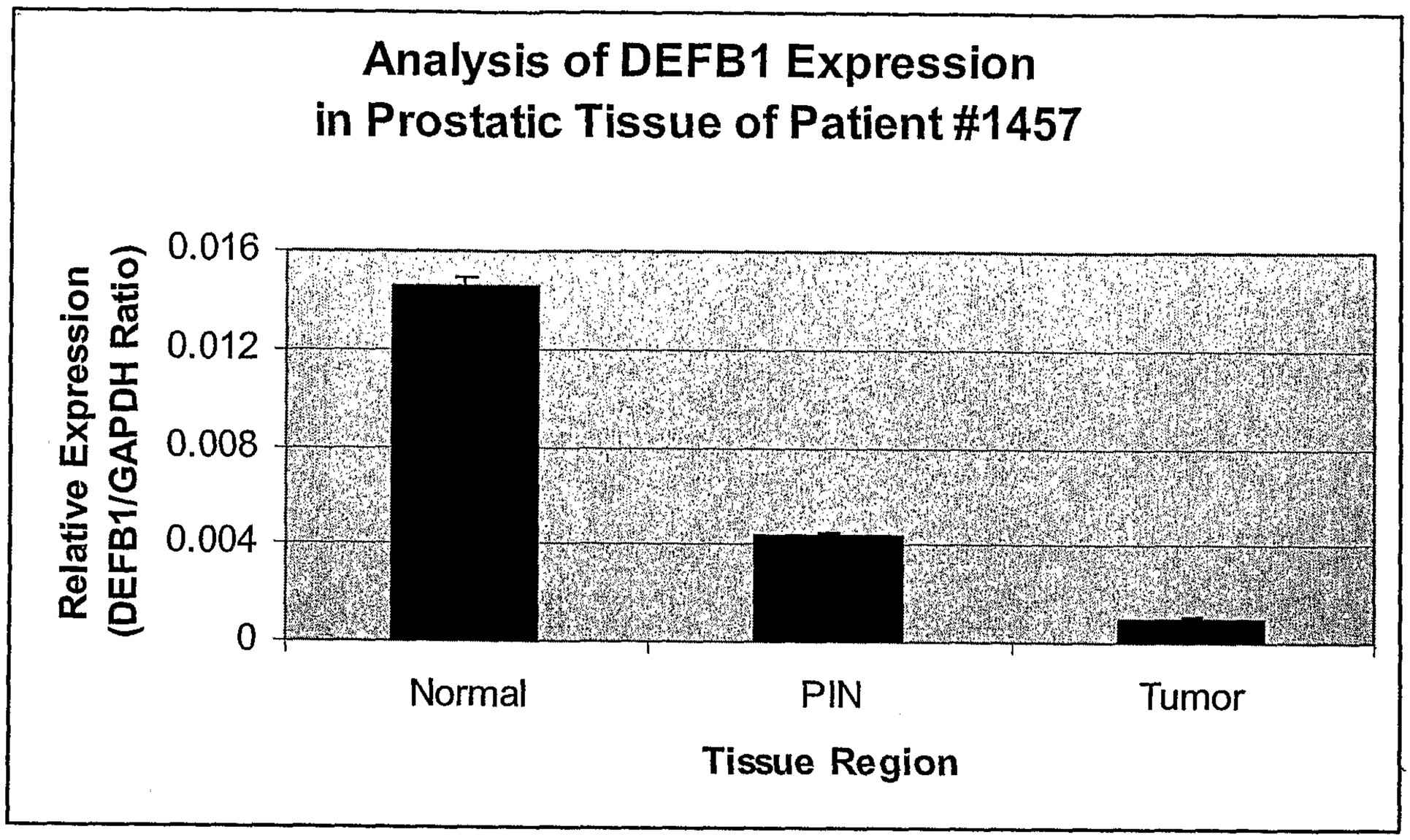
Figure 1.

A.


0.1
0.01
0.001
0.0001
0.00001
Relative Expression (DEFB1/Actin Ratio)
1188
1255
1343
1477
1516
1215
Patient Number

B.


1
0.1
0.01
0.001
0.0001
0.00001
Relative Expression (DEFB1/GAPDH)
Avg of Normal
#1215 Normal
#1215 Tumor
PrEC
PC3
PC3-DEFB1
DU145
DU145-DEFB1
LnCaP
LnCaP-DEFB1


Analysis of DEFB1 Expression
in Prostatic Tissue of Patient #1457
Relative Expression
(DEFB1/GAPDH Ratio)
0.016
0.012
0.008
0.004
0
Normal
PIN
Tumor
Tissue Region

D.

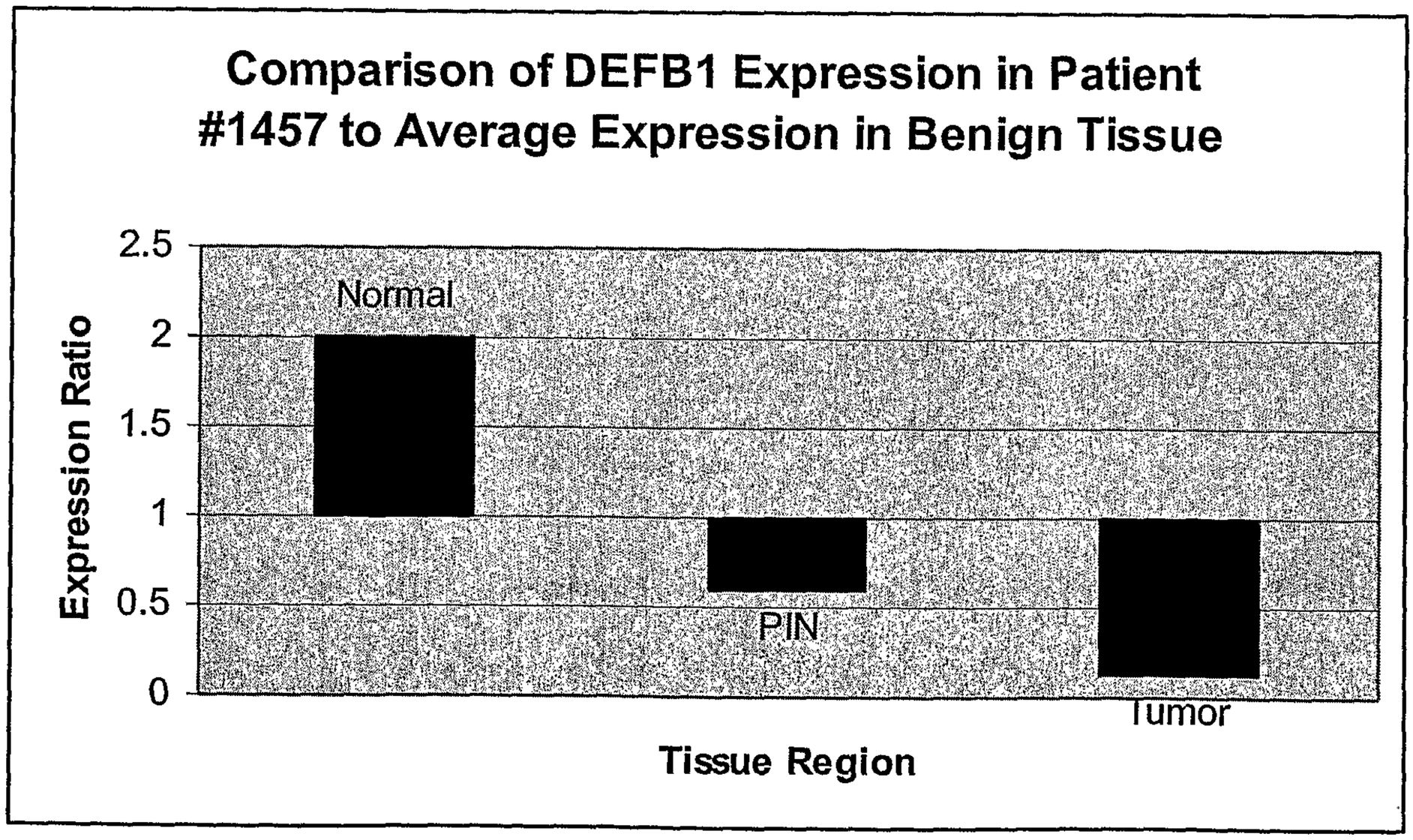
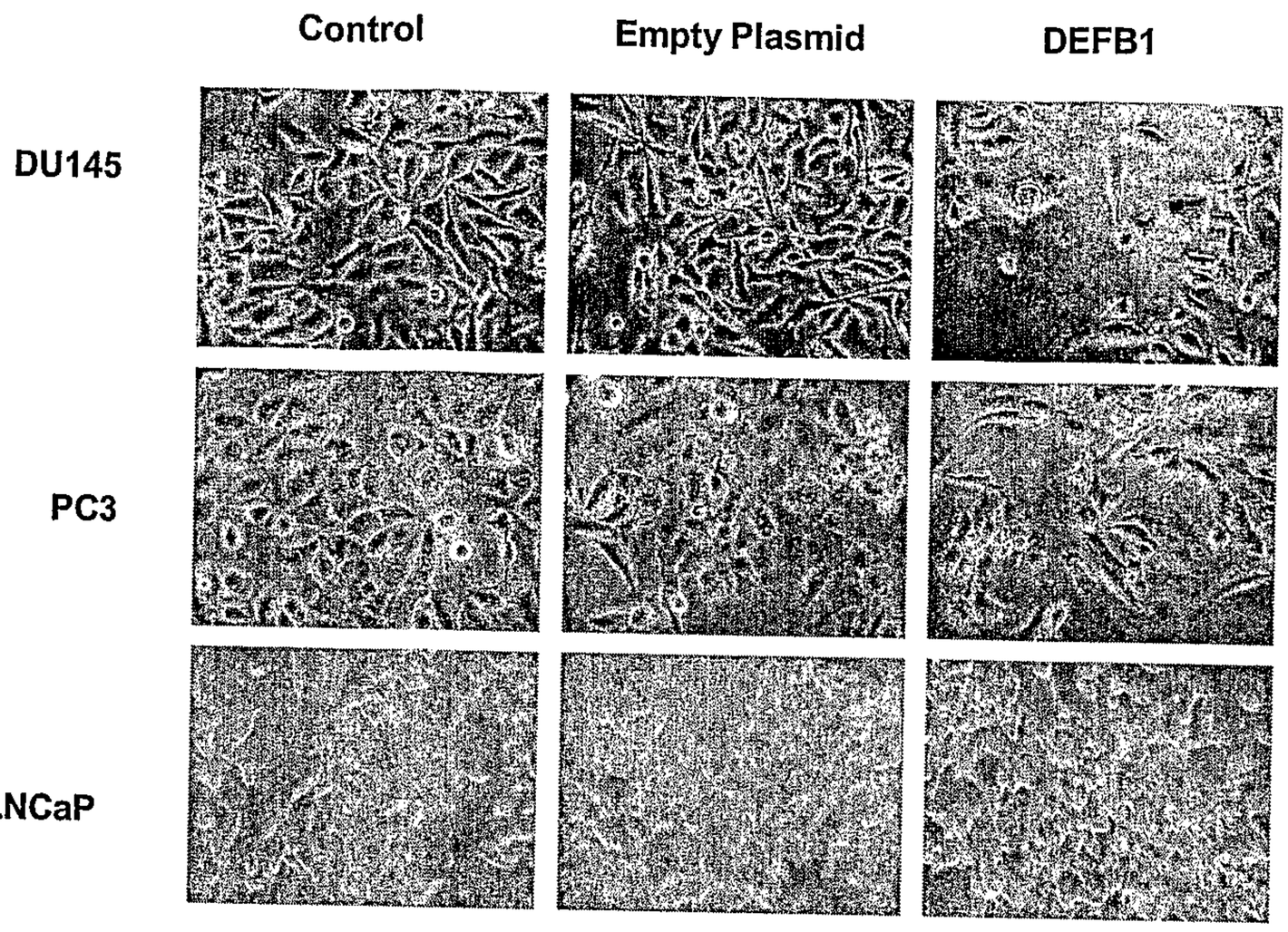

Comparison of DEFB1 Expression in Patient
#1457 to Average Expression in Benign Tissue
Expression Ratio
2.5
2
1.5
1
0.5
0
Normal
PIN
Tumor
Tissue Region

Figure 2.



