PHENOTYPIC REVERSION OF PANCREATIC CARCINOMA CELLS

Inventors: Matthew Pincus, Brooklyn, NY (US); Josef Michl, Little Neck, NY (US)

Correspondence Address:
DILWORTH & BARRESE, LLP
333 EARLE OYINGTON BLVD., SUITE 702
UNIONDALE, NY 11553

Assignee: The Research Foundation of State University of New York, Albany, NY (US)

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Abstract

The present invention provides peptides (including analogs and derivatives thereof) corresponding to residues 96-110 and 35-47 of ras-p21, which peptides have attached thereto a membrane-penetrating leader sequence. The subject peptides, analogs and derivatives thereof are useful in treatment of cancers and have been shown to induce phenotypic reversion of pancreatic cancer cells to non-cancerous cells. Pharmaceutical compositions comprising one or more subject peptides are also provided by the present invention. The present invention further provides replication incompetent Adenovirus (AdV) vectors comprising a promoter sequence and a nucleotide sequence encoding a subject peptide. Methods of treating cancer by administering one or more subject peptides, pharmaceutical compositions, and/or AdV vectors are also provided.
PHENOTYPIC REVERSION OF PANCREATIC CARCINOMA CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is continuation application of U.S. Ser. No. 11/142,051, filed May 31, 2005, which claims the benefit of U.S. Provisional Application Serial No. 60/575,131, filed May 28, 2004, and U.S. Provisional Application Serial No. 60/575,846, filed June 1, 2004, both of which are incorporated by reference herein.

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

[0002] The present invention was funded in part by NIH Grant RO1 CA 42500; the government may have certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Oncogenic ras-p21 protein, but not its wild-type counterpart protein, induces malignant transformation of mammalian cell lines such as NIH 3T3 cells (1) and has been implicated as a major causative factor in a high proportion of human solid tissue tumors (2). In Xenopus laevis oocytes, microinjection of oncogenic (containing Val in place of Gly 12), but not wild-type, p21 induces oocyte maturation (3). Insulin induces oocyte maturation and requires activation of normal cellular ras-p21 (4).

[0004] Several agents that strongly block Val 12-p21-induced oocyte maturation have virtually no effect on insulin-induced maturation (5). Among these agents are specific peptides, identified from molecular modeling studies, that correspond to effector domains from both ras-p21 itself, such as the 35-47, 96-110 and 115-126 sequences (5) and from some of its target proteins such as the ras-binding domain of raf(residues 97-110) (6-8) and the SOS guanine nucleotide exchange protein residues 994-1004) (9,10). These peptide domains were identified as those that change conformation in response to the presence of single oncogenic amino acid substitutions at positions 12 or 61 or multiple substitutions at positions 10, 12 and 59 when the computed average structures for these proteins either alone or in complex with target proteins were superimposed on that for the wild-type protein.

[0005] The finding that these peptides (in addition to other agents) block oncogenic ras-p21 selectively indicates that the oncogenic protein induces mitogenesis by pathways that may overlap with, but are also distinct from, pathways utilized by the wild-type protein. In studies designed to identify pathway differences, it was found, that in oocytes, oncogenic but not insulin-activated wild-type p21 interacts with the transcriptional activating protein, jun and its kinase, jun kinase (JNK) (11,12), and requires the presence of protein kinase C (PKC) (13). In these studies, it was determined that the peptide whose sequence corresponds to p21 residues 96-110, called PNC-2, blocks the interaction of Val 12-p21 with JNK (11,12) in a dose-response curve that superimposes on that for its inhibition of Val 12-p21-induced oocyte maturation (5).

[0006] Additionally, the peptide whose sequence corresponds to p21 residues 35-47, called PNC-7, encompasses a domain of the protein implicated in its interacting with multiple targets including raf p74 protein, GTPase activating protein (GAP) and the guanine nucleotide exchange protein, SOS (reviewed in ref. 5). This peptide strongly inhibits c-raf-induced oocyte maturation but has no effect on oocyte maturation induced by an oncogenic mutant raf lacking the ras binding domain (RBD) in its amino terminal regulatory domain (14). Both PNC-2 and 7 appear to act on different steps on the oncogenic ras-p21 signal transduction pathway. For example, PNC-2 but not PNC-7 interferes with Val 12-p21-JNK interaction (11,12) while PNC-7 but not PNC-2 blocks signal transduction through c-raf (15).

[0007] Since various cancers involve expression of Val 12-p21 protein, as well as other oncogenic proteins, it would be useful to be able to inhibit expression of such proteins. For example, pancreatic cancer is a nearly always fatal disease with a median survival time of only 80-90 days for a patient diagnosed with the disease. Pancreatic cancer is one of the more lethal forms of cancer in numbers of patients killed in the U.S. Less than 4% of patients are alive 5 years from the time of diagnosis. The present invention provides peptides and pharmaceutical compositions comprising such peptides which when administered to pancreatic cancer cells, not only inhibit oncogenic Val 12-p21 but actually cause cancerous cells to phenotypically revert to non-cancerous cells. The present invention is therefore useful in treating various types of cancers which express Val 12-p21 protein and/or other oncogenic proteins. Treatment of ras-induced tumors converts malignant masses into benign ones, allowing for the halting of metastatic disease.

SUMMARY OF THE INVENTION

[0008] The present invention provides peptides comprising at least about ten contiguous amino acids of the amino acid sequence: YREQKRKDSDDVP (SEQ ID NO: 1), or an analog or derivative thereof, wherein said peptide, analog, or derivative thereof comprises a membrane-penetrating leader sequence attached thereto. Preferably, a peptide has the sequence set forth in SEQ ID NO: 1.

[0009] The present invention also provides peptides comprising at least about ten contiguous amino acids of the amino acid sequence: TEEDSYRKQVVD (SEQ ID NO:2) or an analog or derivative thereof wherein said peptide, analog, or derivative thereof comprises a membrane-penetrating leader sequence attached thereto. Preferably, a peptide has the sequence set forth in SEQ ID NO:2.

[0010] The peptides of the present invention, including analogs and derivatives thereof, are useful in treating cancer. Preferably, a peptide, analog or derivative thereof as provided herein has the membrane-penetrating leader sequence located at the carboxy terminal end. In another preferred embodiment, the leader sequence comprises predominantly positively charged amino acid residues. Examples of leader sequences for practicing the present invention include but are not limited to penetratin, Arg9, TAT of HIV1, D-TAT, R-TAT, SV40-NLS, nucleoplasmin-NLS, HIV REV, FHV coat, BMV GAG, HTLV-II (REX), CCMV GAG, P22N, Lambda N, Delta N, yeast PRP6, human U2AF, human C-FOS, human C-JUN, yeast GCN4, or p-vec.

[0011] Also provided by the present invention are pharmaceutical compositions comprising at least one of the subject peptides or analogs or derivatives thereof comprising a membrane-penetrating leader sequence admixed with a pharmaceutically acceptable carrier.

[0012] The present invention also provides methods of treating a patient suffering from cancer. The method comprises administering to said patient a therapeutically effective amount of at least one subject peptide, analog or derivative
thereof comprising a membrane penetrating leader sequence. In another embodiment of the invention, there is provided a method of treating a patient suffering from cancer by administering to said patient a therapeutically effective amount of a subject pharmaceutical composition. Preferably, the cancer to be treated is a ras-induced cancer.