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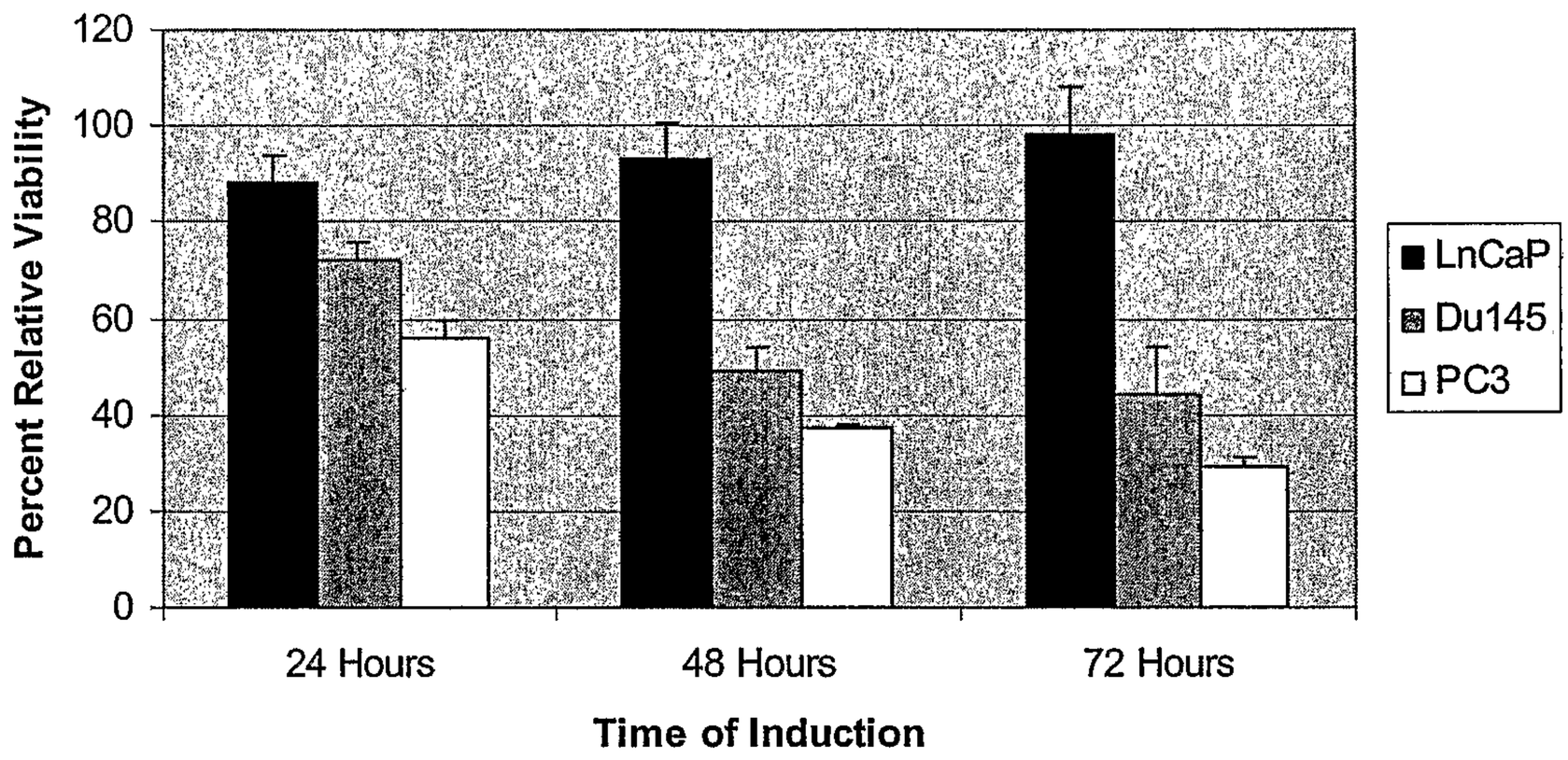
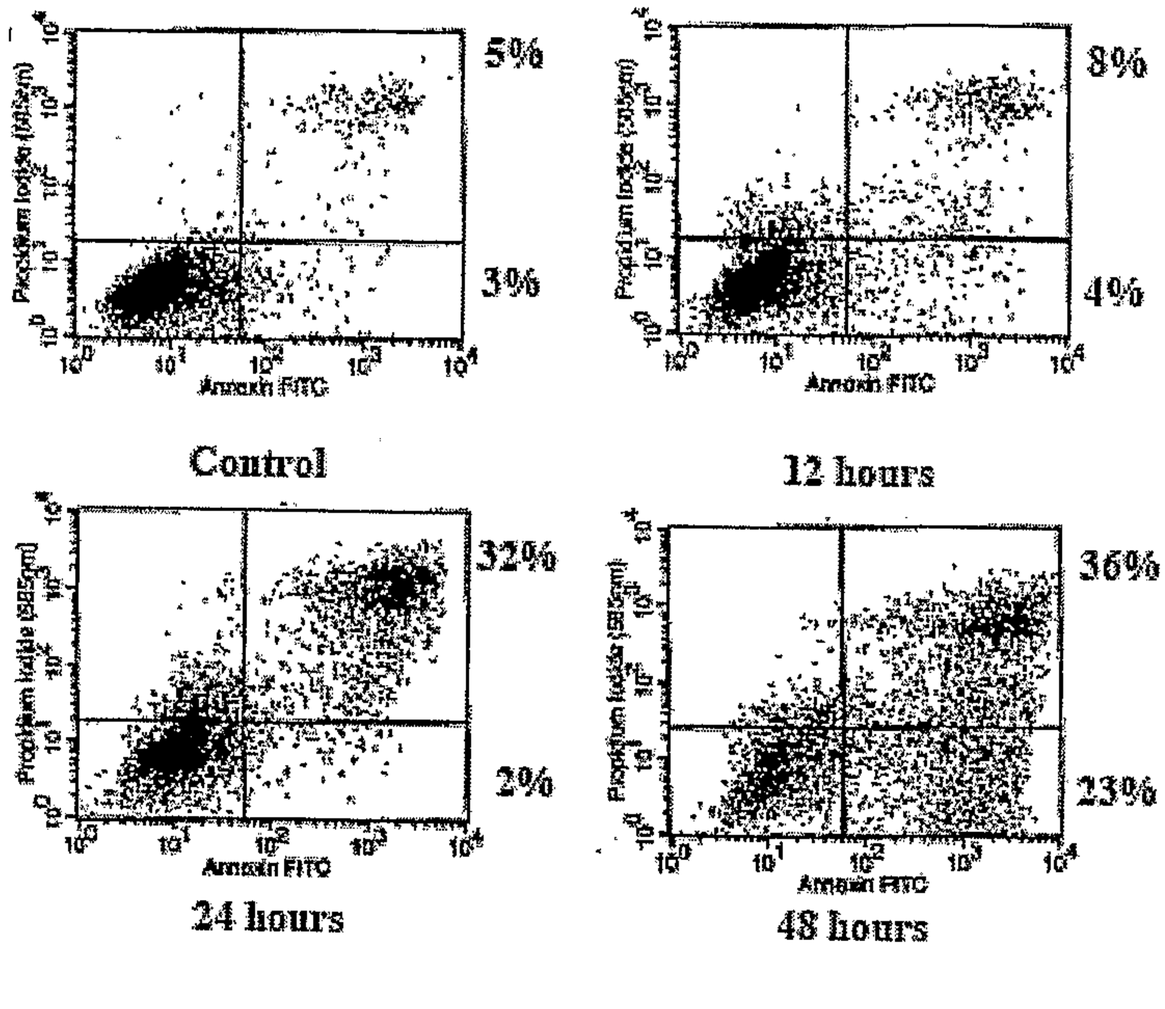

120
100
80
60
40
20
0
Percent Relative Viability
24 Hours
48 Hours
72 Hours
LnCaP
Du145
PC3
Time of Induction

Figure 4.

A.


5%
3%
Control
8%
4%
12 hours
32%
2%
24 hours
36%
23%
48 hours

B.

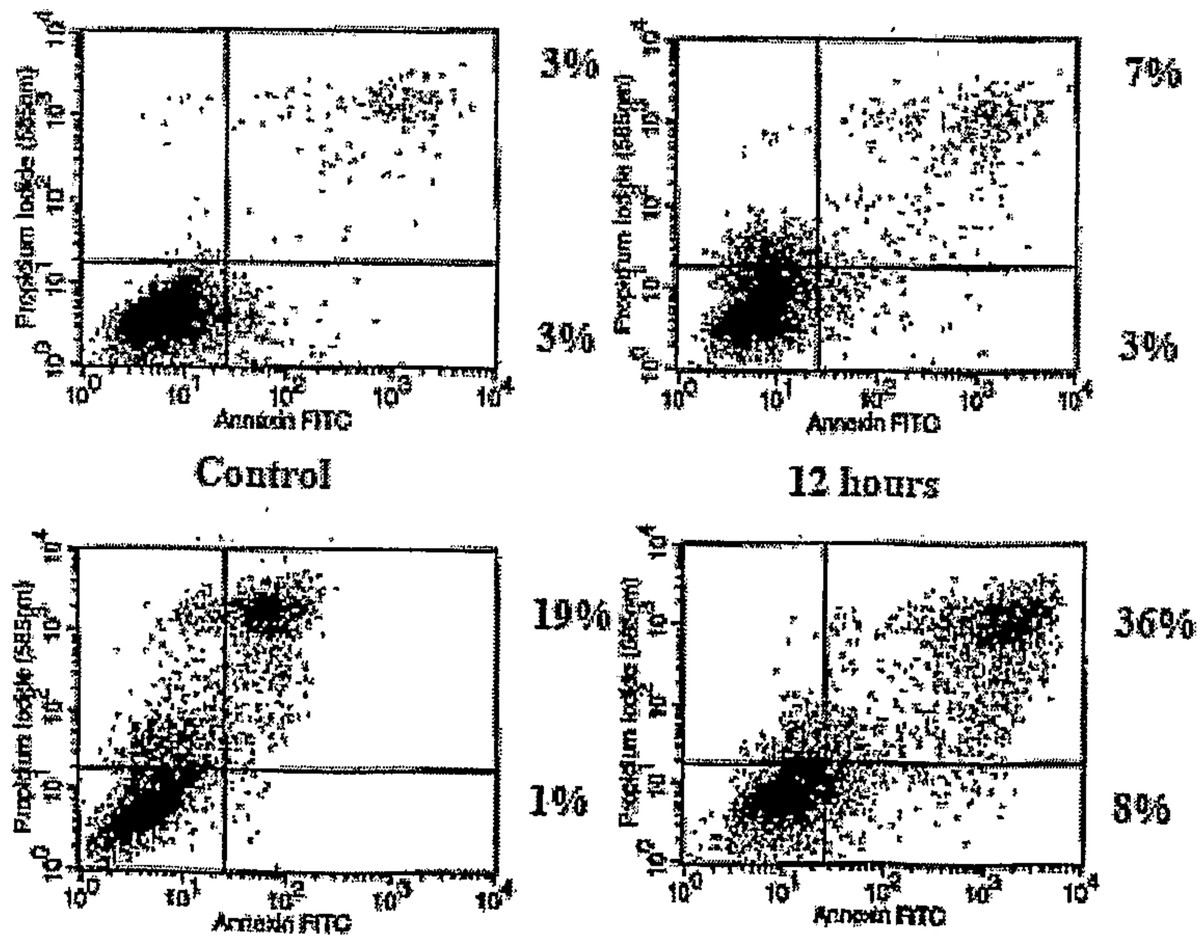

3%
3%
Control
7%
3%
12 hours
19%
1%
24 hours
36%
8%
48 hours

Figure 5.

DU145

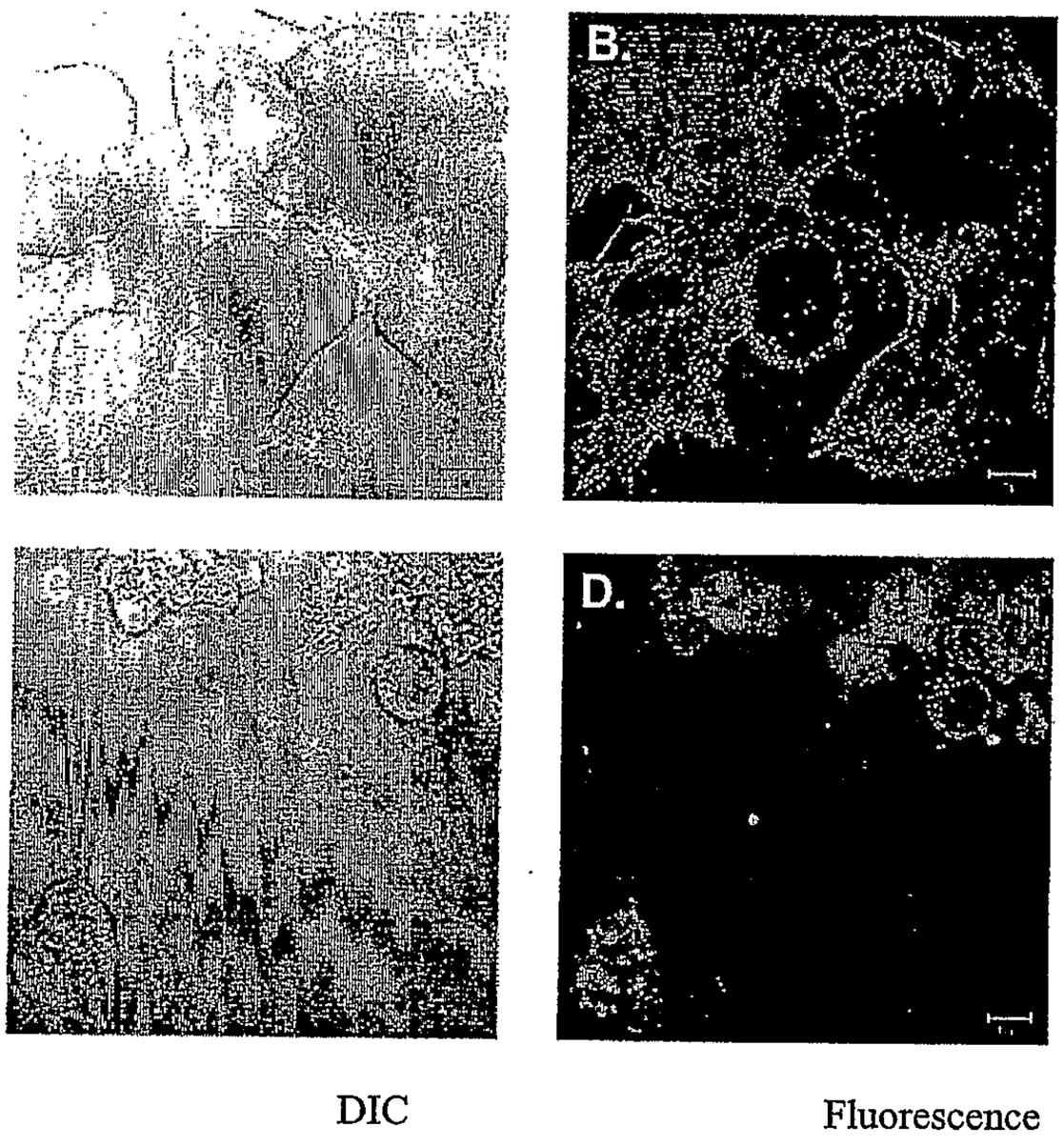


Figure 5.(cont'd)

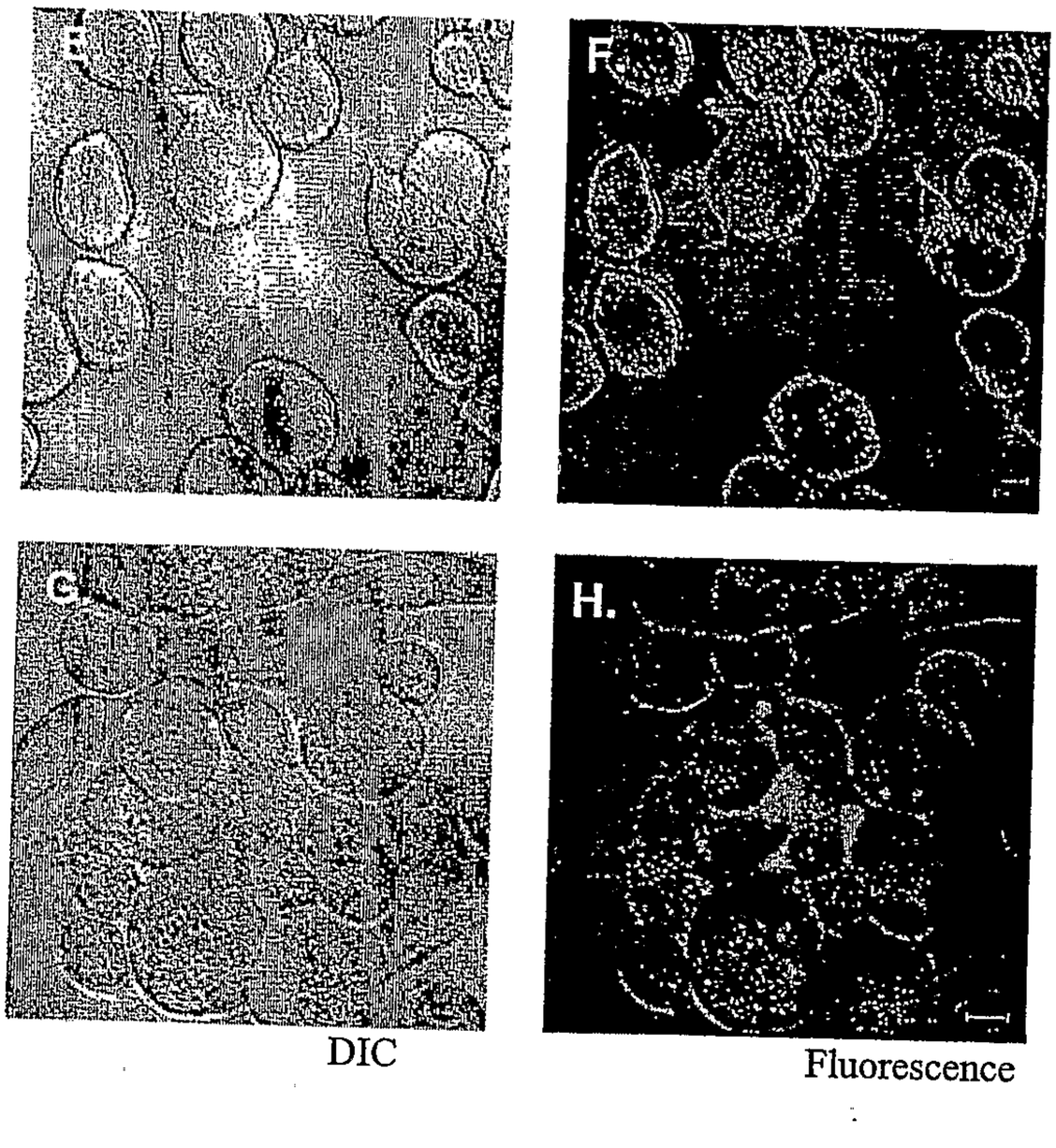
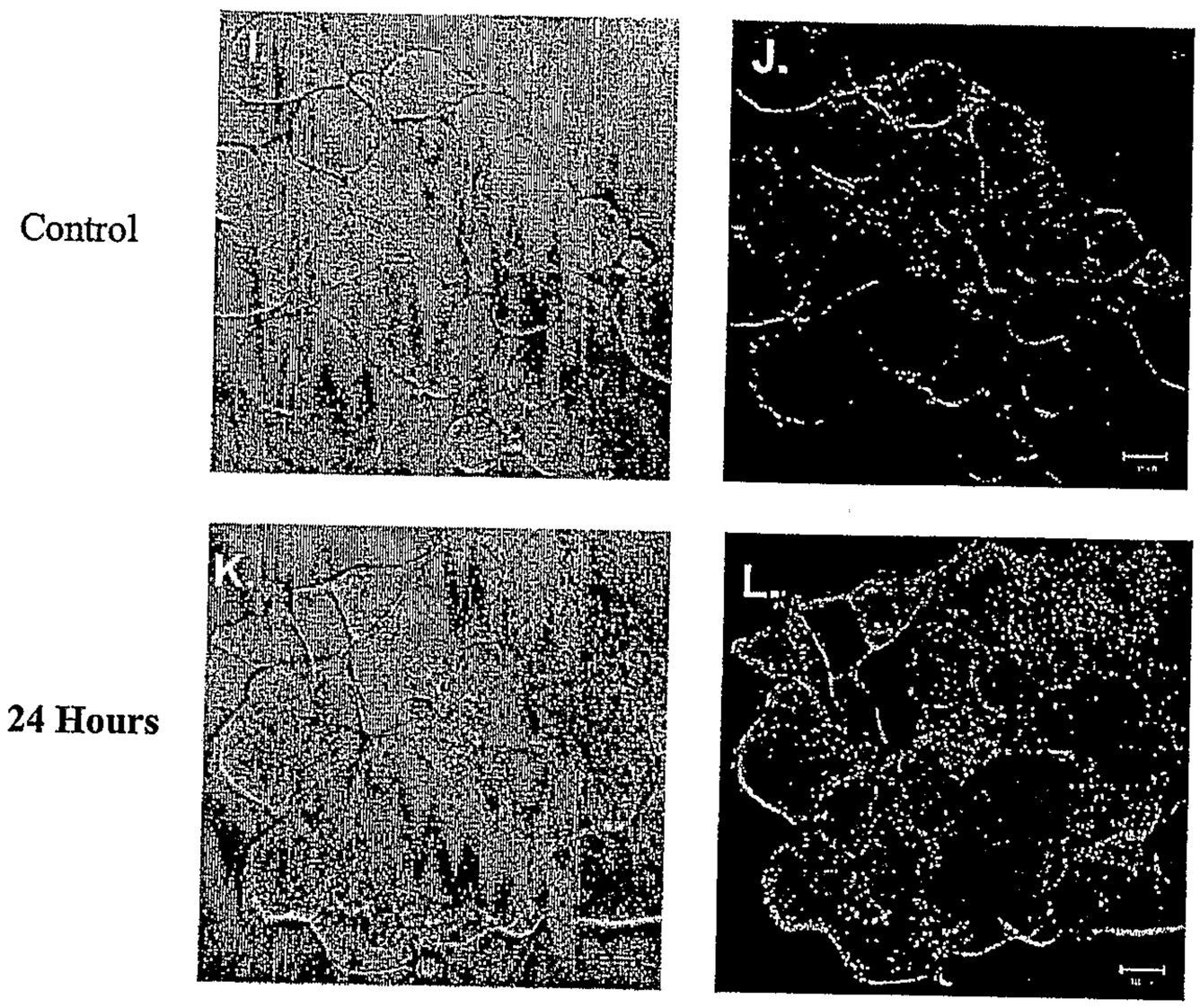