[0013] In still another embodiment of the invention, there is provided a replication incompetent Adenovirus (AdV) vector comprising a promoter sequence operably linked to a nucleotide sequence encoding a peptide, wherein the peptide comprises at least about ten contiguous amino acids of the amino acid sequence: YREQIKVRKDSDDVP (SEQ ID NO: 1), or an analog or derivative thereof. A replication incompetent Adenovirus (AdV) vector comprising a promoter sequence operably linked to a nucleotide sequence encoding a peptide, wherein the peptide comprises at least about ten contiguous amino acids of the amino acid sequence: TIEDSKVRQVVID (SEQ ID NO: 2), or an analog or derivative thereof is also provided. Preferably, the nucleotide sequence further encodes a leader sequence attached to the sequence set forth in SEQ ID NO: 1, 2, or an analog or derivative thereof.

[0014] The present invention also provides a method of treating a patient suffering from cancer by administering to the patient, a therapeutically effective amount of a subject AdV vector. A method of inducing phenotypic reversion of cancerous cells to non-cancerous cells in a subject, by administering to the subject, a therapeutically effective amount of a subject AdV vector is also provided.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0015] FIG. 1A is a photomicrograph of untreated ras-transformed pancreatic cancer (TUC-3) cells.

[0016] FIG. 1B is a photomicrograph of TUC-3 cells treated with X13-leader peptide for two weeks.

[0017] FIG. 1C is a photomicrograph of untreated pancreatic acinar (BMPRA1) cells at confluence.

[0018] FIG. 1D is a photomicrograph of BMPRA1 cells treated with PNC-2-leader peptide, showing no change in morphology or cell viability.

[0019] FIG. 2A is a photomicrograph showing the effects of 100 pg/ml of PNC-2-leader on TUC-3 cells after two weeks of treatment.

[0020] FIG. 2B is a photomicrograph showing the effects of 100 μg/ml of PNC-2-leader on TUC-3 cells after one day of treatment. In the center of the figure, a focus of morphologically revertent cells is shown.

[0021] FIG. 2C is a photomicrograph showing the effects of 100 μg/ml PNC-7-leader peptide on TUC-3 cells after two weeks of treatment.

[0022] FIG. 3A is a photomicrograph taken one week after plating transfected (with PNC-2-expressing plasmid) viable TUC-3 cells in selective media. Foci of reversion can be observed (left and middle of figure). Remaining transformed cells can be seen on the right side of the figure.

[0023] FIG. 3B is a photomicrograph showing that all transfected (with PNC-2-expressing plasmid) TUC-3 cells revert two weeks after transfection and selection of viable cells.

[0024] FIG. 3C is a photomicrograph of TUC-3 cells transfected with PNC-7-expressing plasmid, two weeks after transfection, showing cell and nuclear enlargement. These cells grow sluggishly into stable monolayers.

[0025] FIG. 4A is a photograph of gel blots showing immunoprecipitation of jun-N-terminal kinase (JNK) (lane 6) and MAP kinase (MAPK or ERK) with Ha-ras-p21, immunoprecipitated from oocytes that were induced to maturity by microinjection of oncogenic Val 12-Ha-ras-21 and blotted.

[0026] FIG. 4B is a photograph of gel blots showing immunoprecipitation of jun-N-terminal kinase (JNK) (lane 6) and MAP kinase (MAPK or ERK) with Ha-ras-p21, immunoprecipitated from oocytes that were induced to maturity with insulin, which activates wild-type p210only raf was found to immunoprecipitate with Ha-ras in these oocytes.

**DETAILED DESCRIPTION OF THE EMBODIMENT**

[0027] In accordance with the present invention, it has been surprisingly discovered that peptides designed from molecular modeling studies of the ras-p21 protein induce phenotype reversion of a pancreatic carcinoma cell line but have no effect on normal pancreatic acinar cell growth. The two peptides, designated PNC-2 and PNC-7, block oncogenic ras-induced oocyte maturation but do not block insulin-activated wild type ras-induced maturation.

[0028] Since various cancers involve expression of Val 12-21 protein, inhibition of this protein as well as phenotypic reversion of cancerous cells expressing this protein upon treatment of PNC-2 and/or PNC-7, represents a valuable cancer therapy. One out of every three solid tumors involves expression of Val 12-p21. For example, between 50-75% of colon cancers, greater than 90% of pancreatic cancers, one third of all non-small cell carcinomas of the lung, one fifth of gastric and bladder cancers, as well as many mesotheliomas involve expression of oncogenic ras-p21 protein.

[0029] In accordance with the present invention, the peptides PNC-2, PNC-7, analogs and derivatives of such peptides, pharmaceutical preparations and methods of treatment using PNC-2, PNC-7 peptides, analogs, derivatives thereof and pharmaceutical preparations based thereon, are useful in treating a variety cancers. Preferably, the cancers which are treated with the peptides pharmaceutical compositions and methods of the present invention are ras-induced cancers. Treatment of ras-induced tumors by the compositions of the present invention convert malignant masses into benign ones, allowing for the stopping of metastatic disease.

[0030] In one aspect of the invention, there is provided a peptide comprising at least about ten contiguous amino acids of the amino acid sequence: YREQIKVRKDSDDVP (SEQ ID NO: 1) or an analog or derivative thereof. In a preferred embodiment of the invention, the peptide is designated PNC-2 and comprises the 15 amino acids as set forth in SEQ ID NO: 1.

[0031] In another aspect of the invention, there is provided a peptide comprising at least about ten contiguous amino acids of the amino acid sequence: TIEDSKVRQVVID (SEQ ID NO: 2), or an analog or derivative thereof. In a preferred embodiment of the invention, the peptide is designated PNC-7 and comprises the 15 amino acids as set forth in SEQ ID NO: 2.

[0032] Preferably, the peptides having the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2, or an analog or derivative thereof, are fused to a membrane-penetrating leader sequence. In order to be transported across a cell membrane and effect reversion of cancerous cells to normal phenotype, the leader sequence is preferably positioned at the carboxyl terminal end of the peptide, analog, or derivative thereof. Preferably, the leader sequence comprises predominantly positively charged amino acid residues.
Examples of leader sequences which may be used in accordance with the present invention include but are not limited to penetratin, Arg, TAF of HIV1, D-TAT, R-TAT, SV40-NLS, nucleoplasmin-NLS, HIV REV (34-50), FHV coat (35-40), BMV GAG (7-25), HTLV-II REX (4-16), CCMV GAG (7-25), P22N (14-30), Lambda N (1-22), Delta N (12-29), yeast PRP6, human U2AF, human C-FOS (130-164), human C-JUN (252-279), yeast GCN4, and p-vec. Preferably, the leader sequence is the penetratin sequence from antennapedia protein having the amino acid sequence KKWKMRNQF-WVKVQRF (SEQ ID NO:3).

**[0033]** Pharmaceutical compositions comprising at least one of the subject peptides admixed with a pharmaceutically acceptable carrier are also provided. In addition, methods for treating neoplastic disease (cancer) in a subject i.e., inducing phenotypic reversion of cancerous cells to benign cells in a subject suffering from cancer, are provided. In one embodiment, the method comprises administering to the subject, a therapeutically effective amount of a peptide comprising at least about ten contiguous amino acids of the amino acid sequence: YREQKRKV KDSIDVPR (SEQ ID NO: 1), or an analog or derivative thereof. Preferably, the peptide or analog or derivative thereof is fused to a membrane-penetrating leader sequence and confers a normal phenotype on cancerous cells. Even more preferably, the membrane-penetrating leader sequence is fused to the carboxy terminal end of the peptide, analog, or derivative thereof. The cancer is preferably a ras-induced cancer.

**[0034]** In another embodiment, the method comprises administering to the subject suffering from cancer, a therapeutically effective amount of a peptide having the sequence set forth in TIEDSYRKKVQVTD (SEQ ID NO:2), or an analog or derivative thereof. Preferably, the peptide or analog or derivative thereof is fused to a membrane-penetrating leader sequence and confers a normal phenotype on cancerous cells. Even more preferably, the membrane-penetrating leader sequence is fused to the carboxy terminal end of the peptide, analog, or derivative thereof. The cancer is preferably a ras-induced cancer.