Figure 5.(cont'd)



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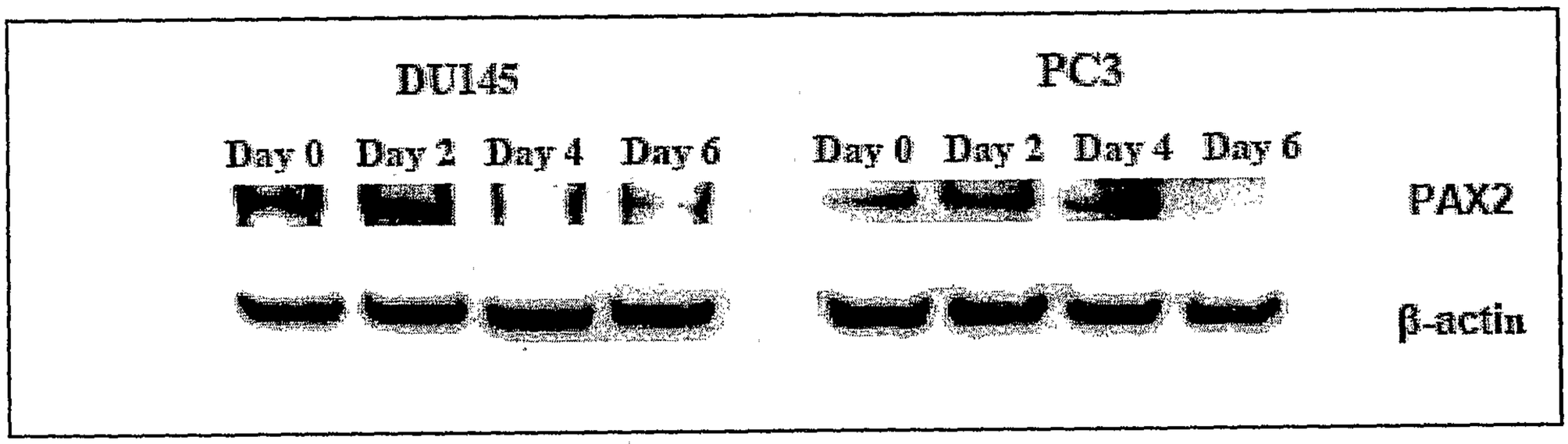

DU145
PC3
Day 0
Day 2
Day 4
Day 6
Day 0
Day 2
Day 4
Day 6
PAX2
β-actin

Figure 7.

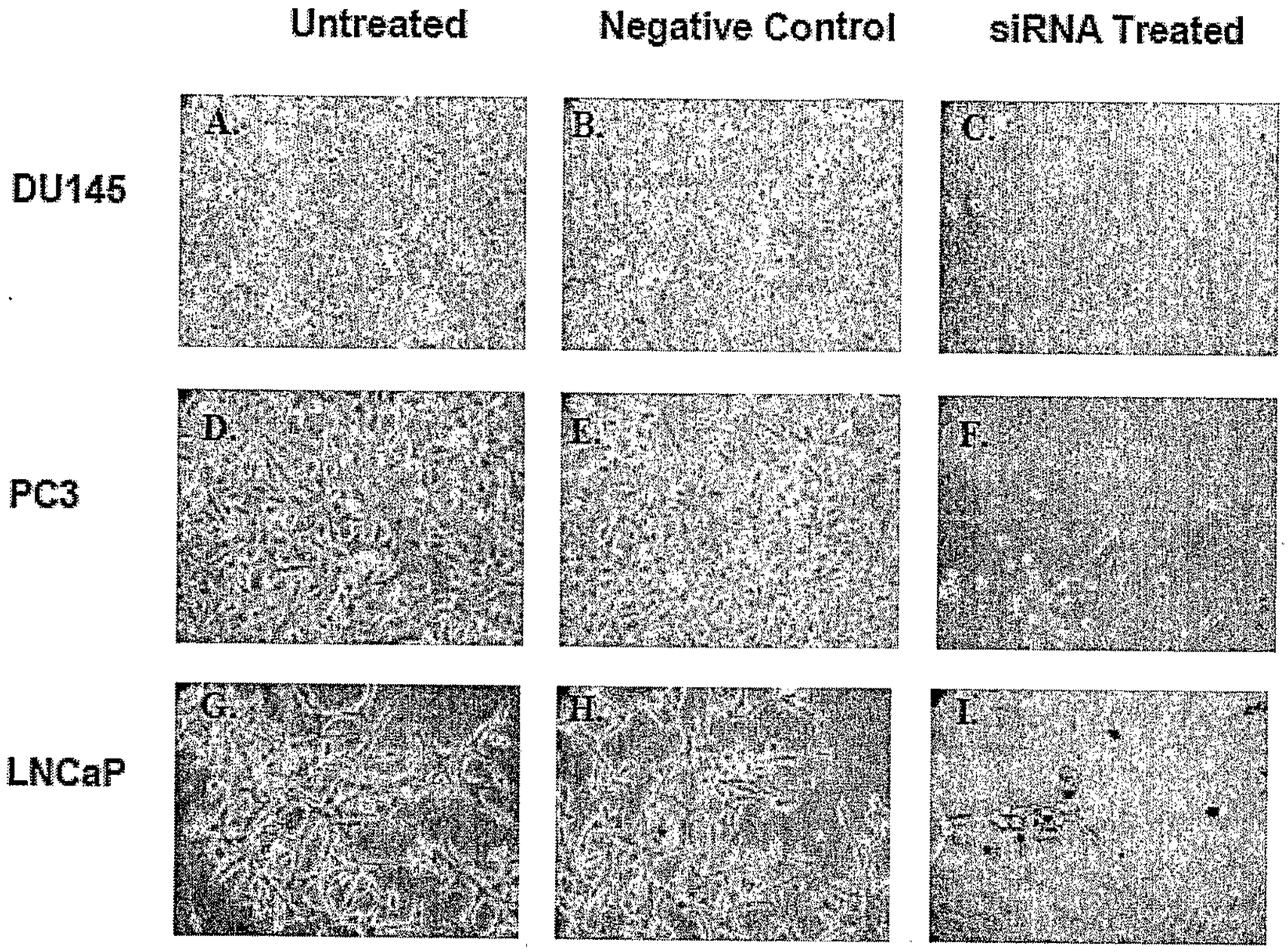

Untreated
Negative Control
siRNA Treated
DU145
PC3
LNCaP
A.
B.
C.
D.
E.
F.
G.
H.
I.

Figure 8.

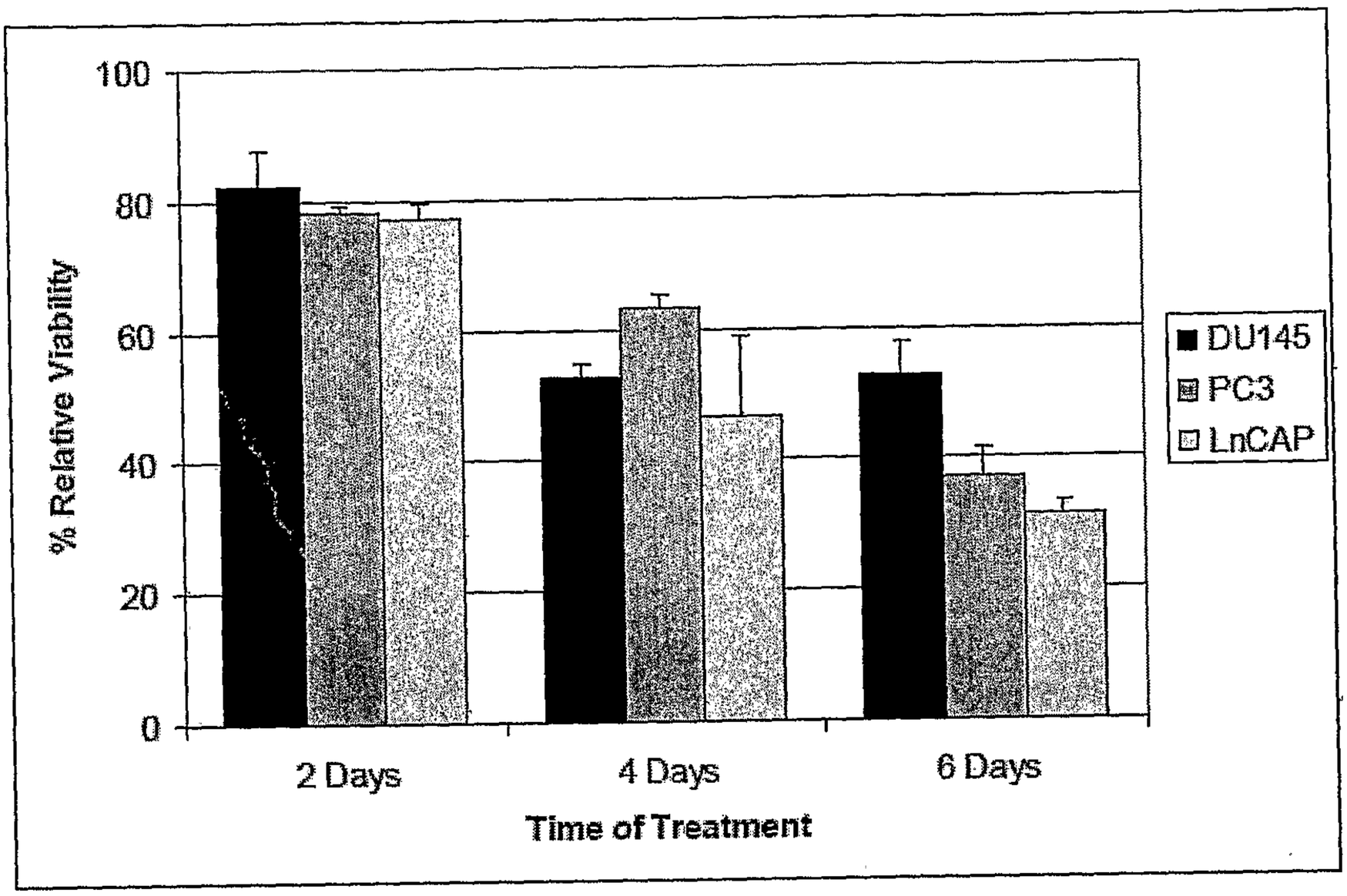

100
80
60
40
20
0
% Relative Viability
2 Days
4 Days
6 Days
Time of Treatment
DU145
PC3
LnCAP

Figure 9.

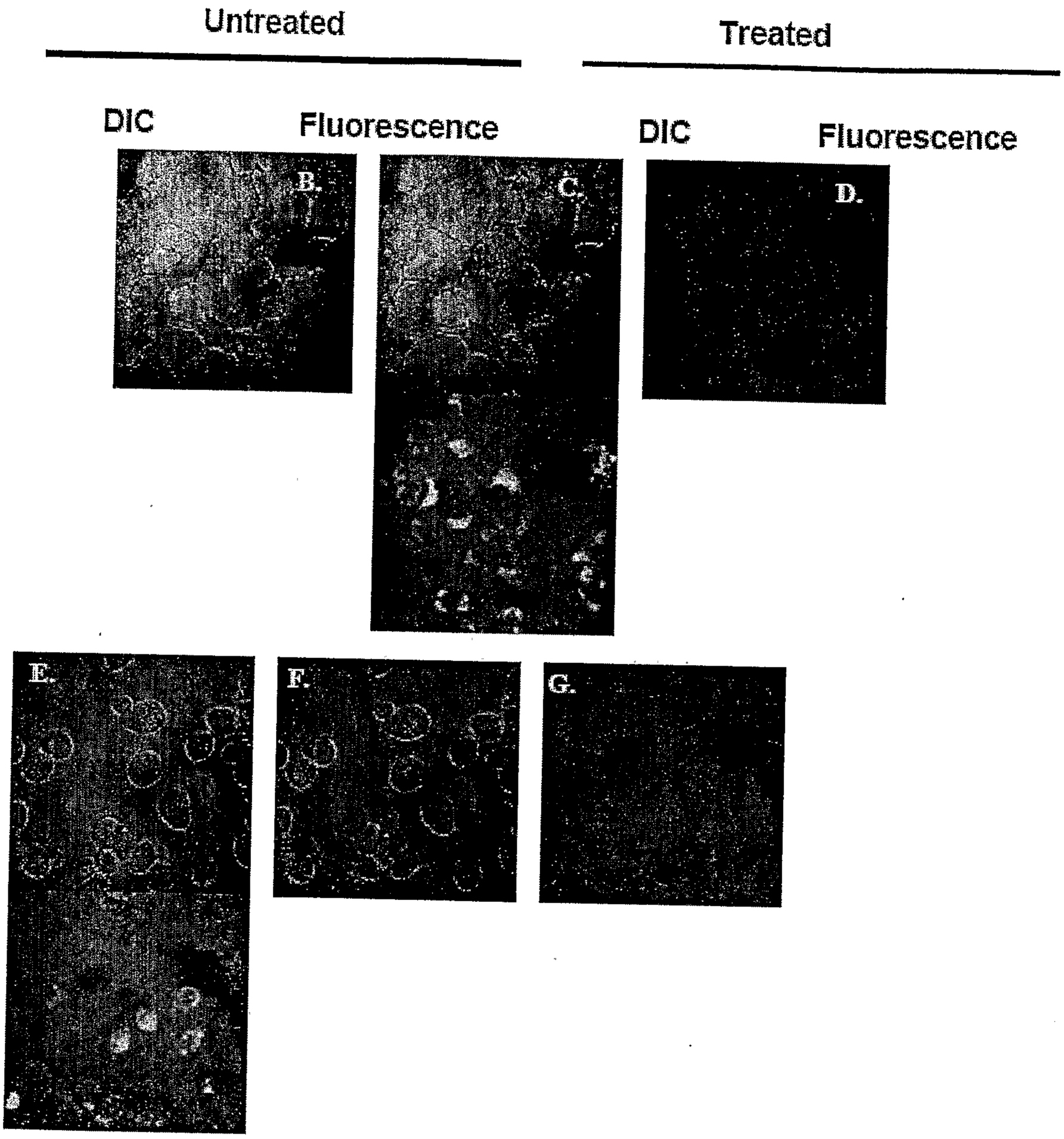


Figure 9. (cont'd)

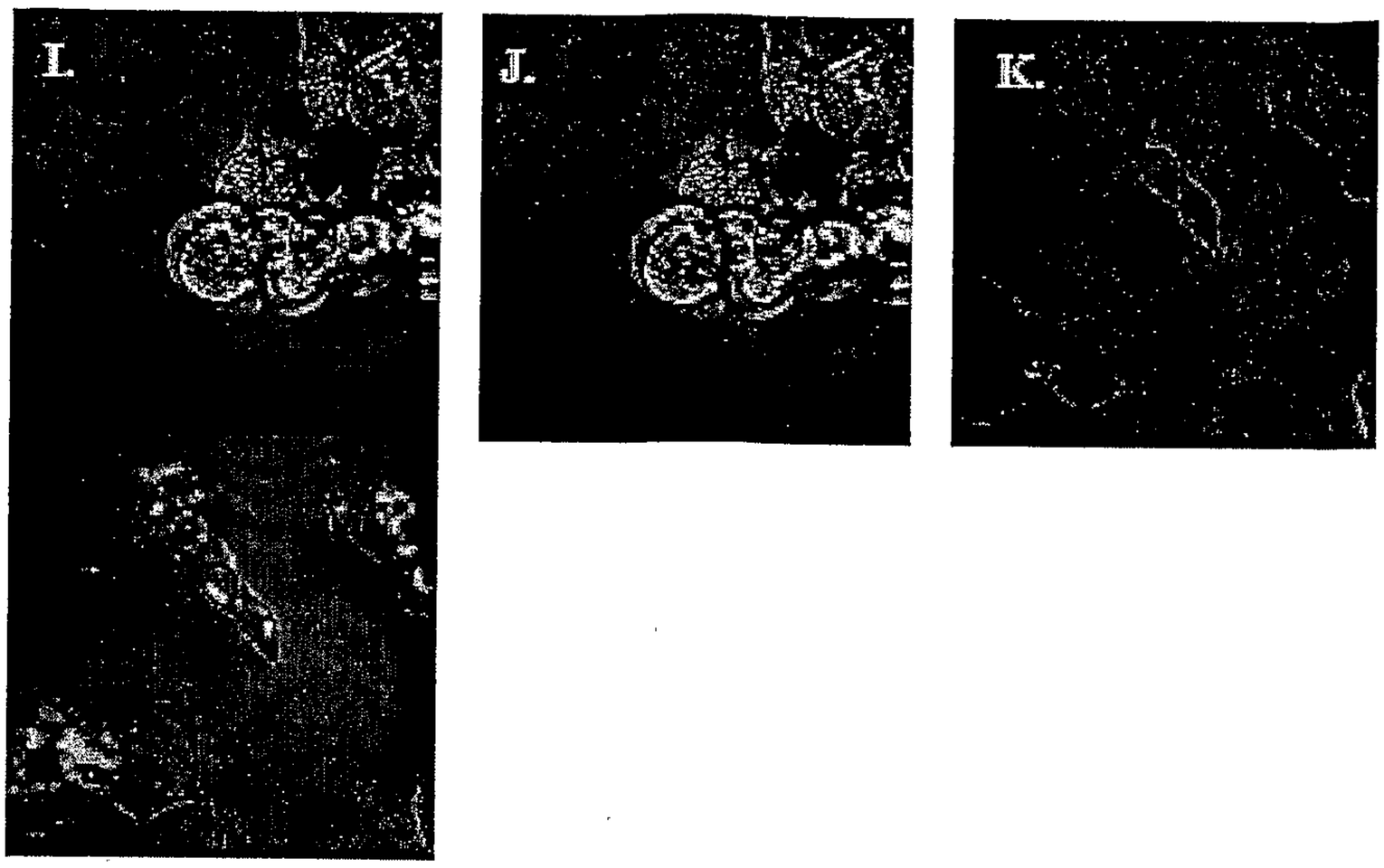
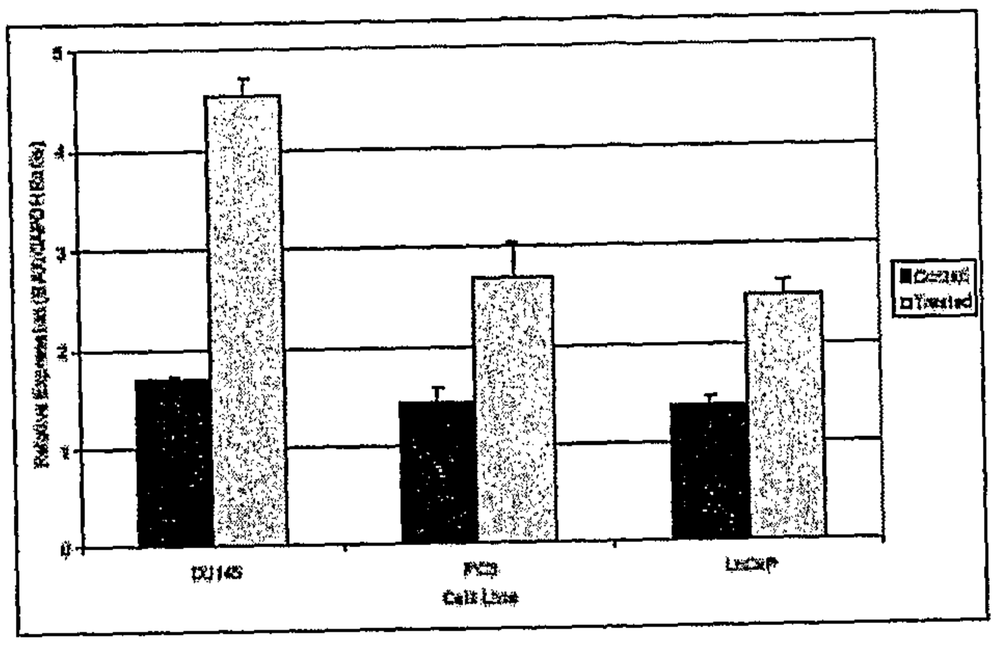
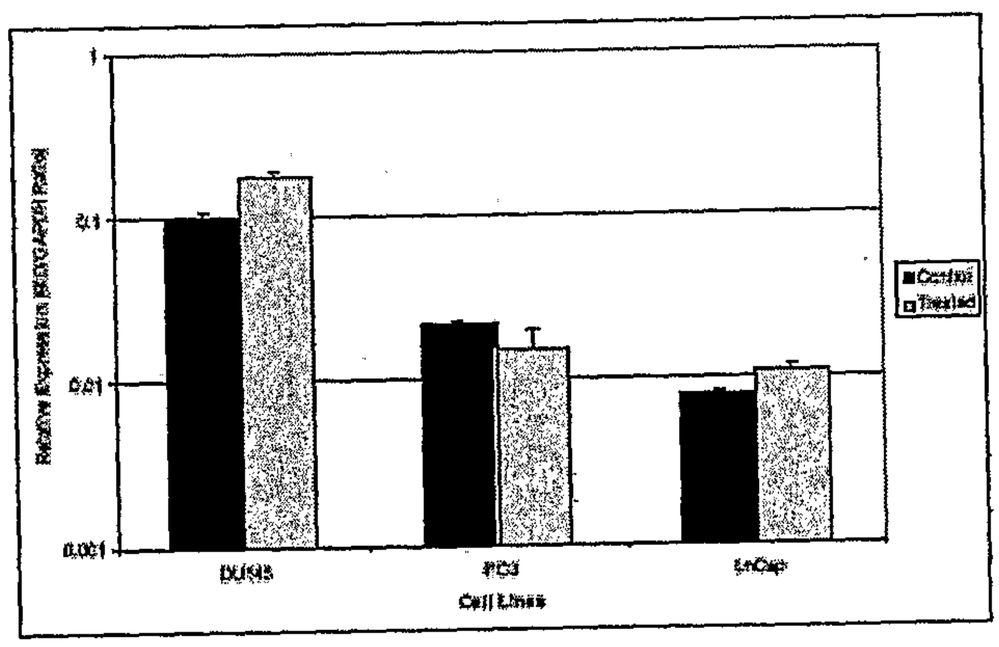

I.
J.
K.

Figure 10.