**[0035]** In still another embodiment of the invention, the method comprises administering to a subject suffering from cancer, a therapeutically effective amount of a mixture of peptides having the sequence set forth in SEQ ID NO: 1 and SEQ ID NO: 2, or analogs or derivatives thereof. Preferably, the peptides or analogs or derivatives thereof are fused to a membrane-penetrating leader sequence and confer a normal phenotype on cancerous cells. Even more preferably, the membrane-penetrating leader sequence is fused to the carboxy terminal end of the peptides, analogs, or derivatives thereof. The cancer is preferably a ras-induced cancer.

**[0036]** Leader sequences which function to import the peptides of the invention into a cell may be derived from a variety of sources. Preferably, the leader sequence comprises predominantly positively charged amino acid residues since a positively charged leader sequence stabilizes the alpha helix of a subject peptide. Examples of leader sequences which may be linked to the peptides of the present invention are described in Futaki, S. et al (2001) Arginine-Rich Peptides, J. Biol. Chem. 276:5836-5840, and include but are not limited to the following membrane-penetrating leader sequences (numbering of the amino acid residues making up the leader sequence of the protein is indicated parenthetically immediately after the name of the protein in many cases):

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penetratin (KEKWKMRNQF-WVKVQRF);  (SEQ ID NO:3)
(Arg)_6 or any poly-R from [R]_4-[-R]_4;  (SEQ ID NO:27)
HIV-1 TAT (47-60) (YRKERRQSERPRPPQ);  (SEQ ID NO:4)
D-TAT (GKRERRQSERPRPPQ);  (SEQ ID NO:5)
R-TAT (GKRERRQSERPRPPQ);  (SEQ ID NO:6)
SV40-NLS (PEKRRKV);  (SEQ ID NO:7)
nucleoplasmin-NLS (KRPQAIQKQQAQKIK);  (SEQ ID NO:8)
HIV REV (34-49) (EGRQARRRNRWRRRQR);  (SEQ ID NO:9)
FHV (35-49) coat- (KEKRRHRRRVR);  (SEQ ID NO:10)
BMV GAG (7-25) (KNTAQRRAAARRHRWTR);  (SEQ ID NO:11)
HTLV-II REX 4-16- (TKRQGRRARHR);  (SEQ ID NO:12)
CCMV GAG (7-25) (-ELRAQRAAARRHRKENTR);  (SEQ ID NO:13)
P22 N (14-29) (NATKRTHRRRKLAIKR);  (SEQ ID NO:14)
LAMBDA N (1-22) (MDAQTNRRRRAAEEQAKAQK);  (SEQ ID NO:15)
Phi N (12-29) (TAKTRKARASLRRARR);  (SEQ ID NO:16)
YRAST PRP6 (129-124) (TRENKRFRQEQMLQKR);  (SEQ ID NO:17)
HUMAN U2AF (SQMTIQAELTVY);  (SEQ ID NO:18)
HUMAN C-POS (139-164) KRRRERKKRQAAGIRRRRSLTD; (SEQ ID NO:19)
HUMAN C-JUN (252-279) (KKRRRERRR1AASERKRKLK); (SEQ ID NO:20)
YRAST GCN4 (KRANIREAARRKRKLQSMH);  (SEQ ID NO:21)
KLAKLKLAKLAKLAK;  (SEQ ID NO:22)
p-vec LILIIQRRQKQAKS.
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**[0037]** Other membrane penetrating leader sequences may also be used. Such sequences are widely available and are described e.g., in Scheller et al. (2000) Eur. J. Biochem. 267:6043-6049, and Elmqist et al., (2001) Exp. Cell Res. 269:237-244.

**[0038]** Preferably, the positively charged leader sequence of the penetratin leader sequence of antennapedia protein is used. This leader sequence has the following amino acid
sequence: KKWKMRRNQFWVKVQRG (SEQ ID NO: 3). Preferably, the leader sequence is attached to the carboxyl terminal end of a subject peptide to enable the synthetic peptide to effect phenotypic reversion of cancerous cells. [0039] Structurally related amino acid sequences may be substituted for the disclosed sequences set forth in SEQ ID NOs: 1 or 2 in practicing the present invention. Amino acid insertional derivatives of the peptides of the present invention include amino and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a pre-determined site in a subject peptide although random insertion is also possible with suitable screening of the resulting product. Deletional variants may be made by removing one or more amino acids from the sequence of a subject peptide. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Typical substitutions are those made in accordance with the following Table 1:

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>Ser</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>Gln; His</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>Glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>Ser</td>
</tr>
<tr>
<td>Gln (Q)</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>Asp</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>Pro</td>
</tr>
<tr>
<td>His (H)</td>
<td>Asn; Gln</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>Leu; Val</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>Ile; Val</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>Arg; Gln; Gln</td>
</tr>
<tr>
<td>Met (M)</td>
<td>Leu; Ile</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>Met; Leu; Tyr</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>Ser</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>Tyr</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>Trp; Phe</td>
</tr>
<tr>
<td>Val (V)</td>
<td>Ile; Leu</td>
</tr>
</tbody>
</table>

[0040] When the synthetic peptide is derivatized by amino acid substitution, the amino acids are generally replaced by other amino acids having like properties such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains and the like. As used herein, the terms “derivative,” “analogue,” “fragment,” “portion” and “like molecule” refer to a subject peptide having the amino acid sequence as set forth in SEQ ID NOs: 1 or 2, having an amino acid substitution, insertion, addition, or deletion, as long as said derivative, analogue, fragment, portion, or like molecule retains the ability to enter and effect phenotypic reversion of cancer cells, while having no effect on normal, non-cancerous cells.

[0041] The synthetic peptides of the present invention may be synthesized by a number of known techniques. For example, the peptides may be prepared using the solid-phase technique initially described by Merrifield (1963) in J. Am. Chem. Soc. 85:2149-2154. Other peptide synthesis techniques may be found in M. Bodanszky et al. Peptide Synthesis: John Wiley and Sons, 2d Ed., (1976) and other references readily available to those skilled in the art. A summary of polypeptide synthesis techniques may be found in J. Sturart and J. S. Young, Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, III., (1984). Peptides may also be synthesized by solution methods as described in The Proteins, Vol. 1, 3d Ed., Neurath, H. et al., Eds., pp. 105-237, Academic Press, New York, N.Y. (1976). Appropriate protective groups for use in different peptide syntheses are described in the texts listed above as well as in J. F. W. McMinnie, Protective Groups in Organic Chemistry, Plenum Press, New York, N.Y. (1973). The peptides of the present invention may also be prepared by chemical or enzymatic cleavage from larger portions of the ras-p21 protein or from the full-length ras-p21 protein. Likewise, leader sequences for use in the synthetic peptides of the present invention may be prepared by chemical synthesis or enzymatic cleavage from larger portions or the full-length proteins from which such leader sequences are derived.

[0042] Additionally, the peptides of the present invention may also be prepared by recombinant DNA techniques. For most amino acids used to build proteins, more than one coding nucleotide triplet (codon) can code for a particular amino acid residue. This property of the genetic code is known as redundancy. Therefore, a number of different nucleotide sequences may code for a particular subject peptide. The present invention also contemplates use of a deoxyribonucleic acid (DNA) molecule that defines a gene coding for, i.e., capable of expressing a subject peptide or a chimeraic peptide from which a peptide of the present invention may be enzymatically or chemically cleaved.