A.



B.



C.

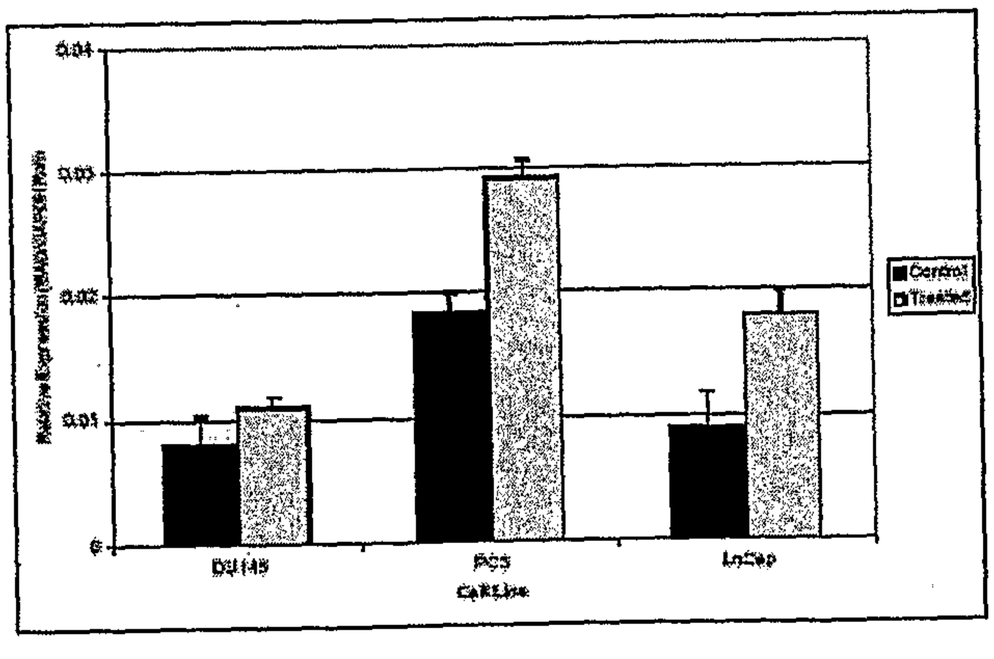


Figure 11.

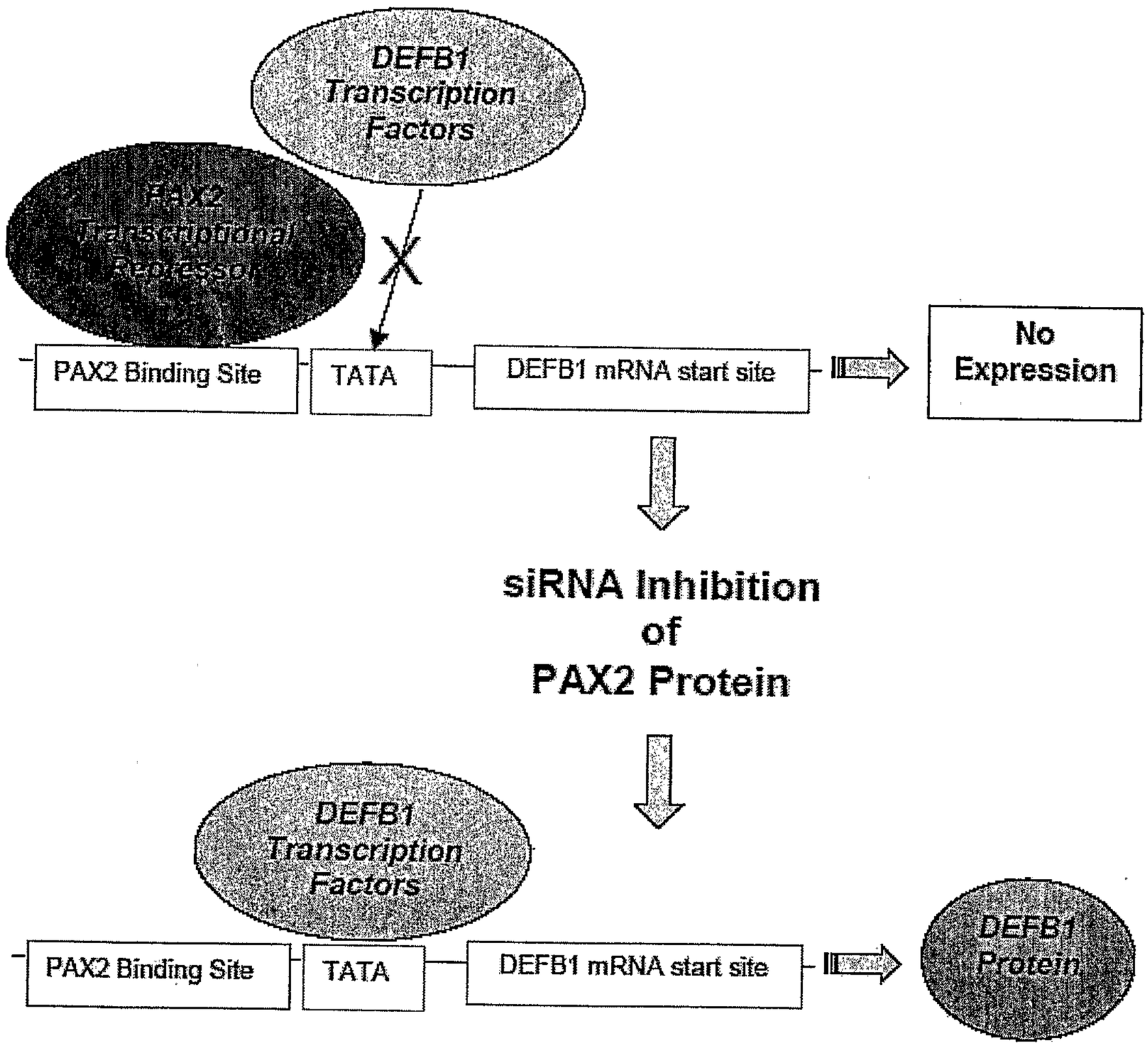

DEFB1 Transcription Factors
PAX2 Transcriptional Repressor
PAX2 Binding Site
TATA
DEFB1 mRNA start site
No Expression
siRNA Inhibition of PAX2 Protein
DEFB1 Transcription Factors
PAX2 Binding Site
TATA
DEFB1 mRNA start site
DEFB1 Protein

Figure 12.

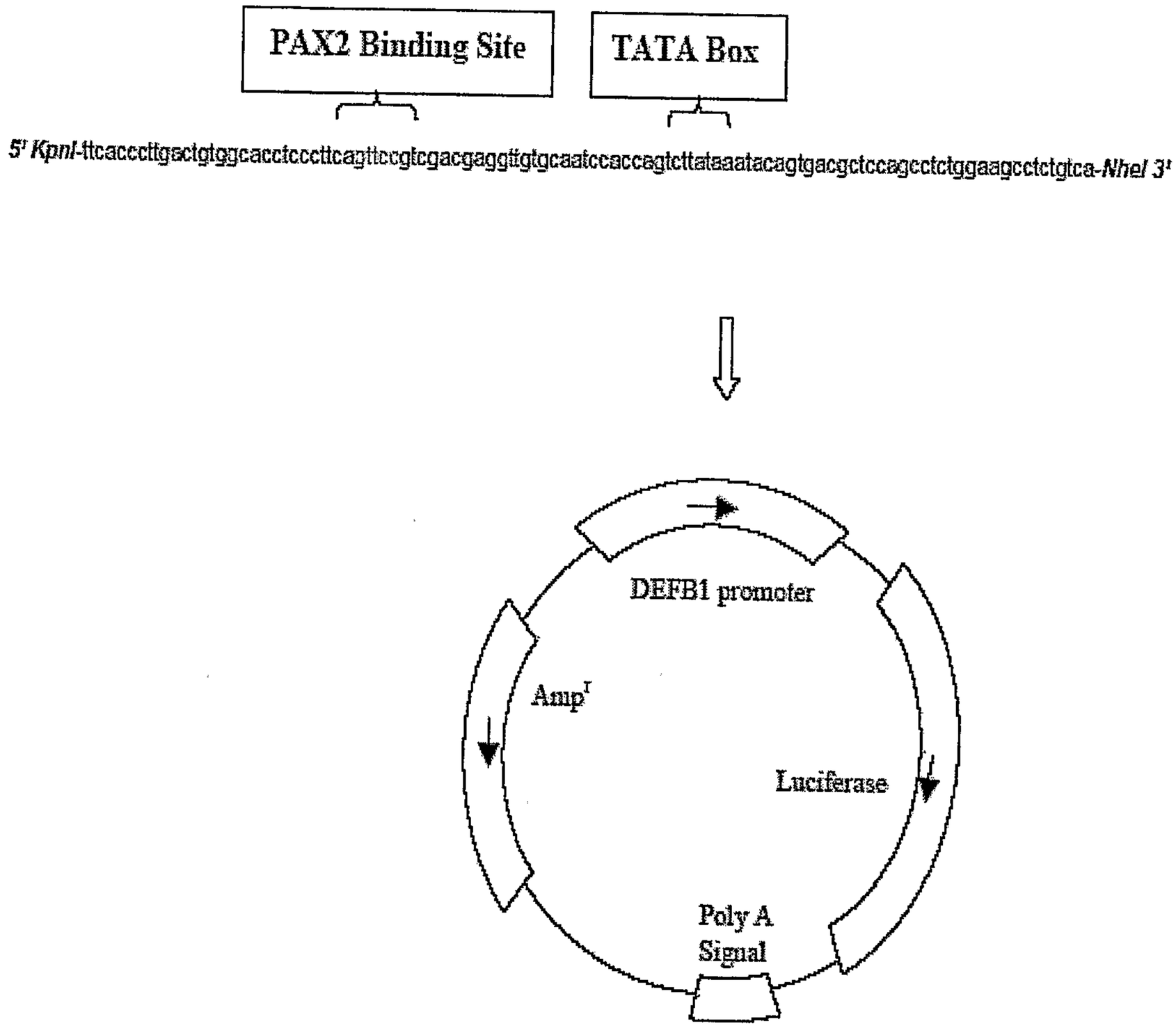

PAX2 Binding Site
TATA Box
5' KpnI-ttcacccttgactgtggcacctcccttcagttccgtcgacgaggttgtgcaatccaccagtcttataaatacagtgacgctccagcctctggaagcctctgtca-NheI 3'
DEFB1 promoter
Amp^r
Luciferase
Poly A
Signal

Figure 13.

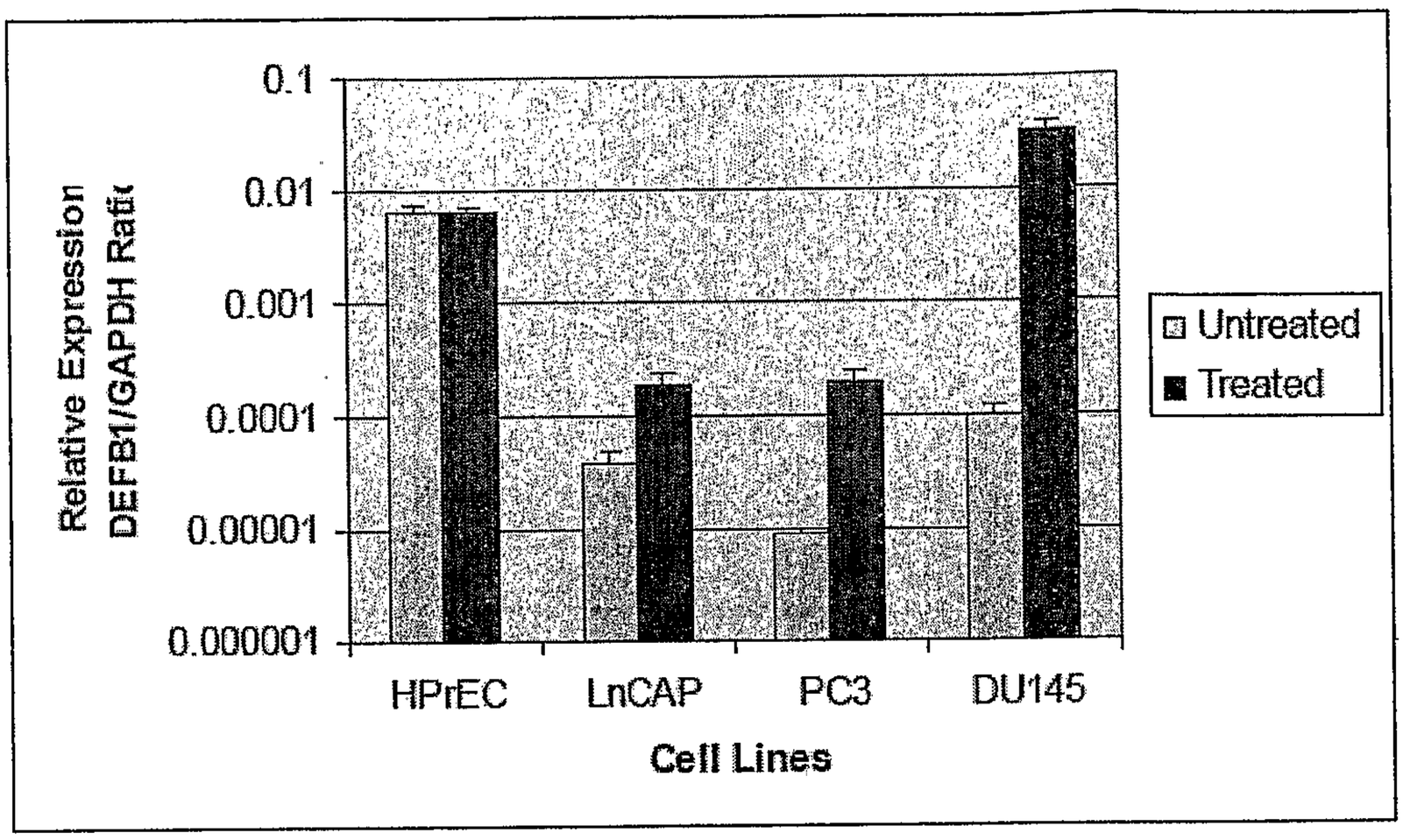

0.1
0.01
0.001
0.0001
0.00001
0.000001
Relative Expression
DEFB1/GAPDH Ratio
HPrEC
LnCAP
PC3
DU145
Cell Lines
Untreated
Treated

Figure 14.

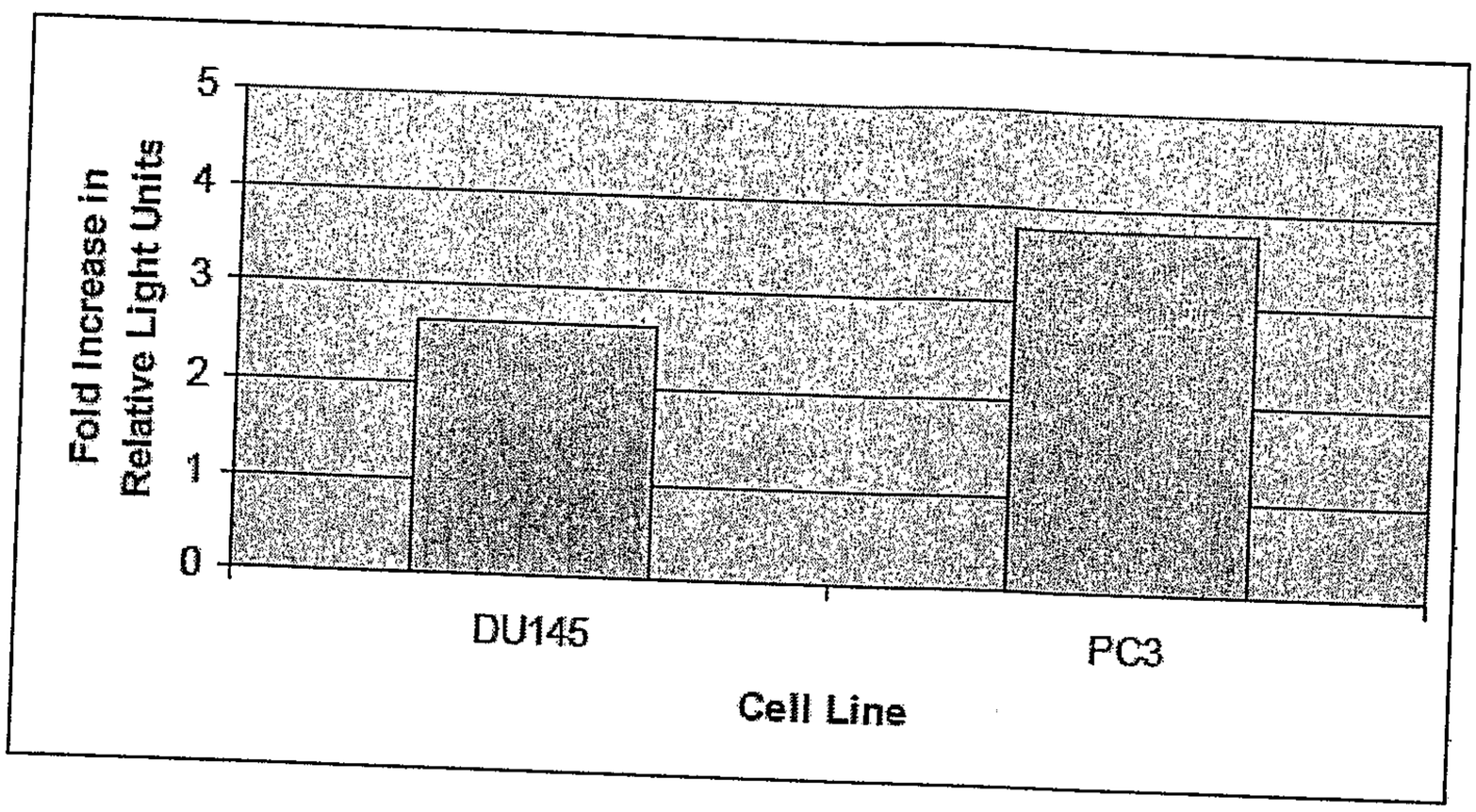

Fold Increase in Relative Light Units
0
1
2
3
4
5
DU145
PC3
Cell Line

Figure 15.

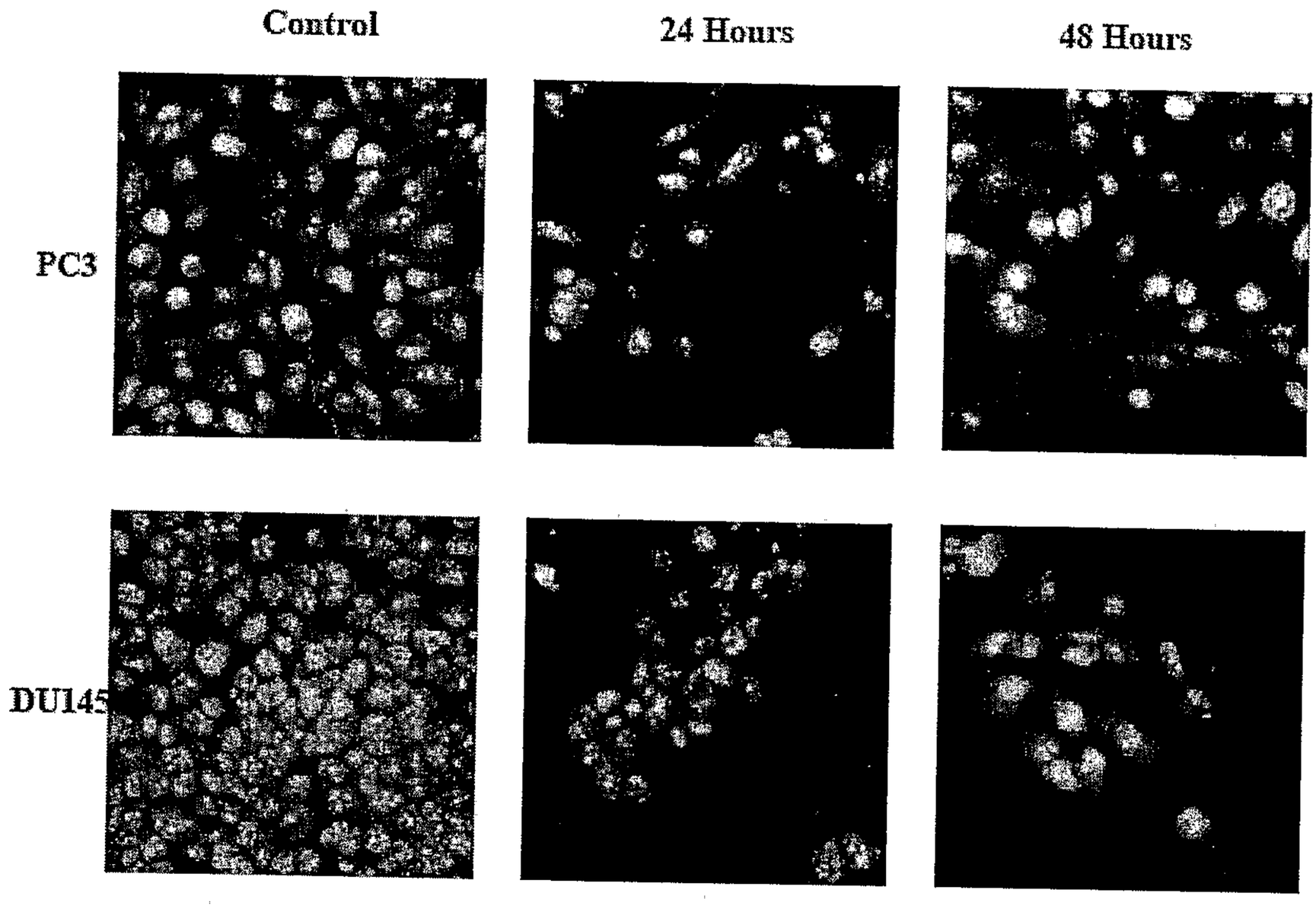


Figure 16.