[0043] Consistent with the observed properties of the peptides of the invention, the subject peptides may be used to induce phenotypic reversion of neoplastic or malignant cells, i.e., cancer cells in animals, preferentially humans. The synthetic peptides of the present invention are thus administered in an effective amount to convert malignant cells or masses into benign cells or masses in a subject animal or human. Reversion of cancerous cells or masses into benign cells or masses would have an additional benefit of halting metastasis and the spread of metastatic disease.

[0044] The synthetic peptides of the present invention may be administered preferably to a human patient as a pharmaceutical composition containing a therapeutically effective dose of at least one synthetic peptide according to the present invention together with a pharmaceutically acceptable carrier. The term “therapeutically effective amount” or “therapeutically effective amount” means the dose needed to produce an individual, phenotypic reversion of neoplastic or malignant cells, i.e., cancer cells to benign or non-cancerous cells.

[0045] Preferably, compositions containing one or more of the synthetic peptides of the present invention are administered intravenously for the purpose of treating neoplastic or malignant disease such as cancer. Examples of different cancers which may be effectively treated using one or more the peptides of the present invention include but are not limited to: breast cancer, prostate cancer, lung cancer, cervical cancer, colon cancer, melanoma, pancreatic cancer and all solid tissue tumors (epithelial cell tumors) and cancers of the blood including but not limited to lymphomas and leukemias. Preferably, the cancer to be treated in accordance with the present invention is a ras-induced cancer such as colon cancer, pancreatic cancer, non-small cell carcinoma of the lung, gastric cancer, bladder cancer and mesotheliomas. Most preferably the cancer to be treated is pancreatic cancer.

[0046] Administration of the synthetic peptides of the present invention may be by oral, intravenous, intranasal, suppository, intraperitoneal, intramuscular, intradermal or
subcutaneous administration or by infusion or implantation. When administered in such manner, the synthetic peptides of the present invention may be combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of the other ingredients, except that they must be pharmaceutically acceptable, efficacious for their intended administration, cannot degrade the activity of the active ingredients of the compositions, and cannot impede importation of a subject peptide into a cell. The peptide compositions may also be impregnated into transdermal patches, or contained in subcutaneous inserts, preferably in a liquid or semi-liquid form which patch or insert time-releases therapeutically effective amounts of one or more of the subject synthetic peptides.

[0047] The pharmaceutical forms suitable for injection include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The ultimate solution form in all cases must be sterile and fluid. Typical carriers include a solvent or dispersion medium containing, e.g., water buffered aqueous solutions, i.e., biocompatible buffers, ethanol, polyols such as glycerol, propylene glycol, polyethylene glycol, suitable mixtures thereof, surfactants or vegetable oils. Sterilization may be accomplished utilizing any art-recognized technique, including but not limited to filtration or addition of antibacterial or antifungal agents. Examples of such agents include paraben, chlorbutanol, phenol, sorbic acid or thimerosal. Isotonic agents such as sugars or sodium chloride may also be incorporated into the subject compositions.

[0048] As used herein, a “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic agents and the like. The use of such media and agents are well known in the art.

[0049] Production of sterile injectable solutions containing the subject synthetic peptides is accomplished by incorporating one or more of the subject synthetic peptides described hereinabove in the required amount in the appropriate solvent with one or more of the various ingredients enumerated above, as required, followed by sterilization, preferably filter sterilization. In order to obtain a sterile powder, the abovementioned solutions are vacuum-dried or freeze-dried as necessary.

[0050] Inert diluents and/or assimilable edible carriers and the like may be part of the pharmaceutical compositions when the peptides are administered orally. The pharmaceutical compositions may be in hard or soft shell gelatin capsules, be suspended into tablets, or may be in an elixir, suspension, syrup or the like.

[0051] The subject synthetic peptides are thus compounded for convenient and effective administration in pharmaceutically effective amounts with a suitable pharmaceutically acceptable carrier in a therapeutically effective dosage. Examples of a pharmaceutically effective amount include peptide concentrations in the range from about at least about 25 µg/ml to at least about 300 µg/ml.

[0052] A precise therapeutically effective amount of synthetic peptide to be used in the methods of the invention applied to humans cannot be stated due to variations in stage of neoplastic disease, tumor size and aggressiveness, the presence or extent of metastasis, etc. In addition, an individual’s weight, gender, and overall health must be considered and will affect dosage. It can be generally stated, however, that the synthetic peptides of the present invention be administered in an amount of at least about 10 mg per dose, more preferably in an amount up to about 1000 mg per dose. Since the peptide compositions of the present invention will eventually be cleared from the bloodstream, re-administration of the pharmaceutical compositions is indicated and preferred.

[0053] The synthetic peptides of the present invention may be administered in a manner compatible with the dosage formulation and in such an amount as will be therapeutically effective. Systemic dosages depend on the age, weight, and condition of the patient and the administration route. An exemplary suitable dose for the administration to adult humans ranges from about 0.1 to about 20 mg per kilogram of body weight. Preferably, the dose is from about 0.1 to about 10 mg per kilogram of body weight.

[0054] In accordance with the present invention, there is also provided a method of treating neoplastic disease. The method comprises administering to a subject in need of such treatment, a therapeutically effective amount of a synthetic peptide hereinbefore described, including analogs and derivatives thereof. Thus for example, in one embodiment, an effective amount of a peptide comprising at least about ten contiguous amino acids as set forth in SEQ ID NO: 1 or an analog or derivative thereof, fused on its carboxy terminal end to a leader sequence may be administered to a subject. An effective amount of a peptide having the amino acid sequence as set forth in SEQ ID NO:2 or an analog or derivative thereof, fused on its carboxy terminal end to a leader sequence may also be administered to a subject. In accordance with a method of treatment, a mixture of synthetic peptides may be administered. Thus, for example, in addition to administering one of the peptides, or analogs or derivatives thereof hereinbefore described in an effective amount, mixtures of two or more peptides or analogs or derivatives hereinbefore described may be administered to a subject.

[0055] In another aspect of the present invention, there are provided expression vehicles comprising replication incompetent Adenovirus (AdV) and having a promoter sequence operably linked to a coding sequence for a subject peptide, e.g., nucleotide sequences encoding those peptides described above i.e., SEQ ID NO: 1, SEQ ID NO: 2, or analogs or derivatives thereof as described fully above. As described above, more than one triplet (codon) can code for a particular amino acid residue. Table 2 shows the different combinations of codons which may be used to encode the amino acid sequence set forth in SEQ ID NO: 1. Table 3 shows the different combinations of codons which may be used to encode the amino acid sequence set forth in SEQ ID NO: 2. The amino acid sequence of SEQ ID NO: 11 is shown in the top line of Table 2 in bold. The amino acid sequence of SEQ ID NO: 2 is shown in the top line of Table 3 in bold.

<table>
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<tr>
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<th>Q</th>
<th>I</th>
<th>K</th>
<th>R</th>
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<td>GAC</td>
<td>GCC</td>
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</table>

| COT | ATA | CGA| GTA | TCA | GTA | CCA | CUC | CGC | TCG | CGG | CGA | AGA | AGT |

In the above tables, the codons are listed for each amino acid sequence.
With respect to using nucleotide sequences encoding an analog or derivative of the amino acid sequences set forth in SEQ ID NOS: 1 or 2, one skilled in the art can refer to a table of the Genetic Code to select appropriate codons.

A number of different classes of Ad vectors exist, and may be used in the methods of the present invention. Such Ad vectors are described in the literature and are readily available. See refs. 26 and 27. For example, in accordance with the present invention, an Ad vector may be used wherein the E1 and/or E3 genes have been removed, allowing the introduction of up to about 6.5 kb of transgene under the control of a heterologous promoter. See ref. 28. The defective E1 viruses may be propagated in an E1-complementing cell line, such as 293A cells, which cells provided the E1 gene in trans.

Alternatively, an Ad vector may be used which in addition to lacking the E1 and E3 genes, also lack the E2 genes. See e.g., refs. 29 and 30.

In addition, helper-dependent (HD) or gutted vectored deleted of most or all Ad coding sequences may be used in accordance with the present invention. Such vectors have great potential as gene transfer vectors for gene therapy since long term expression of therapeutic genes have been observed in mice as well as monkeys. The production of these gutted vectors in tissue culture requires a complementing helper virus to provide the proteins required for growth and assembly of the gutted vector in trans. See refs. 31-33. The disclosures of these papers and all references cited herein, are incorporated by reference as if fully set forth.

As discussed above, in the present application directed to viral therapy of neoplastic disease, e.g., cancer, where the goal of the therapy is clearance of the target tissue, a host anti-Ad immune response targeting the vector infected cells is considered desirable. Thus, a gutted Ad vector may not be as preferred as some of the earlier generation vectors which elicit a stronger immune response in the host.

An Ad vector may be based on a two-plasmid system, an entry plasmid and a destination vector made from E1 and E3 gene deleted adenoviral genome that contains a promoter operably linked to a nucleotide sequence encoding one of the peptides described above (SEQ ID NOS: 1or 2) as well as analogs or derivatives thereof. The two-plasmid system is thoroughly described in refs. 28, 34, and 35. The E1 and E3 gene deletions prevent the virus from replicating in cells that do not express E1 and E3 proteins.

For example, the entry plasmid contains the gene encoding a subject peptide which plasmid is cloned into the AdV via a lambda recombination reaction. The replication incompetent vector may be propagated in 293 A cells, which are bioengineered human embryonic kidney cells transformed by AdV genomic DNA (Wang et al., 2000). This cell line supplements the deficient genes required for viral replication.

The replication incompetent AdV vectors of the present invention can be constructed using standard recombinant DNA methods. Standard techniques for the construction of vectors are well-known to those of ordinary skill in the art and can be found in references such as Sambrook, Fritsch and Maniatis, 1989, or any of a number of laboratory manuals on recombinant DNA technology that are widely available. A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments and can be readily determined by the skilled artisan. There are a number of different promoters which may be operably linked to the nucleotide sequences encoding a subject peptide. The promoter should function in the cells of a subject undergoing viral therapy with a subject AdV vector. There are a number of widely available promoters which may be used in the AdV vectors of the present invention. Examples of such promoters include, but are not limited to: CMV, SV40, RSV, LTR, beta-actin, EF-1 alpha, Gal-E1b, U6C, beta-Casein, EM-7, EF, TEF1, CMV-2 and Bsd. In a preferred embodiment, the promoter is CMV.

The recombinant vectors may then be subsequently built into intact viruses using standard methods such as that described in ref. 36, which is incorporated by reference herein as if fully set forth. Other references which describe rebuilding recombinant vectors into intact viruses include ref. 37, also incorporated by reference herein as if fully set forth.

Once a subject AdV vector is constructed, it may be used to treat patients suffering from different types of cancer. Therapy of neoplastic disease (cancer) may be accomplished by administering to a patient suffering from such disease a composition comprising the adenovirus vectors of the present invention. A human patient or nonhuman mammal suffering from a carcinoma may be treated by administering an effective antineoplastic dosage of a subject vector. The subject AdV vectors comprising a promoter operably linked to a nucleotide encoding a subject peptide are useful in treating a number of different cancers including but not limited to breast cancer, prostate cancer, lung cancer, cervical cancer, colon cancer, melanoma, pancreatic cancer, all solid tissue tumors (epithelial cell tumors) and cancers of the blood including but not limited to lymphomas and leukemias. In a preferred embodiment, the cancer to be treated is pancreatic cancer.

Suspensions of infectious adenovirus particles may be applied to neoplastic tissue by various routes, including intravenous, intraperitoneal, intramuscular, subdermal, and topical. Other routes include inhalation as a mist (e.g., in treating lung cancer) or direct application such as by swabbing a tumor site, e.g., cervical carcinoma, or during surgery if necessary. An adenovirus suspension may also be administered by infusion, e.g., into the peritoneal cavity for treating ovarian cancer. Other suitable routes include direct injection into a tumor mass, such as a breast tumor, via enema (colon cancer) or catheter in the case of bladder cancer.

The actual dosage may vary from patient to patient based on the age, weight, type and progression of cancer, location of tumor(s), presence of metastases, and overall condition of the patient. It can generally be said, however, that an adenovirus suspension containing about 10^9 to about 10^10 or more virion particles per ml may be administered. Re-administration of the AdV vector suspension may be performed as necessary.
The AdV vectors of the present invention may be admixed in a sterile composition containing a pharmacologically effective dosage of one or more subject AdV vectors. Generally speaking, the composition will comprise about $10^8$ to about $10^9$ or more AdV particles in an aqueous suspension. The sterile composition is usually an aqueous solution such as e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. Such compositions may contain pharmaceutically acceptable auxiliary substances e.g., to mimic physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The compositions may also comprise excipients that enhance infection of cells by the subject AdV vectors.

The following examples further illustrate the invention, and are not meant in any way to limit the scope thereof.

**EXAMPLE I**

**Materials and Methods**

Peptides. Three peptides, attached to the penetratin leader sequence, KKKKRMRRQFWKVQRG, designated as “leader,” on their carboxyl terminal ends, were synthesized by solid phase methods: the two-ras-p21 peptides corresponding to p21 residues 35-47 (TIEDYSYRKQVVID (SEQ ID NO:2)) and 96-110 (YREQIKRVDSDVP (SEQ ID NO:1)), denoted as PNC-7 and PNC-2, respectively; and the negative control X13 sequence (from mammalian cytochrome P450 (MRPSTGRKRMIGE)). With the penetratin sequence attached to their carboxyl terminal ends, each of these peptides is denoted as PNC-7-leader, PNC-2-leader and X13-leader, respectively. All peptides were purified to 95% purity.

**Plasmids.** Construction of the plasmids that express the H-ras Val12-p21 peptide sequence 96-110 (PNC-2) and the control X13 peptide from mammalian cytochrome P450 has been described (21). The nucleotide sequences for PNC-2 and X13 peptides are given in ref. 21. The nucleotide sequences, including the 5’ sticky end, used to encode the PNC-7 peptide were 5’T CGA GCC ACC ATG GGG ACC GAG GAT TCT TAC AGA AAA CAA GTG GTT ATA GAT TAA C (SEQ ID NO: 24) and 3’-CGG TGG TAC CCC TGG TAT TCT CTA AGA ATG TCT TTT GTT CAC CAA TAT CTA AIT GGG CC (SEQ ID NO:25).

Briefly, all of the oligonucleotides (plus and minus strands) encoding each sequence (PNC-2, PNC-7 and X13) and including a NotI (5’) and KpnI (3’) restriction site were synthesized by solid phase methods; sequential degradation of each oligonucleotide confirmed its sequence. These oligonucleotides were then incorporated into the pOPRSV/1 MCS vector from the Lac switch II isopropylthiogalactoside (IPTG)-inducible mammalian expression system from Stratagene (La Jolla, Calif.) by cutting this vector with KpnI and NotI and then ligating the oligomers into the plasmid with T4 ligase overnight at 4°C. The vectors containing the cloned oligonucleotides were transfected into DH5α competent cells (Gibco-BRL, Grand Island, N.Y.) and spread on LBamp plates for overnight incubation. Colonies from each plate were selected and grown at 37°C in 5 ml of LBamp liquid medium. DNA was prepared by the Qiagen (Valencia, Calif.) miniprep procedure, cut with KpnI/NotI, and run on 2 percent agarose/TAE to estimate the size of the inserts. Clones with the correct size DNA inserts were regrown in 500 ml LBamp overnight at 37°C, and plasmids were then purified by the Qiagen maxiprep method. An aliquot of each positive DNA was sequenced using T3 or T7 primers.

We note that, in our former paper describing these plasmids, an error occurred in the 5’ nucleotide sequence encoding PNC-2. This sequence should have read: upper

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5’-GGGAGAGGAGGCTACGGGACGATCGAAGGATGCGAAGCGG
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We also note that in several prior publications (16, 17, 20), we have developed two cell lines, one a normal contact-inhibited line of rat pancreatic acinar cells, called BMRPA1.430 (BMRPA1) cells and the other a pancreatic acinar carcinoma obtained by transfection of BMRPA1 cells with a plasmid containing an activated human K-ras oncogene [single base mutation at codon 12, valine substitution for the wild type glycine in the ras protein (K-ras<sup>val12</sup>); a kind gift of Dr. M. Perucho (CIBR, La Jolla, Calif.)] and a neurokinin resistance gene. BMRPA1 cells have an epithelial cell phenotype, form acinar structures in culture, have no c-ki-ras nor p53 mutations, are unable to grow in anchorage-independent conditions and do not form tumors in Nu/Nu mice (17).

In addition, they phenotypically maintain differentiated cell functions such as continued enzyme production and activation of zymogen secretion by secretagogue. On the other hand, ras-transformed BMRPA1 or TUC-3 cells, selected after transfection for their basis resistance to G418 and the overexpression of K-ras<sup>val12</sup>, no longer display an epithelial cell phenotype and acinar cell functions; they grow significantly faster than BMRPA1 cells, have a transformed spindle cell phenotype and form colonies under anchorage-independent conditions in vitro and tumors in vivo in nude mice.

**Peptide Incubation Experiments.** Approximately 300,000 cells (either BMRPA1 or TUC-3) were plated in each of six wells and were allowed to adhere overnight. In one set of experiments, the initial medium consisted of DMEM with 10% fetal bovine serum that contained no peptide. In another set of experiments, the initial medium contained peptide. In the first set, media containing peptide was added after 24 hours; in both sets, after the first 24 hours, the media was changed every 24 hours and always contained peptide at a particular concentration. Cells were observed daily for three weeks for changes in morphology and growth characteristics. Peptides were present at concentrations of 1, 10, 50, 100 and 1000 μg/ml.

**Transfection Experiments.** Approximately 300,000 TUC-3 cells were plated overnight in a six-well dish and were allowed to adhere overnight. To three wells, 5.5 μg of either PNC-2 or PNC-7 plasmid were added and, to the other three wells, 5.6 μg of X13 plasmid were added. To each of these wells, Superfect transfection agent (Qiagen) was added, using the Qiagen protocol, to enhance transfection efficiencies. We found that a 1:2 ratio of plasmid DNA to Superfect reagent gave the highest transfection efficiencies when compared with 1:5 and 1:10 ratios. Treated cells were then plated in selective medium containing 100 μg/ml G418 and 200 μg/ml of ampicillin together with 1 mM isopropylthiogalactoside (IPTG). The cells were washed and the medium changed
every 24 hours. Viable cells were observed for morphology and growth characteristics over a two-week period. **[0077]**

**Example II**

**Results**

**[0078]** Effects of Peptides on TUC-3 and BMRPA1 Cells. **FIGS. 1A and 1C** show the morphology of untreated TUC-3 pancreatic carcinoma cells and their normal counterpart BMRPA1 pancreatic acinar cells, respectively. The former are non-contact-inhibited and do not form monolayers but are “heaped up” on one another with considerable plasmomorphism between cells and indistinct cell boundaries. The latter form contact-inhibited monolayers with well-defined cell boundaries. Panel B in FIG. 1 shows that incubation of the X13 leader control peptide with TUC-3 cells for two weeks has no effect on their transformed morphologies. As expected, incubation of this control peptide with BMRPA1 cells has no effect (not shown). Incubation of BMRPA1 cells with PNC-2 leader peptide likewise has no effect on the morphology of these cells (Panel D in FIG. 1).

**[0079]** Effects of PNC-2-Leader and PNC-7-Leader on TUC-3 Cells. Treatment of TUC-3 cells with PNC-2-Leader (100 ng/ml) for 1 week results in a change in cell morphology as shown in FIG. 2A. As can be seen in this figure, the cells appear very similar to BMRPA1 cells (FIG. 1C); the cells grow into contact-inhibited monolayers and show distinct cell boundaries. This effect was achieved at concentrations as low as 1 μg/ml. At this low concentration, complete phenotypic reversion was achieved after two weeks. After one day of treatment, foci of acinar cellular differentiation appear; an example of a focus of revertant cells is shown in FIG. 2B.

**[0080]** Treatment of TUC-3 cells with PNC-7-leader peptide at concentrations of 100 and 200 μg/ml likewise resulted in phenotypic reversion of the cells as shown in FIG. 2C for cells growing in culture. In contrast to the results obtained with PNC-2-leader peptide, complete reversion after two weeks of incubation of TUC-3 cells with PNC-7-leader was achieved only at concentrations $\geq$ 100 μg/ml.

**[0081]** Transfection of TUC-3 cells with Inducible Plasmids Encoding PNC-2 and X13 Peptides. Since both PNC-2 and X13 leader peptides induce phenotypic reversion while X13 leader control peptide does not, we conclude that induction of reversion is specific to the two ras-p21 peptides and that the leader sequence, besides enabling membrane penetration, does not contribute to the induction of phenotypic reversion. To test the latter conclusion further, i.e. that PNC-2 and PNC-7 peptides alone, without the leader sequence, can induce phenotypic reversion, we prepared plasmids encoding these and the negative control X13 sequences and transfected them into TUC-3 cells. In a previous publication, we described the preparation of these plasmids which simultaneously confer G418 and ampicillin resistance under the lac promoter (21). We co-microinjected these plasmids with Val 12-p21 protein into Xenopus laevis oocytes and found that oocytes injected with either PNC-2 or PNC-7 but not X13 plasmid, in the presence of isopropylthiogalactose (IPTG), did not undergo maturation (21). When we transfected each of these plasmids into TUC-3 cells growing in the selective medium, viable cells expressing X13 peptide continued to grow in the presence of IPTG and exhibited the transformed morphology shown in Panel A of FIG. 1.

**[0082]** On the other hand, during a period of two weeks post-transfection with PNC-2 plasmid, all viable TUC-3 cells became progressively differentiated as shown in panel A (after 1 week) and B (after 2 weeks) of FIG. 3. As can be seen in panel A of FIG. 3, after one week, many cells adopted the untransformed phenotype (center and left of panel A) while some cells exhibited the transformed phenotype (right side of figure). At the end of two weeks, all cells exhibited the morphology shown in panel B of FIG. 3. As can be seen in this figure, the cells have distinct cell boundaries and exhibit the same morphology as untransformed BMRPA1 cells in growth phase. These cells eventually grew into contact-inhibited monolayers with a morphology that was the same as shown in FIG. 1, panel C.

**[0083]** Transfection of TUC-3 cells with PNC-7 plasmid exhibited the phenotype shown in Panel C of FIG. 3. These cells, which are seen to be enlarged with enlarged nuclei but have distinct cell boundaries, grew only sluggishly to confluence, and strongly resemble viable revertant cells that resulted from the treatment of TUC-3 cells with the anti-protein kinase C inhibitor, CYP 2151 (16). These cells fail to grow in soft agar (16).

**[0084]** Morphologically Revertant Cells Do Not Form Tumors in Nude Mice. To test whether morphologically revertant cells were functionally revertant, 5x10^6 cells treated for two weeks with 100 μg/ml PNC-2-leader peptide were explanted subcutaneously into each of five nude mice while the same number of untreated TUC-3 cells were concomitantly similarly explanted. The results, shown in Table 4, indicate that morphologically revertant cells fail to form tumors up to two months after reversion while untreated cells form tumors rapidly (within 1 week). At three weeks, all of the nude mice injected with untreated TUC-3 cells were found to have large primary nodules and multiple other nodules and metastatic cancer, with ascites and other sites. Similar results (not shown) to those obtained with PNC-2 leader peptide-treated TUC-3 cells were obtained for morphologically revertant cells resulting from TUC-3 cells treated with PNC-7 leader peptide.

**[0085]** Both PNC-2 and PNC-7 peptides block mitogenic signaling by oncopgenic ras-p21 in oocytes but have little effect on signaling by insulin-activated wild-type cellular ras-p21 (5). This finding suggested to us that growth of mammalian cells transformed by oncopgenic ras-p21 can be selectively blocked by these peptides without affecting normal growth processes.

**[0086]** Both PNC-2 and PNC-7 peptides induce 100 percent phenotypic reversion of ras-transformed pancreatic (TUC-3) cancer cells and have no apparent effects on the growth of the normal counterpart BMRPA1 cell line. This effect is specific since neither the X13-leader control peptide nor the plasmid encoding it has any effect on TUC-3 cell proliferation. That the PNC-2 and 7 sequences and not the leader sequence, are responsible for this effect is supported by the absence of any effect on TUC-3 cells of the X13-leader peptide and by the finding that the plasmids encoding PNC-2
and PNC-7 without the leader sequence induces the same observed phenotypic reversion.

A surprising finding is that the phenotypic reversion induced by both peptides occurs over a prolonged period of time (120 days), as revealed by the absence of any tumor growth of these cells when explanted into nude mice. Since the half-lives of these peptides is expected to be much shorter than two months, their effects are not likely to be caused by their continuing presence. Significantly, the prolonged reversion effect appears to be independent of the site of action of these peptides since PNC-2 blocks oncogenic ras-p21-JNK interactions (5,11,12) while PNC-7 blocks oncogenic ras-p21-ref interactions (14,15).

It is possible that both peptides activate rapid expression of other proteins that interfere with oncogenic ras-induced cell proliferation. This type of effect has been observed in human pancreatic carcinoma cells induced to revert by the agent azagrocin that is known to induce expression of the ras reversion gene (rca) (22,23) and which also selectively blocks oncogenic ras-p21-induced oocyte maturation (13). Another possibility is that each peptide, by blocking signal transduction unique to the oncogenic ras-p21-induced pathway, allows other inhibitory processes continuously to deactivte critical elements in this pathway.

The activity of both PNC-2 and PNC-7 peptides contrasts with that of another oncogenic-ras-p21-specific inhibitor, the staurosorosine derivative, CGP 41 251, that selectively inhibits protein kinase C (PKC)(24). This agent blocks oncogenic ras-p21-induced oocyte maturation but has much less effect on insulin-activated wild-type ras-p21-induced maturation (13). In contrast to the results with PNC-2 and PNC-7, this agent induces both necrosis and phenotypic reversion of TUC-3 cells (16) and is cytototoxic to BMRPA1 cells, although surviving cells grow rapidly into stable monolayers (16). Cytotoxicity of CGP 41 251 may be due to its blocking critical PKC-dependent cell processes that may not be involved in cell proliferation.

In prior studies, it had been found that PKC and JNK require each others presence on the oncogenic ras-p21 signal transduction pathway (25). In addition, PNC-2 synergizes with CGP 41 251 in TUC-3 cells in that it significantly lowers its IC50 for induction of cytototoxicity to a level that is not toxic to BMRPA1 cells (16). This finding suggests the possibility that PNC-2, which blocks ras-p21-induced activation of JNK (5), inhibits the mutual PKC-JNK activation cycle thereby removing an important activation process, resulting in facilitation of inhibition by CGP 41 251.

Evidently PNC-2 and PNC-7 exert a more selective effect that is specific to the oncogenic ras-p21 pathway, hence the lack of cytototoxicity of these peptides. This finding indicates that these peptides are useful in the treatment of ras-induced human tumors.

<table>
<thead>
<tr>
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<th>TUC-3 Cells</th>
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</tr>
<tr>
<td>14</td>
<td>11.7 ± 2.3</td>
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</tr>
<tr>
<td>21</td>
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<table>
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<th>Time (days)</th>
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<tbody>
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<td>0.0</td>
</tr>
<tr>
<td>56</td>
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</table>

* An amount of 5 x 10^6 TUC-3 cells was injected into the posterior cervical fat pad of each of 5 nude mice, and the same number of TUC-3 cells treated for 2 weeks with PNC-2-leader peptide was injected into the posterior cervical fat pad of another 5 nude mice.

**Expressed as the means ±SD for 4 mice in each group.**

*Multiple nodules and tumor metastasis with ascites occurred in all five mice at this time. Further observations were therefore discontinued.

**TABLE 4-continued**

Growth of TUC-3 Cells and Morphologically Reverted TUC-3 Cells Treated with PNC-2 Peptide Explanted into Nude Mice.*

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>TUC-3 Cells</th>
<th>PNC-2-Treated TUC-3</th>
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*An amount of 5 x 10^6 TUC-3 cells was injected into the posterior cervical fat pad of each of 5 nude mice, and the same number of TUC-3 cells treated for 2 weeks with PNC-2-leader peptide was injected into the posterior cervical fat pad of another 5 nude mice.

**Expressed as the means ±SD for 4 mice in each group.**

*Multiple nodules and tumor metastasis with ascites occurred in all five mice at this time. Further observations were therefore discontinued.

**EXAMPLE III**

PNC-2 and PNC-7 Block the Interaction of JNK and MAP Kinase with Val12-ras p21 Inside the Cell

In these experiments, the Ha-ras form of Val 12-p21 was injected into oocytes (100 µg/ml, 50 µl per oocyte) either alone or together with inhibitory p21 peptide (residues 96-110 shown in this figure). Mature oocytes (non-matured oocytes were used with inhibitory p21 96-110 peptide since it strongly inhibits maturation) were collected after 24 hours (about 50% maturation, approximately 100 oocytes) and subjected to lysis in buffer consisting of 0.3 M LiCl, 50 mM HEPES, pH 7.6, 1 mM EGTA, 1 mM dithiothreitol (DTT), 2 mM MgCl2, 50 mM NPP, 1 mM sodium vanadate, and an inhibitor 'cocktail' consisting of 1 µg/ml each of the protease inhibitors: peptatin, leupeptin and aprotinin, and the phosphatase inhibitors: 1 mM sodium orthovanadate and 5 mM sodium fluoride). The lysate was centrifuged for 15 min at 17000xg at 4°C, and the supernatant was either used directly. The lysates were then subjected to immunoprecipitation using an anti-Ha-ras antibody (Calbiochem). In this procedure, cell lysate was first pre-cleared by incubation with 50 µl of protein A beads for 1 hr at room temperature, followed by centrifugation. Anti-Ha-ras antibody was added to the lysate such that 0.1 µg antibody was added per 250 µg of pre-cleared lysate protein. A volume of 25 µl protein A agarose beads (Sigma) was then added to the incubation mixture, and the resulting mixture was incubated overnight at 4°C, after which the mixture was centrifuged, and the immunoprecipitate was washed three times with 0.5 ml of kinase buffer as described above. Immunoprecipitates were subjected to SDS-PAGE as described above in the preceding paragraph and blotted with anti-Ha-ras (1:2000 with 0.25% BSA), anti-raf (Calbiochem, San Diego, Calif.), diluted 1:2000 with 0.25% BSA, anti-JNK polyclonal antibody (1:2000), anti-MEK (Calbiochem) and anti-MAPK, diluted 1:2000 with 0.25% BSA. All incubations were performed as described in the preceding paragraph, i.e., for 12 hr at 4°C, after which the membranes were washed three times with Tris-buffered saline with Triton (TBS-T) and incubated with anti-rabbit secondary antibody (Pierce, Rockford, Ill.) at 1:2000 dilution. Detection was accomplished using the ECL chemiluminescence detection kit (Pierce). An identical set of experiments was performed with oocytes incubated for 24 hr with 10 µg/ml insulin (Sigma, St. Louis, Mo.).

**FIG. 4A** shows the results of injected Val 12-p21 forming a complex with raf, MEK, JNK and MAPK (ERK).
Oocytes that matured after being injected with Val 12-Ha-ras-p21 were lysed and immunoprecipitated with anti-Ha-ras antibody. The immunoprecipitate was blotted with anti-raf (lane 2), anti-MEK (lane 4), anti-JNK (lane 6) and anti-MAPK (lane 8). Oocytes were also injected with Val 12-p21 and ras-p21 inhibitory peptide 96-110, labeled as PNC-2, lysed and subjected to immunoprecipitation with anti-Ha-ras. These immunoprecipitates were then blotted with anti-raf (lane 1), anti-MEK (lane 3), anti-JNK (lane 5) and anti-MAPK (lane 7). As can be seen in this figure, ras, MEK, JNK and MAPK all co-precipitate with Ha-Val 12-ras-p21. On the other hand, the presence of the two inhibitory peptides, none of these proteins precipitated with Val 2-ras-p21 although there is still some binding to raf.

[0094] The same experiment, the results of which are shown in FIG. 4A was performed on oocytes that were induced to mature with insulin. FIG. 4B shows blots for raf (A), MEK (B), JNK (C) and MAPK (D). The first lane for each of these four sets presents the results for the blots of whole cell lysate to demonstrate the presence of each protein. The second lane in each set of blots shows the results of blotting for each protein in the anti-Ha-ras-p21 immunoprecipitate. As can be seen in this figure, only raf co-precipitates with endogenous Ha-ras-p21 in the oocytes. Thus oncogenic, but not activated wild-type, ras-p21 forms a large complex with vital mitogenic signal transducing proteins and induces activation of ras-MAPK-MAP kinase (MAPK or ERK) and JNK-Jun pathways while insulin-activated wild-type p21 (at least the Ha-ras form) forms a complex only with raf.

REFERENCES


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What is claimed is:
1. A peptide comprising at least about ten contiguous amino acids of the amino acid sequence: YREQIKRVKDS- DDVP (SEQ ID NO:1), or an analog or derivative thereof, wherein said peptide, analog, or derivative thereof comprises a membrane-penetrating leader sequence attached thereto.
2. A peptide comprising at least about ten contiguous amino acids of the amino acid sequence: TIEDSYRKQVVID (SEQ ID NO:2) or an analog or derivative thereof wherein said peptide, analog, or derivative thereof comprises a membrane-penetrating leader sequence attached thereto.
3. The peptide, analog or derivative thereof of claim 1 or 2 wherein the membrane-penetrating leader sequence is located at the carboxy terminal end of the peptide, analog, or derivative thereof.
4. The peptide, analog or derivative thereof according to claim 1 or 2 wherein the leader sequence comprises predominantly positively charged amino acid residues.
5. The peptide, analog or derivative thereof according to claim 1 or 2 wherein the leader sequence is at least one of penetratin, Arg_{55}, Tat of HIV-1, D-Tat, R-Tat, SV-40-NLS, nucleoplasmin-NLS, HIV REV, FHV coat, BMV GAG, HTLV-II (REX), CCMV GAG, P22N, Lambda N, Delta N, yeast PRP6, human U2AF, human C-FO8, human C-JUN, yeast GCN4, or p-vec.
6. The peptide, analogue, or derivative thereof of claim 5 wherein the penetratin leader sequence has the amino acid sequence: KKWKMRNRQFWVKVQRG (SEQ ID NO:3).
7. A pharmaceutical composition comprising at least one of the peptides, or analogs or derivatives thereof comprising a membrane-penetrating leader sequence according to claim 1 or 2 admixed with a pharmaceutically acceptable carrier.
8. A pharmaceutical composition comprising at least one of the peptides, analogs, or derivatives thereof comprising a membrane-penetrating leader sequence according to claim 1 admixed with a pharmaceutically acceptable carrier.
9. A pharmaceutical composition comprising at least one of the peptides, analogs, or derivatives thereof comprising a membrane-penetrating leader sequence according to claim 1 admixed with a pharmaceutically acceptable carrier.
10. A method of treating a patient suffering from cancer, said method comprising administering to said patient a therapeutically effective amount of at least one peptide, analog or derivative thereof comprising a membrane penetrating leader sequence according to claim 1 or 2.
11. A method of treating a patient suffering from cancer, said method comprising administering to said patient a therapeutically effective amount of at least one peptide, analog, or derivative thereof comprising a membrane penetrating leader sequence according to claim 3.
12. A method of treating a patient suffering from cancer, said method comprising administering to said patient a therapeutically effective amount of at least one peptide, analog, or derivative thereof comprising a membrane penetrating leader sequence according to claim 4.
13. A method of treating a patient suffering from cancer, said method comprising administering to said patient a therapeutically effective amount of the pharmaceutical composition of claim 7.
14. A method of treating a patient suffering from cancer, said method comprising administering to said patient a therapeutically effective amount of the pharmaceutical composition of claim 8.
15. The method of claim 10 wherein the treatment results in phenotypic reversion of cancerous cells into non-cancerous cells.
16. The method of claim 11 wherein the treatment results in phenotypic reversion of cancerous cells into non-cancerous cells.
17. The method of claim 12 wherein the treatment results in phenotypic reversion of cancerous cells into non-cancerous cells.
18. The method of claim 13 wherein the treatment results in phenotypic reversion of cancerous cells into non-cancerous cells.
19. The method of claim 14 wherein the treatment results in phenotypic reversion of cancerous cells into non-cancerous cells.
20. A replication incompetent Adenovirus (AdV) vector comprising a promoter sequence operably linked to a nucleotide sequence encoding a peptide, wherein the peptide comprises at least about ten contiguous amino acids of the amino acid sequence: YREQIKRVKDSDDVP (SEQ ID NO: 1), or an analog or derivative thereof.
21. A replication incompetent Adenovirus (AdV) vector comprising a promoter sequence operably linked to a nucleotide sequence encoding a peptide, wherein the peptide comprises at least about ten contiguous amino acids of the amino acid sequence: TIEDSYRKQVVID (SEQ ID NO: 2), or an analog or derivative thereof.
22. A method of treating a patient suffering from cancer, said method comprising administering to the patient, a therapeutically effective amount of the AdV vector of claim 20 or 21.
23. A method of inducing phenotypic reversion of cancerous cells to non-cancerous cells in a subject, said method comprising administering to the subject, a therapeutically effective amount of the AdV vector of claim 20 or 21.
24. The method of claim 23 wherein the cancerous cells are colon cancer cells, pancreatic cancer cells, non-small cell carcinoma of the lung, gastric cancer cells, bladder cancer cells or mesothelioma cells.
25. The method of claim 10 wherein the cancer is a ras-induced cancer.
26. The method of claim 25 wherein the ras-induced cancer is colon cancer, pancreatic cancer, non-small cell carcinoma of the lung, gastric cancer, bladder cancer or mesothelioma.

* * * * *