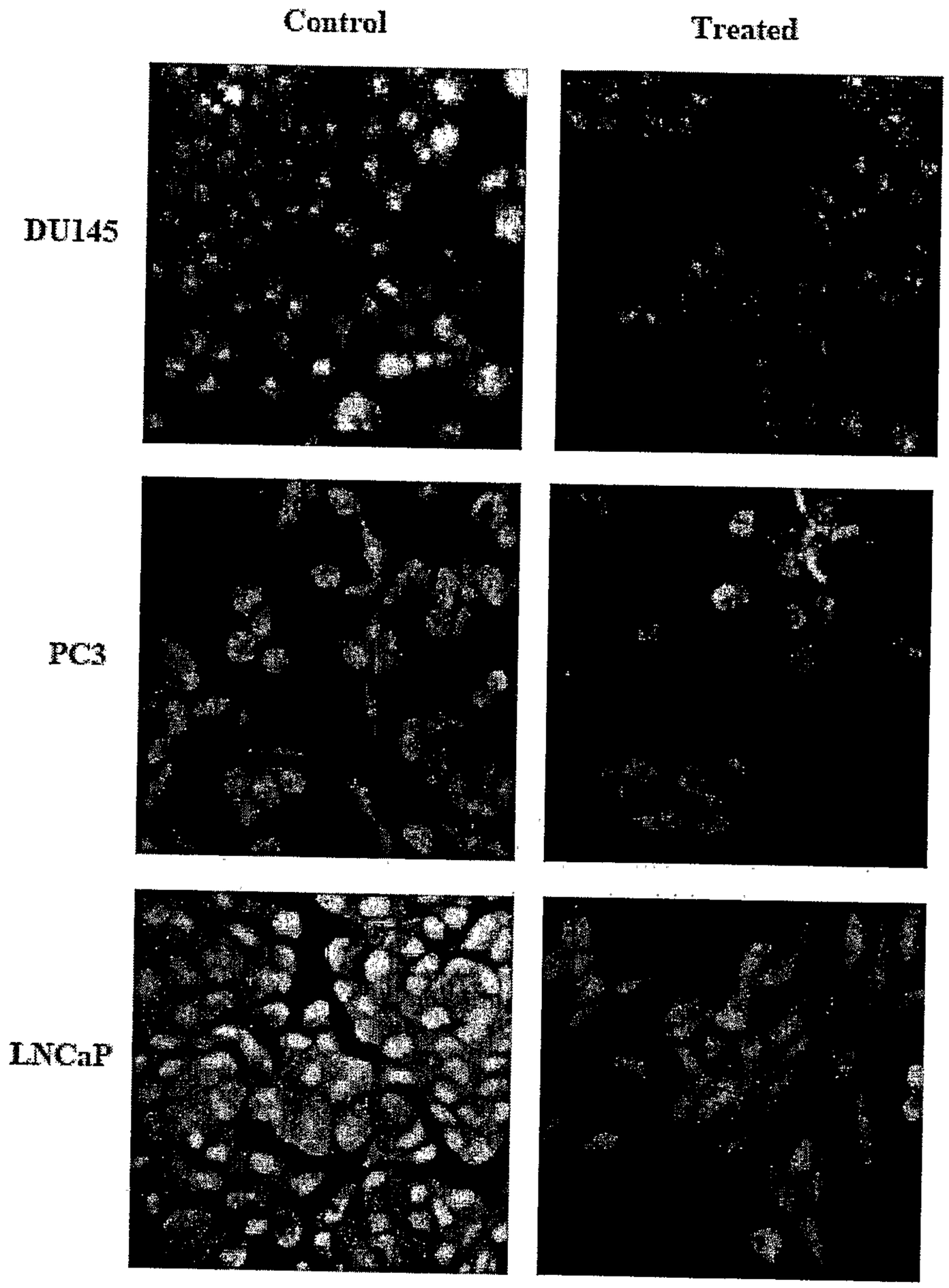


Figure 17.

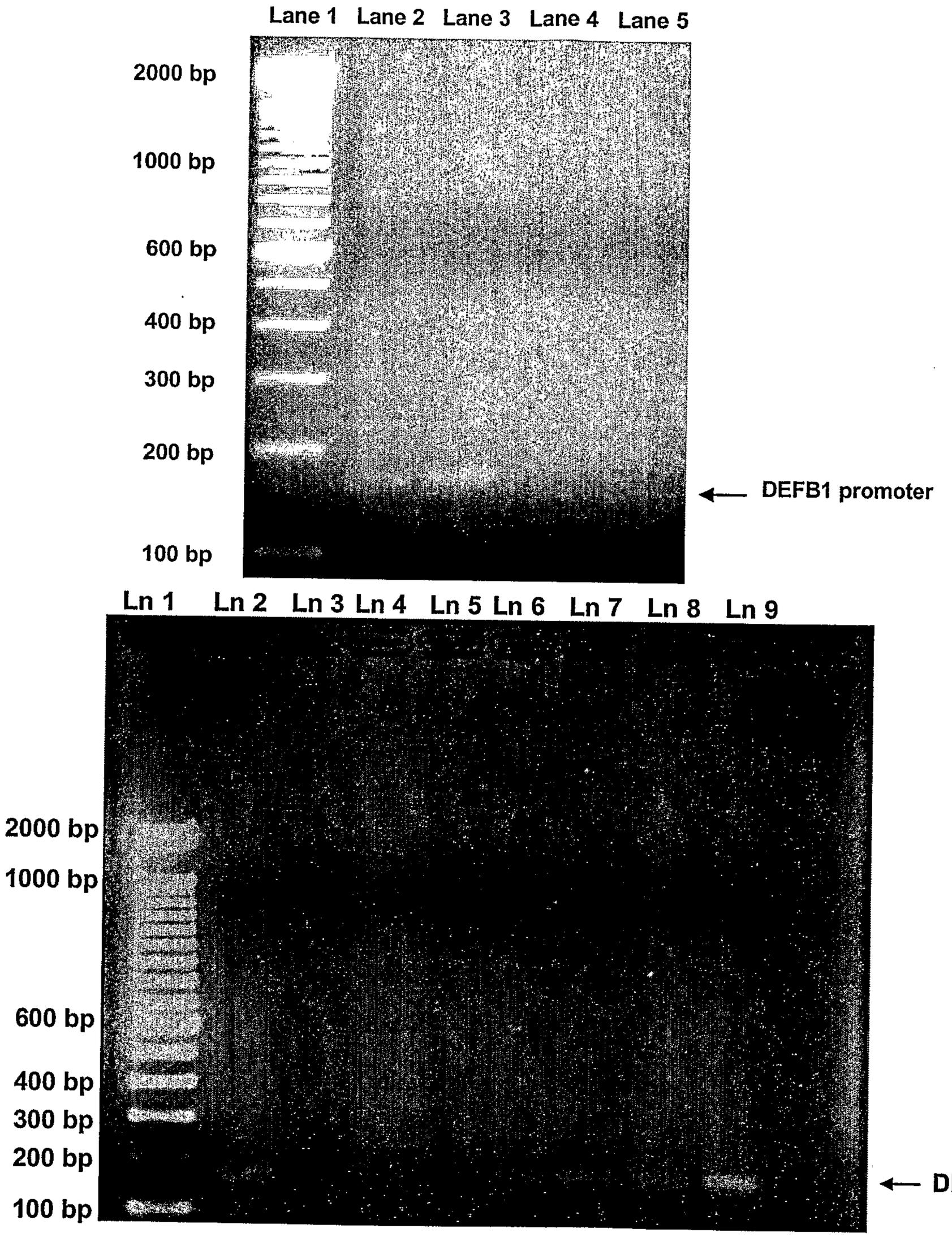

Lane 1
Lane 2
Lane 3
Lane 4
Lane 5
2000 bp
1000 bp
600 bp
400 bp
300 bp
200 bp
100 bp
DEFB1 promoter
Ln 1
Ln 2
Ln 3
Ln 4
Ln 5
Ln 6
Ln 7
Ln 8
Ln 9
2000 bp
1000 bp
600 bp
400 bp
300 bp
200 bp
100 bp
DEFB1 160 bp

Figure 18.

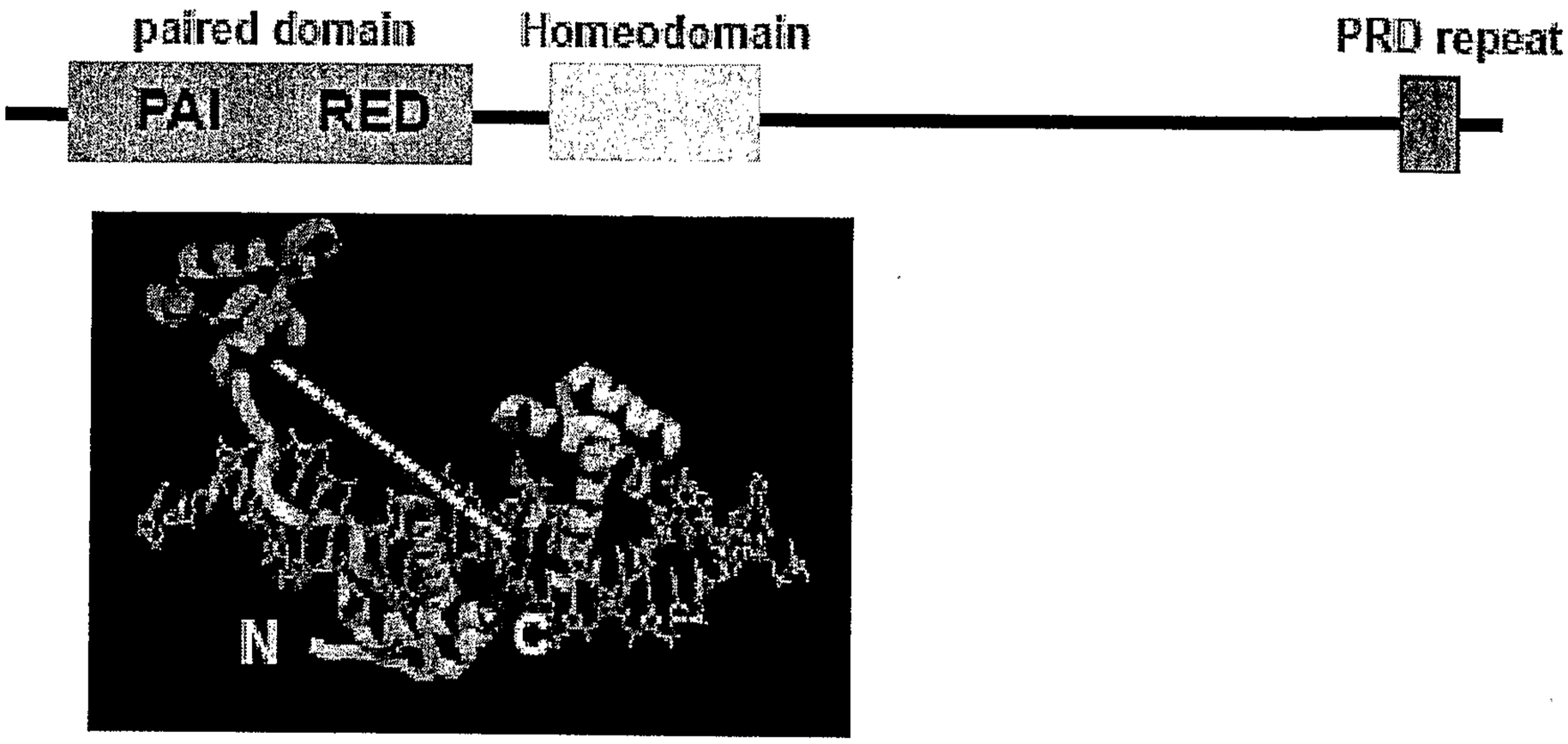

paired domain
Homeodomain
PRD repeat
PAI
RED
N
C
TCAAGCGTGACTAATTG
C
N

Figure 19: