(54) Title: INHIBITION OF TUMORIGENIC PROPERTIES OF MELANOuma Cellaa

(57) Abstract: The invention relates to a variety of compositions and methods for inhibiting one or more tumorigenic properties of melanoma cells. The invention also includes methods for identifying compositions which are useful for this purpose and for making pharmaceutical composition from compositions identified in this manner. The invention includes compositions and methods for enhancing expression of E-cadherin expression in melanoma cells and compositions and methods for inhibiting expression of Mel-CAM (MUC18), the β3 subunit of the (αvβ3) vitronectin receptor, or both, in melanoma cells. Other tumorigenic properties of melanoma cells which can be inhibited using the compositions and methods of the invention include melanocyte growth, melanocyte proliferation, melanocyte and invasiveness of melanocytes.
INHIBITION OF TUMORIGENIC PROPERTIES
OF MELANOMA CELLS

STATEMENT REGARDING FEDERALLY SUPPORTED
RESEARCH AND DEVELOPMENT

This research was supported in part by U.S. Government funds
(National Cancer Institute grants numbers CA76674, CA25874), and the U.S.
Government may therefore have certain rights in the invention.

BACKGROUND OF THE INVENTION

Melanoma is a relatively common cancer, the incidence of which is
generally believed to be increasing. Primary treatment of melanoma is by excision of
the melanomonic lesion in patients in whom the cancer has remained localized, although
intensive gamma radiation therapy has also been performed with limited success. The
prognosis for patients whose melanoma has metastasized is much less favorable.
Although systemic chemotherapeutic (e.g. dacarbazine or nitrosoureas), vaccine (e.g.
BCG vaccine), and other immunotherapeutic (e.g. interleukin-2, interferon alpha, and
lymphokine-activated killer cell) therapies have been employed, none of these
therapies has proven widely successful for treatment of metastasized melanoma.

In the basal layer of the epidermis of normal patients, individual
melanocytes congregate with groups (e.g. five or six) of keratinocytes. Melanin
produced by the melanocyte of a group is transferred to keratinocytes in the group by
way of dendritic melanocyte-to-keratinocyte structures. Keratinocytes proliferate
rapidly in the epidermis, produce keratin, and eventually die and are sloughed off in the
outer portion of the epidermis. Melanocytes, in contrast, remain localized in the basal
layer of the epidermis, and normally proliferate when stimulated by extracorporeal
radiation (e.g. sunlight). In patients afflicted with melanoma proliferation of
melanocytes is uncontrolled.

Growth, proliferation, dendricity, and cell-surface molecule composition
of melanocytes are normally under the control of basal layer-type keratinocytes
(Herlyn et al., 1987, Cancer Res. 47:3057-3061; Valyi-Nagy et al., 1993, Lab. Invest.
69:152-159; Shih et al., 1994, Am. J. Pathol. 145:837-845). Melanoma cells are refractory to the regulatory controls normally exerted by keratinocytes, and therefore proliferate in an uncontrolled manner. Isolated and cultured melanocytes lose their normal phenotype, but regain it upon co-culture with basal layer-type keratinocytes. The homeostatic effects of basal layer-type keratinocytes upon melanocytes appears to require cell-to-cell contact. Furthermore, only basal layer-type keratinocytes exert these effects upon melanocytes. None of differentiated keratinocytes, dermal fibroblasts, and carcinoma cells effect homeostasis of melanocytes.

The mechanism(s) underlying the homeostasis-inducing effects of basal level-type keratinocytes upon melanocytes has not previously been understood. Elucidation of the biological mechanism(s) by which such homeostasis-inducing effects are induced would permit effective diagnosis, prevention, and therapy of melanoma, including metastatic melanoma. Given the limited benefit accruing from prior art melanoma detection, prevention, and treatment methods, a critical need remains for improved methods. The present invention satisfies these needs.

SUMMARY OF THE INVENTION

The invention relates to a method of inhibiting a tumorigenic property of a melanoma cell. The method comprises providing to the cell an isolated polypeptide consisting of at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

In one aspect, the cell is a human cell.

In another aspect, the cell is present in the body of a human patient.
Also included in the invention is a method of inhibiting a tumorigenic property of a melanoma cell. This method comprises enhancing expression of E-cadherin in the cell.

In one aspect, expression of E-cadherin in the cell is enhanced by providing to the cell an expressible vector comprising an isolated polynucleotide encoding at least a portion of E-cadherin.

In another aspect, the nucleotide sequence of the isolated polynucleotide is at least 90% homologous with SEQ ID NO: 2, and wherein the amino acid sequence of the portion is at least 75% identical to SEQ ID NO: 1.

Further included is a method of inhibiting a tumorigenic property of a melanoma cell, wherein the method comprises providing to the cell a peptidomimetic of a polypeptide consisting of at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bel-2, Bax, and Bad.

In addition, there is provided a method of inhibiting a tumorigenic property of a melanoma cell, wherein the method comprises providing to the cell an isolated polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic
helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

The invention also relates to a method of inhibiting a tumorigenic property of a melanoma cell. The method comprises providing to the cell a peptidomimetic of a polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

In addition, the invention relates to a method of determining whether a polypeptide or peptidomimetic is effective for inhibiting a tumorigenic property of a melanoma cell. The method comprises providing a test polypeptide or peptidomimetic to a test melanoma cell, wherein the test polypeptide or peptidomimetic is derived from a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad; and comparing the tumorigenic property of the test melanoma cell with the same property of a control melanoma cell to which the test polypeptide or peptidomimetic is not provided, whereby if the tumorigenic property of the test melanoma cell is inhibited relative to the same property of the control melanoma cell, then the test polypeptide or peptidomimetic is effective for inhibiting a tumorigenic property of a melanoma cell.
In one aspect, the tumorigenic property is selected from the group consisting of cell growth, cell proliferation, and tissue invasiveness.

In another aspect, the test melanoma cell is maintained in the presence of the test polypeptide or peptidomimetic for a period of at least one hour prior to comparing the tumorigenic property of the test melanoma cell with the same property of the control melanoma cell.

In addition, the invention relates to a method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The method comprises providing a test polypeptide or peptidomimetic to a test melanoma cell, wherein the test polypeptide or peptidomimetic is derived from a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad; comparing the tumorigenic property of the test melanoma cell with the same property of a control melanoma cell to which the test polypeptide or peptidomimetic is not provided; and if the tumorigenic property of the test melanoma cell is inhibited relative to the same property of the control melanoma cell, combining the test polypeptide or peptidomimetic with a pharmaceutically acceptable carrier to form the pharmaceutical composition.

The invention further includes a method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of the composition for at least one hour; and comparing Mel-CAM expression in the test melanoma cell with Mel-CAM expression in a control melanoma cell which is not maintained in the presence of the composition, whereby a lower level of Mel-CAM expression in the test
melanoma cell than in the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of a melanoma cell.

In one aspect, Mel-CAM expression is assessed by assessing the amount of intracellular mRNA encoding Mel-CAM.

In another aspect, Mel-CAM expression is assessed by assessing the amount of cellular Mel-CAM protein.

In yet another aspect, Mel-CAM expression is assessed by assessing the amount of cell-surface Mel-CAM protein.

In a preferred embodiment, the amount of cell-surface Mel-CAM protein is assessed using a labeled anti-Mel-CAM antibody.

The invention further includes a method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of the composition for at least one hour; and comparing αvβ3 expression in the test melanoma cell with αvβ3 expression in a control melanoma cell which is not maintained in the presence of the composition, whereby a lower level of αvβ3 expression in the test melanoma cell than in the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of a melanoma cell.

In one aspect, αvβ3 expression is assessed by assessing the amount of the amount of intracellular mRNA encoding the β3 subunit of αvβ3.

In another aspect, αvβ3 expression is assessed by assessing the amount of cellular αvβ3 protein.

In yet another aspect, αvβ3 expression is assessed by assessing the amount of cell-surface αvβ3 protein.

In a preferred embodiment, the amount of cell-surface αvβ3 protein is assessed using a labeled anti-αvβ3 antibody.

Further included in the invention is a method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of the composition for at least one hour; and comparing Mel-CAM and αvβ3 expression in the test
melanoma cell with Mel-CAM and αvβ3 expression in a control melanoma cell which is not maintained in the presence of the composition, whereby a lower level of Mel-CAM and αvβ3 expression in the test melanoma cell than in the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of a melanoma cell.

The invention additionally includes a method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of a test composition for at least one hour; comparing Mel-CAM expression in the test melanoma cell with Mel-CAM expression in a control melanoma cell which is not maintained in the presence of the test composition; and if a lower level of Mel-CAM expression is detected in the test melanoma cell than in the control melanoma cell, then combining the test composition with a pharmaceutically acceptable carrier to form the pharmaceutical composition.

Also provided in the invention is a method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of a test composition for at least one hour; comparing αvβ3 expression in the test melanoma cell with αvβ3 expression in a control melanoma cell which is not maintained in the presence of the test composition; and if a lower level of αvβ3 expression is detected in the test melanoma cell than in the control melanoma cell, then combining the test composition with a pharmaceutically acceptable carrier to form the pharmaceutical composition.

Additionally included in the invention is a method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of a test composition for at least one hour; comparing Mel-CAM and αvβ3 expression in the test melanoma cell with Mel-CAM and αvβ3 expression in a control melanoma cell which is not maintained in the presence of the test composition; and if a lower level of Mel-CAM and αvβ3 expression is detected in the test melanoma cell than in the control
melanoma cell, then combining the test composition with a pharmaceutically acceptable carrier to form the pharmaceutical composition.

The invention also includes a method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of basal layer keratinocytes and in the presence of the composition for at least one hour; and comparing gap junction formation between the test melanoma cell and the basal layer keratinocytes with gap junction formation between a control melanoma cell and basal layer keratinocytes maintained in the absence of the composition, whereby a greater degree of gap junction formation for the test melanoma cell than for the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of a melanoma cell.

Further included in the invention is a method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The method comprises maintaining a test melanoma cell in the presence of basal layer keratinocytes and in the presence of a test composition for at least one hour; comparing gap junction formation between the test melanoma cell and the basal layer keratinocytes with gap junction formation between a control melanoma cell and basal layer keratinocytes maintained in the absence of the test composition; and if a greater degree of gap junction formation is detected for the test melanoma cell than for the control melanoma cell, then combining the test composition with a pharmaceutically acceptable carrier to form the pharmaceutical composition.

The invention also includes a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The composition comprises an active agent admixed with a pharmaceutically acceptable carrier, wherein the active agent is an expressible vector comprising an isolated polynucleotide encoding at least a portion of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper
motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

The invention also includes a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The composition comprises an active agent admixed with a pharmaceutically acceptable carrier, wherein the active agent is an isolated polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

In addition, the invention includes a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell. The composition comprises an active agent admixed with a pharmaceutically acceptable carrier, wherein the active agent is a peptidomimetic of a polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.
BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiment(s) which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:

Figure 1 is a bar graph which indicates the degree of adhesion to keratinocytes exhibited by melanoma cells which were transduced with either LacZ/Ad5 or E-cad/Ad5, as described herein. In the right-most data set, E-cad/Ad5-transduced melanoma cells were contacted with a monoclonal antibody which specifically binds with E-cadherin prior to contacting the cells with keratinocytes.

Figure 2 is a graph which depicts the ratio of melanocytes to keratinocytes in cultures which initially comprised melanocytes and keratinocytes at a ratio of 1:10 (i.e. 0.1). Data obtained from cultures in which the melanocytes were non-transformed, non-melanoma melanocytes are indicated by filled triangles. Data obtained from cultures in which the melanocytes were melanoma cells transduced with E-cad/Ad5 are indicated by filled circles. Data obtained from cultures in which the melanocytes were melanoma cells transduced with LacZ/Ad5 are indicated by filled squares.

Figure 3, comprising Figures 3A, 3B, 3C, 3D, 3E, and 3F, is a series of images of tissue samples taken from human skin reconstructions into which transduced melanoma cells were incorporated. Samples from reconstructions comprising melanoma cells transduced with E-cad/Ad5 are shown in Figures 3A, 3C, and 3E. Samples from reconstructions comprising melanoma cells transduced with LacZ/Ad5 are shown in Figures 3B, 3D, and 3F. Tissue samples in Figures 3A, 3B, 3C, and 3D are stained with hematoxylin and eosin. Tissue samples in Figures 3E and 3F are stained using the commercial apoptosis detection kit described herein. Arrows in Figures 3C and 3E indicate melanoma cells comprising an apoptotic body. Magnification is 100x in Figures 3A and 3B, and is 250x in Figures 3C, 3D, 3E, and 3F.
**Figure 4**, comprising Figures 4A, 4B, and 4C, depicts the nucleotide sequence of a human cDNA encoding E-cadherin (GenBank Accession No. Z13009; Bussemakers et al., 1993, Mol. Biol. Rep. 17:123-128).

**Figure 5** depicts the amino acid sequence of human E-cadherin (GenBank Accession No. Z13009; Bussemakers et al., 1993, Mol. Biol. Rep. 17:123-128).

**DETAILED DESCRIPTION**

The present invention is based on the discovery that enhanced expression in melanocytes of the cell-to-cell adhesion receptor designated E-cadherin, induces diminished expression of the melanoma-associated cell surface proteins designated Mel-CAM (MUC18) and αvβ3, when the melanoma cells are cocultured with keratinocytes. These cell surface proteins are expressed on melanoma cells but are expressed at a much lower level, or not at all, on non-tumorigenic melanocytes. The present invention is also based on the discovery that enhanced expression of E-cadherin in melanoma cells restores keratinocyte control over melanoma cell proliferation, growth, and dendricity - even over highly aggressive and metastatic melanoma cells. Thus, it has been discovered in the present invention that expression of E-cadherin in melanoma cells mediates resumed control of melanoma cell proliferation by keratinocytes.

In the present discussion, it is important to distinguish between the use of the terms melanocyte and melanoma cell. As used herein, a “melanocyte” is a normal non-tumorigenic cell; however, a “melanoma cell” is a non-normal, tumorigenic melanocyte. A tumorigenic melanocyte (i.e., a melanoma cell) is one which exhibits uncontrolled growth and invasive characteristics which are not evident in melanocytes.

Under natural conditions, melanocytes express E-cadherin on their surface, but melanoma cells do not (Hsu et al., 1996, J. Invest. Dermatol. Symp. Proc. 1:188-194); melanoma cells express N-cadherin, melanocytes do not. Melanoma cells express greater amounts of Mel-CAM and αvβ3 than do melanocytes. Both cell types
express α-catenin, β-catenin, and plakoglobin (Ozawa et al., 1992, J. Cell Biol. 116:989-996; Knudsen et al., 1995, J. Cell Biol. 130:67-77). Thus, several proteins including those listed above, and potentially others, are believed to be involved in transmission of physiologically relevant signals from keratinocytes to melanocytes, wherein the signals facilitate control exerted by keratinocytes over melanocyte growth. Proteins which are believed to be important in this regard are E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad. Until the present invention, it was unclear which, if any, of these proteins is able to induce or inhibit one or more tumorigenic properties in a melanocyte.

The data presented herein demonstrate that enhanced expression of E-cadherin in melanoma cells facilitates the keratinocyte-induced inhibition of tumorigenic properties of the melanoma cells. Expression of E-cadherin in a melanoma cell results in reduced expression of Mel-CAM and αvβ3 in the cell; when the melanoma cell is cocultured with keratinocytes, the invasiveness of the cell is reduced, and apoptosis of melanoma cells which are invasive is induced.

The present invention thus includes a method of inhibiting a tumorigenic property of a melanoma cell by inducing enhanced expression of E-cadherin in the cell or by providing to the cell a compound which mimics the effect of enhanced E-cadherin expression. When a cell so treated is cocultured with a keratinocyte, the cell looses its tumorigenic properties.

The invention further includes assays which identify compositions (e.g. polypeptides derived from E-cadherin or another protein, such as α-catenin, β-catenin, or plakoglobin, which is involved in transmitting signals from keratinocytes to melanocytes) for inhibiting a tumorigenic property of a melanoma cell and
compositions identified using such assays. Compositions so identified are useful for treatment and prevention of melanoma. In addition, the invention includes diagnostic methods for identifying a melanoma cell in a biological sample, for example, a sample which is obtained from a human patient.

5 Definitions

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "antisense oligonucleotide" means a nucleic acid polymer, at least a portion of which is complementary to a nucleic acid which is present in a normal cell or in an affected cell. The antisense oligonucleotides of the invention preferably comprise between about fourteen and about fifty nucleotides. More preferably, the antisense oligonucleotides comprise between about twelve and about thirty nucleotides. Most preferably, the antisense oligonucleotides comprise between about sixteen and about twenty-one nucleotides. However, it will be appreciated that the antisense oligonucleotides may comprises as few as eight and as many as three hundred contiguous nucleotides in length. The antisense oligonucleotides of the invention include, but are not limited to, phosphorothioate oligonucleotides and other modifications of oligonucleotides. Methods for synthesizing oligonucleotides, phosphorothioate oligonucleotides, and otherwise modified oligonucleotides are well known in the art (U.S. Patent No: 5,034,506; Nielsen et al., 1991, Science 254: 1497).

The term “antisense” refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule.
The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

A "tumorigenic property" of a melanoma cell is a phenotype which is exhibited by a melanoma cell and which is not exhibited, or which is exhibited to a different degree, in a (non-tumorigenic) melanocyte of the same animal. The tumorigenic property confers upon the melanoma cell the ability to form tumors, and generally is the result of uncontrolled growth of a melanoma cell, wherein the melanoma cell exhibits invasive characteristics which are not evident in melanocytes.

A tumorigenic property of a melanoma cell is "inhibited" by a composition or treatment if, upon provision of the composition or the treatment to the cell, the tumorigenic property is made more similar to the property (or lack thereof) in a melanocyte of the same animal than it is in the absence of the composition or treatment. For example, if the level of expression of telomerase activity in the melanoma cell is increased following treatment with the composition, then the composition inhibits a tumorigenic property of the melanoma cell.

A composition is "effective for inhibiting" a tumorigenic property of a melanoma cell if upon provision of the composition to an animal, the property is made more similar to the property (or lack thereof) in a non-tumorigenic melanocyte of the same animal.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a
genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

"Homology" and "identity" as used interchangeably herein, refers to nucleotide sequence similarity between two regions of the same nucleic acid strand or between regions of two different nucleic acid strands. When a nucleotide residue position in both regions is occupied by the same nucleotide residue, then the regions are homologous at that position. A first region is homologous to a second region if at least one nucleotide residue position of each region is occupied by the same residue. Homology between two regions is expressed in terms of the proportion of nucleotide residue positions of the two regions that are occupied by the same nucleotide residue. By way of example, a region having the nucleotide sequence 5'-'ATTGCC-3' and a region having the nucleotide sequence 5'-TATGCC-3' share 50% homology. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residue positions of each of the portions are occupied by the same nucleotide residue. More preferably, all nucleotide residue positions of each of the portions are occupied by the same nucleotide residue.

Similarly, homology or identity between two polypeptides or peptidomimetics refers to amino acid sequence similarity between two regions of the same polypeptide or between regions of two different polypeptides. Homology between two regions is expressed in terms of the proportion of amino acid residue positions of the two regions that are occupied by the same amino acid residue. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the amino acid residue positions of each of the portions are occupied by the same amino acid residue. More preferably, all amino acid residue positions of each of the portions are occupied by the same amino acid residue.

An "expressible vector" is a polynucleotide vector which comprises a first polynucleotide encoding a gene product (i.e. an RNA or a protein) operably linked
with a promoter/regulatory sequence, whereby the gene product can be produced in a cell when the expressible vector is provided thereto.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be constitutive, inducible, or one which expresses the gene product in a tissue specific manner.

A "constitutive" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living human cell under most or all physiological conditions of the cell.

An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living human cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living human cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

Expression of a protein in a cell is "enhanced" by a composition or treatment if expression of the protein is greater in the presence of the composition or treatment than in its absence.

A "portion" of a polypeptide means at least 4, 6, 8, 12, 20, 50, 100, 200, or 400 or more consecutive amino acid residues of the polypeptide.

A polypeptide is "derived" from a protein if the polypeptide comprises or consists of a portion which is at least 50%, 60%, 70%, 80%, 90%, or 98% or more identical to a corresponding portion of the protein. Preferably, the portion of the polypeptide is 100% identical to the corresponding portion of the protein.
A peptidomimetic is "derived" from a protein if it in some way mimics a portion of the protein.

"Dendricity" of a melanocyte refers to the number of extensions exhibited by the melanocyte, the individual or collective length of extensions exhibited by the melanocyte, or the proportion of cell mass or cell surface area of the melanocyte which is present in the form of extensions. It is understood that dendrites represent a branch of cellular protoplasm which occurs in the form of an extension of the protoplasm. The thickness, length, geometry, structure, and number of dendrites varies even among cells of the same type. Dendrites transduce and reinforce signals, release hormones, small peptides, and second messenger compounds, and retain their plasticity due to the presence of microtubules, intermediate filaments, and actin therein.

"Invasiveness" of a melanocyte, as used herein, refers to the ability of a melanocyte to leave the tissue site at which it originated and proceed to proliferate at a different site in the body.

As used herein, the term "pharmaceutically acceptable carrier" means a chemical composition with which the active ingredient may be combined and which, following the combination, can be used to administer the active ingredient to a subject.

As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition and which is not deleterious to the subject to which the composition is to be administered.

Description

The present invention is based on the discovery that enhancement of E-cadherin expression in a melanoma cell alters the manner in which the melanoma cell communicates with surrounding keratinocytes, thereby facilitating the inhibition of tumorigenic properties of the melanoma cell. Among the tumorigenic properties of melanoma cells that are reduced or eliminated are cell growth, proliferation, invasiveness, absence of E-cadherin expression, and diminished or absent cell-surface expression of melanoma-associated antigens such as Mel-CAM (a.k.a. MUC18) and the β3 subunit of the vitronectin receptor. In short, enhancement or commencement of
E-cadherin expression in melanoma cells induces those cells to behave like normal melanocytes when the cells are cocultured with keratinocytes.

While not wishing to be bound by any particular theory of operation, it is believed that expression of E-cadherin renders melanoma cells susceptible to physiological control signals which keratinocytes normally exert upon melanocytes. Such controls may be exerted upon melanocytes either by a biological activity catalyzed by E-cadherin protein of the melanocytes or by a protein to which E-cadherin is able to indirectly transmit a signal (e.g. by way of intracellular signaling proteins such as α-catenin, β-catenin, or plakoglobin). Alternatively, the interaction between E-cadherin and a keratinocyte enables the keratinocyte to affect the biological activity of another melanocyte protein (e.g. N-cadherin) which generates or transmits a control signal to melanoma-associated melanocyte proteins.

The present invention therefore relates to both methods and compositions for enhancing E-cadherin expression in melanoma cells and to methods and compositions for mimicking generation or transmission of keratinocyte-derived melanocyte control signals. E-cadherin expression may be enhanced, for example, by providing an expressible vector to melanoma cells or by providing a composition to a melanoma cell which enhances expression of the endogenous gene encoding E-cadherin. Generation, transmission, or both, of a keratinocyte-derived melanocyte control signal may be mimicked by providing to the cell a polypeptide or a nucleic acid encoding the same, or a peptidomimetic of the same, wherein the nucleic acid is in an expressible vector, wherein the polypeptide (or nucleic acid encoding the same), or peptidomimetic has or mimics the amino acid sequence of a portion of a signal generation or transmission protein (e.g. a portion of E-cadherin, N-cadherin, α-catenin, β-catenin, or plakoglobin), which protein is normally exposed or activated upon interaction of melanoma-expressed E-cadherin with a keratinocyte.

The present invention further relates to methods for identifying compounds (e.g. polypeptides, peptidomimetics, and nucleic acids encoding polypeptides) which are effective for inhibiting a tumorigenic property of a melanoma cell and to methods for preparing pharmaceutical compositions comprising such
compounds. In addition, the invention includes pharmaceutical compositions made using such methods.

Thus, in one aspect, the invention relates to a method of inhibiting a tumorigenic property of a melanoma cell (e.g. a human melanoma cell). This method comprises inducing enhanced expression of E-cadherin in the cell. Expression of E-cadherin may be enhanced either by enhancing expression of E-cadherin from the endogenous gene of the melanoma cell or by supplementing the genome of the melanoma cell with an expressible vector comprising an isolated polynucleotide encoding E-cadherin (e.g. an expressible vector having a nucleotide sequence comprising SEQ ID NO: 2). This method may be used either in vitro (e.g. for the purpose of screening compounds which are effective to induce enhanced expression of E-cadherin) or in vivo (e.g. for the purpose of treating a subject afflicted with melanoma).

It is understood that enhanced expression of E-cadherin in a melanoma cell may be induced by providing to the cell an expressible vector comprising an isolated polynucleotide encoding less than the entire amino acid sequence of E-cadherin. Preferably, of course, the expressible vector encodes the entire sequence (e.g. comprises the amino acid sequence SEQ ID NO: 1). However, the expressible vector may encode only a portion of E-cadherin, such as at least 4, 6, 8, 12, 20, 50, 100, 200, or 400 or more consecutive amino acid residues of E-cadherin. Thus, when the melanoma cell is a human melanoma cell, the expressible vector preferably encodes a polypeptide having an amino acid sequence consisting of at least 4, 6, 8, 12, 20, 50, 100, 200, or 400 or more consecutive amino acid residues of SEQ ID NO: 1, and preferably from about 6 to about 70 consecutive amino acid residues.

It is furthermore understood that enhanced expression of E-cadherin in a melanoma cell may be induced by providing to the cell an expressible vector comprising an isolated polynucleotide which is not necessarily identical to a known E-cadherin cDNA. For example, the vector may comprise an isolated polynucleotide which is at least 50%, 60%, 70%, 80%, 90%, 95%, or 98% or more homologous with at least twelve consecutive nucleotide residues of SEQ ID NO: 2. Preferably the amino
acid sequence of the portion of E-cadherin encoded by the vector is at least 75%, 80%, 85%, 90%, 95%, or 98% or more identical to at least four consecutive amino acid residues of SEQ ID NO: 1. In a contemplated embodiment, the vector comprises an isolated polynucleotide which is at least 90% homologous with at least twelve consecutive nucleotide residues of SEQ ID NO: 2, and the amino acid sequence encoded by these twelve residues is at least 75% homologous with a portion of SEQ ID NO: 1.

The invention also relates to a method of inhibiting a tumorigenic property of a melanoma cell by providing to the cell an isolated polypeptide which mimics generation or transmission of a physiological signal which is normally induced following interaction of a keratinocyte with E-cadherin of a melanocyte. The polypeptide may, for example, comprise or consist of at least 4, 6, 8, 12, 20, 50, 100, 200, or 400 or more consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, N-cadherin, α-catenin, β-catenin, other catenins, and plakoglobin. Of course, the protein may be a protein which is not included in the aforementioned list of proteins. For example, the polypeptide may comprise a portion of an armadillo repeat-containing protein (e.g. any of several catenins known to comprise one or more armadillo repeat motifs). Portions of other proteins are also included within the scope of the invention including, for example, portions of axins, glycogen synthase kinases, APC, PKB/Akt, Lef/TCF proteins, POU proteins, Ets proteins, E-box binding proteins, proteins of the myc family, proteins having one or more leucine zipper motifs, proteins having one or more homeobox motifs, and proteins which modulate apoptosis (e.g. bcl-2, bax, bad, and the like).

It is understood that the polypeptide need not have an amino acid sequence that is 100% identical to the corresponding portion of the protein. For example, the polypeptide may have non-homologous amino acid residues on either or both ends thereof, or it may comprise only a portion (e.g. 4, 6, 8, 12, 20, 50, 100, 200, or 400 or more amino acid residues) which is at least 50%, 60%, 70%, 80%, 90%, or 98% or more homologous with the corresponding portion of the protein.
The invention also relates to a method of inhibiting a tumorigenic property of a melanoma cell by providing to the cell a peptidomimetic of a polypeptide which mimics generation or transmission of a physiological signal which is normally induced following interaction of a keratinocyte with E-cadherin of a melanocyte. The polypeptide may, for example, comprise or consist of at least 4, 6, 8, 12, 20, 50, 100, 200, or 400 or more consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, N-cadherin, α-catenin, β-catenin, or plakoglobin.

It will be appreciated, of course, that the peptides may incorporate amino acid residues which are modified without affecting biological activity (i.e. 'peptidomimetics' may be used in place of the peptides described herein). For example, the termini of the peptide may be derivatized to include blocking groups, i.e. chemical substituents suitable to protect and/or stabilize the N- and C-termini from "undesirable degradation", a term meant to encompass any type of enzymatic, chemical or biochemical breakdown of the compound at its termini which is likely to affect the function of the compound, i.e. sequential degradation of the compound at a terminal end thereof.

Blocking groups include protecting groups conventionally used in the art of peptide chemistry which will not adversely affect the in vivo activities of the peptide. For example, suitable N-terminal blocking groups can be introduced by alkylation or acylation of the N-terminus. Examples of suitable N-terminal blocking groups include C1-C5 branched or non-branched alkyl groups, acyl groups such as formyl and acetyl groups, as well as substituted forms thereof, such as the acetamidomethyl (Acm) group. Desamino analogs of amino acids are also useful N-terminal blocking groups, and can either be coupled to the N-terminus of the peptide or used in place of the N-terminal residue. Suitable C-terminal blocking groups, in which the carboxyl group of the C-terminus is either incorporated or not, include esters, ketones or amides. Ester or ketone-forming alkyl groups, particularly lower alkyl groups such as methyl, ethyl and propyl, and amide-forming amino groups such as primary amines (-NH2), and mono- and di-alkylamino groups such as methylamino, ethylamino, dimethylamino, diethylamino, methylethylamino and the like are examples
of C-terminal blocking groups. Descarboxylated amino acid analogues such as agmatine are also useful C-terminal blocking groups and can be either coupled to the peptide's C-terminal residue or used in place of it. Further, it will be appreciated that the free amino and carboxyl groups at the termini can be removed altogether from the peptide to yield desamino and descarboxylated forms thereof without affect on peptide activity.

Other modifications can also be incorporated without adversely affecting the ability of the peptide to inhibit a tumorigenic property of a melanoma cell, and these include, but are not limited to, substitution of one or more of the amino acids in the natural L-isomeric form with amino acids in the D-isomeric form. Thus, the peptide may include one or more D-amino acid residues, or may comprise amino acids which are all in the D-form. Retro-inverso forms of peptides in accordance with the present invention are also contemplated, for example, inverted peptides in which all amino acids are substituted with D-amino acid forms.

Acid addition salts of the present invention are also contemplated as functional equivalents. Thus, a peptide in accordance with the present invention treated with an inorganic acid such as hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, and the like, or an organic acid such as an acetic, propionic, glycolic, pyruvic, oxalic, malic, malonic, succinic, maleic, fumaric, tartaric, citric, benzoic, cinnamic, mandelic, methanesulfonic, ethanesulfonic, p-toluenesulfonic, salicylic and the like, to provide a water soluble salt of the peptide is suitable for inhibition of a tumorigenic property of a melanoma cell.

In the methods of the invention described below, various test compounds are added to a melanoma cell, and the effect of the test compound on the cell is assessed. When there is an effect of the test compound on the cell, the test compound is determined to be one which may be useful for reducing the tumorigenicity of the melanoma cell. When this is the case, in effect, the test compound confers upon the melanoma cell properties which better resemble melanocytes rather than melanoma cells.
The invention includes a method of determining whether a polypeptide or peptidomimetic is effective for inhibiting a tumorigenic property of melanoma cells. This method comprises providing a test polypeptide or peptidomimetic to a test melanoma cell. Test polypeptide or peptidomimetic is derived from a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad. In the method of the invention, the tumorigenic property of the test melanoma cell is then compared with the same property of a control melanoma cell to which the test polypeptide or peptidomimetic is not provided. If the tumorigenic property of the test melanoma cell is inhibited relative to the same property of the control melanoma cell, then the test polypeptide or peptidomimetic is effective for inhibiting a tumorigenic property of melanoma cells. Preferably, the protein is E-cadherin, and more preferably, the protein is human E-cadherin (e.g. a protein having the amino acid sequence SEQ ID NO: 1). In one embodiment, the test melanoma cell is maintained in the presence of the test polypeptide or peptidomimetic for a period of at least one hour prior to comparing the tumorigenic property of the test melanoma cell with the same property of the control melanoma cell. If the tumorigenic property of the test melanoma cell is inhibited relative to the same property of the control melanoma cell, then this is an indication that the polypeptide or peptidomimetic may be combined with a pharmaceutically acceptable carrier to form the pharmaceutical composition which is useful for inhibiting a tumorigenic property of a melanoma cell.

The invention further includes a method of determining whether a composition is effective for inhibiting a tumorigenic property of melanoma cells. This method comprises maintaining a test melanoma cell in the presence of the composition for at least one hour; and comparing E-cadherin expression in the test melanoma cell
with E-cadherin expression in a control melanoma cell which is not maintained in the presence of the composition. A greater level of E-cadherin expression in the test melanoma cell than in the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of melanoma cells. This is also an indication that the composition may be combined with a pharmaceutically acceptable carrier and used as a pharmaceutical composition for inhibiting a tumorigenic property of melanoma cells in an animal. In this method, E-cadherin expression may be assessed by assessing the amount of intracellular mRNA encoding E-cadherin, by assessing the amount of cellular E-cadherin protein, or by assessing the amount of cell-surface E-cadherin protein, for example. The amount of cell-surface E-cadherin protein may be assessed using, for example, an anti-E-cadherin antibody which is labeled with a detectable label.

In another aspect of the invention, there is provided a different method of determining whether a composition is effective for inhibiting a tumorigenic property of melanoma cells. This method comprises maintaining a test melanoma cell in the presence of the composition for at least one hour; and comparing the level of expression of Mel-CAM, αvβ3, and/or the level of expression of both genes, in the test melanoma cell with the expression of these genes in a control melanoma cell which is not maintained in the presence of the composition. A lower level of expression of Mel-CAM, αvβ3, and/or a lower level of expression of both genes in the test melanoma cell compared with the level of expression of these genes in the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of melanoma cells. This is also an indication that the composition may be combined with a pharmaceutically acceptable carrier and used as a pharmaceutical composition for inhibiting a tumorigenic property of melanoma cells in an animal. Mel-CAM expression may, for example, be assessed by assessing the amount of intracellular mRNA encoding Mel-CAM, by assessing the amount of cellular Mel-CAM protein, or by assessing the amount of cell-surface Mel-CAM protein (e.g. by using a labeled anti-Mel-CAM antibody). αvβ3 expression may be assessed by assessing αvβ3 protein expression per se or by assessing expression of β3 protein. For example, αvβ3
expression may be assessed by assessing the amount of intracellular mRNA encoding the β3 subunit of αvβ3. αvβ3 expression may be assessed by assessing the amount of cellular αvβ3 protein or by assessing the amount of cell-surface αvβ3 protein (e.g. using a labeled anti-αvβ3 antibody).

While not wishing to be bound by theory, it is believed that compounds which affect a lower level of expression of both Mel-CAM and αvβ3 are more likely to be effective in inhibiting a tumorigenic property of a melanoma cell. This is because it is believed that such compounds are likely to act at the level of a master switch which turns off expression of both genes, the master switch being one which is essential for the tumorigenic properties of the melanoma cell. However, the method of the invention should not be limited to any requirement that both genes are turned off by any given test compound, but instead should be construed as encompassing the inhibition of expression of at least one of these genes, and preferably, inhibition of both genes.

The invention relates to an additional method of determining whether a composition is effective for inhibiting a tumorigenic property of melanoma cells. This method comprises maintaining a test melanoma cell in the presence of basal layer keratinocytes and in the presence or absence of the composition for at least one hour; and comparing gap junction formation between the test melanoma cell and the basal layer keratinocytes with gap junction formation between a control melanoma cell and basal layer keratinocytes maintained in the absence of the composition. A greater degree of gap junction formation in the test melanoma cell than in the control melanoma cell is an indication that the composition is effective for inhibiting a tumorigenic property of melanoma cells. This is also an indication that the composition may be combined with a pharmaceutically acceptable carrier and used as a pharmaceutical composition for inhibiting a tumorigenic property of melanoma cells in an animal. Gap junction formation can be assessed, for example, using well known microscopic methods.

The invention also relates to pharmaceutical compositions for inhibiting a tumorigenic property of a melanoma cell. The compositions comprise an active agent
admixed with a pharmaceutically acceptable carrier. The active agent is selected from
(i) an expressible vector comprising an isolated polynucleotide encoding at least a
portion of a protein selected from the group consisting of E-cadherin, α-catenin, β-
catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt,
an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box
binding protein, a protein of the myc family, a protein having one or more leucine
zipper motifs, a homeobox protein, a protein having one or more homeobox motifs,
Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing
protein, a protein of the dishevelled and frizzled family of proteins, a protein which
modulates apoptosis, Bcl-2, Bax, and Bad, and the like); (ii) an isolated polypeptide
having an amino acid sequence which is at least 75% identical with at least four
consecutive amino acid residues of a protein selected from the group consisting of E-
cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase
kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets
protein, an E-box binding protein, a protein of the myc family, a protein having one or
more leucine zipper motifs, a homeobox protein, a protein having one or more
homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine
zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a
protein which modulates apoptosis, Bcl-2, Bax, and Bad, and the like); (iii) a
peptidomimetic of a polypeptide having an amino acid sequence which is at least 75%
identical with at least four consecutive amino acid residues of a protein selected from
the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an
axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain
containing protein, an Ets protein, an E-box binding protein, a protein of the myc
family, a protein having one or more leucine zipper motifs, a homeobox protein, a
protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic
helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and
frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad;
and, an antisense molecule which is capable of affecting expression of any of the
aforementioned genes.
The invention encompasses the preparation and use of medicaments and pharmaceutical compositions comprising one or more of these active agents as an active ingredient. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. Administration of one of these pharmaceutical compositions to a subject is useful for inhibiting a tumorigenic property of a melanoma cell in the subject, as described elsewhere in the present disclosure. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, parenteral, topical, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

It is understood that the ordinarily skilled physician will readily determine and prescribe an effective amount of the compound to inhibit a tumorigenic property of a melanoma cell in the subject. In so proceeding, the physician or veterinarian may, for example, prescribe a relatively low dose at first, subsequently
increasing the dose until an appropriate response is obtained. It is further understood, however, that the specific dose level for any particular subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the stage of the melanoma being treated. It is contemplated that the amount of active ingredient to be administered to a mammal, preferably, a human, will vary depending upon any number of factors including, but not limited to, the age, weight, and general state of health of the human and the extent of the melanoma disease. Thus, the amount of active ingredient to be administered may be as little as one microgram or as much as 100 milligrams or even one gram per kilogram of body weight of the mammal.

Another aspect of the invention relates to a kit comprising a pharmaceutical composition of the invention and an instructional material. As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which is used to communicate the usefulness of the pharmaceutical composition of the invention for inhibiting a tumorigenic property of a melanoma cell in a subject. The instructional material may also, for example, describe an appropriate dose of the pharmaceutical composition of the invention. The instructional material of the kit of the invention may, for example, be affixed to a container which contains a pharmaceutical composition of the invention or be shipped together with a container which contains the pharmaceutical composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the pharmaceutical composition be used cooperatively by the recipient.

The invention also includes a kit comprising a pharmaceutical composition of the invention and a delivery device for delivering the composition to a subject. By way of example, the delivery device may be a squeezable spray bottle, a metered-dose spray bottle, an aerosol spray device, an atomizer, a dry powder delivery device, a self-propelling solvent/powder-dispensing device, a syringe, a needle, a
tampon, or a dosage measuring container. The kit may further comprise an instructional material as described herein.

The invention is now described with reference to the following Example. This Example is provided for the purpose of illustration only, and the invention should in no way be construed as being limited to this Example, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example

Induction of E-Cadherin Expression

in Melanoma Cells Restores Regulatory Dominance of Keratinocytes

Over the Malignant Cells

The experiments presented in this Example demonstrate that expression of E-cadherin by melanocytes is necessary in order for growth, proliferation, and invasiveness of the melanocytes to be maintained under the control of keratinocytes.

The materials and methods used in the experiments presented in this Example are now described.

Normal human melanocytes, keratinocytes, fibroblasts, and primary and metastatic melanoma cells were isolated and cultured as described (Hsu et al., 1996, In: Human Cell Culture Protocols, Humana Press Inc., Totowa, NJ, pp. 9-20; Boyce et al., 1985, J. Tissue Cult. Meth. 9:83-93). Melanocytes were mixed with keratinocytes at ratios of 1:5 to 1:10, and the mixtures were then seeded into 8-well chambers slides (Lab-Tek™, Nunc, Inc., Naperville, IL). The mixtures were maintained for four days, and then the cells were fixed with 3% (b/b) paraformaldehyde and permeabilized with a solution of 0.5% NP-40 detergent (nonidet P-40; (octylphenoxy)polyethoxycethanol; Sigma Chemical Co., St. Louis, MO). Fixed and permeabilized cells were subjected to double immunofluorescence analysis. In this analysis, the cells were contacted with a suspension of an antibody designated Mel-5 (Signet, Dedham, MA) which binds specifically with TRP-1an melanoma cells, and were then contacted with a suspension of Cy3™-conjugated (i.e. fluorescently-labeled) goat anti-mouse IgG (obtained from
Jackson Immuno Research Laboratories, Inc., West Grove, PA). Next, the fixed and permeabilized cells were contacted with a biotinylated monoclonal antibody designated SAP (Hsu et al., 1998, Am. J. Pathol. 153:1435-1442) which binds specifically with the β3 subunit of the vitronectin receptor, and then with an antibody which binds specifically with Mel-CAM (Shih et al., 1994, Cancer Res. 54:2514-2520). The cells were then contacted with streptavidin conjugated with fluorescein isothiocyanate (FITC; Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Following these treatments, all melanoma cells were labeled with Cy3, and melanoma cells which expressed either or both of Mel-CAM and the β3 subunit of the vitronectin receptor were also labeled with FITC. For cell growth experiments, cell culture slides were counter-stained using Hœchst reagent (bisbenzimide; a cell nucleus stain; Sigma Chemical Co., St. Louis, MO). As a negative control, normal mouse serum was used in place of primary (i.e. anti-Mel-CAM and anti-β3 antibodies. Cell growth on the slides was monitored by counting cells in high power (250x) microscopic fields. The ratio of keratinocytes (KC) to melanocytes (MC) was determined using the following equation:

\[
\frac{KC}{MC} = \frac{\text{Number of Hœchst-positive nuclei}}{\text{Number of red cells}} - \frac{\text{Number of red cells}}{\text{Number of red cells}}
\]

Melanoma cells (cell lines WM115 and three others; i.e. cell lines which did not express E-cadherin) were transduced using an adenovirus vector which comprised a polynucleotide encoding E-cadherin (vector E-cad/Ad5) or a polynucleotide encoding LacZ protein (vector LacZ/Ad5). Vector E-cad/Ad5 was generated by transducing E1-positive 293 cells with a recombinant shuttle vector comprising full-length human E-cadherin cDNA and the vector pAd.CMV-Link.1 wherein the region E3 was deleted in the dl7001 human adenoviral DNA. The vector LacZ/Ad5 was constructed similarly, using a DNA encoding LacZ in place of the E-cadherin cDNA. Melanoma cells were transduced by maintaining the cells with 20 plaque forming units per cell of the selected vector for two hours in serum-free Dulbecco's modified Eagle's medium (DMEM). After 48 hours, the vector-treated cells
were incubated for four hours with a 10 milligram per milliliter solution of the fluorescent dye Dil (Molecular Probes, Eugene, OR). The cells were detached from their growth substrate by contacting the substrate for 30 minutes with a 0.01% (w/v) suspension of trypsin in 1 millimolar Ca-HEPES-buffered salt solution (HBSS) at 37 °C. Under these conditions, cadherins are specifically protected from proteolytic digestion. E-cadherin expression was detected using flow analysis/cell sorting procedure in which cells were contacted with a fluorescently-labeled anti-E-cadherin antibody.

For adhesion-blocking experiments, melanoma cells infected with E-cad/Ad5 were contacted at 4°C for 30 minutes with a 5 microgram per milliliter suspension of a monoclonal antibody designated SHE78-7 (Sigma Chemical Co., St. Louis, MO), which binds specifically with E-cadherin. The cells were rinsed with HBSS and resuspended in an assay medium comprising 1% (w/v) bovine serum albumin and 1 millimolar calcium in HBSS. A total of about 2 x 10^5 cells in a volume of 400 microliters was added to differentiated keratinocyte monolayers in 4-well chamber slides. Keratinocyte differentiation in the monolayers had been induced prior to contact with the melanoma cell suspension by treatment of the cells with 2 millimolar calcium for 1 hour, as described (Valyi-Nagy et al., 1993, Lab. Invest. 69:152-159). The melanoma cell suspension was maintained in contact with the monolayers for 1 hour, in order to allow adherence of melanoma cells to keratinocytes. After this period, non-adherent cells were removed, and the slices were fixed using 3% (v/v) paraformaldehyde. The numbers of adherent cells per high power (250x) microscopic field were assessed in triplicate wells using a fluorescence microscope. Statistical analyses were performed using the Student's t-test.

Invasiveness of melanoma cells was assessed in artificial skin reconstructs, as described (Hsu et al., 1998, Am. J. Pathol., 153:1435-1442). Briefly, human foreskin dermal fibroblasts suspended in rat tail collagen were placed onto a pre-cast collagen gel and allowed to constrict the collagen for six days. E-cad/Ad5-transduced, LacZ/Ad5-transduced, and non-transduced melanoma cells (cell line 1205Lu) were then mixed with epidermal keratinocytes at a 1:5 ratio and seeded onto
the surface of the dermal constructs. Five days later, cultures were lifted to the air-liquid interface in order to allow stratification of epidermal keratinocytes. Ten days thereafter, the reconstructs were harvested, fixed using paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Apoptosis in the reconstructs was assessed using the ApopTag™ in situ apoptosis detection kit (Oncor, Gaithersburg, MD) according to the manufacturer's instructions.

The results of the experiments presented in this Example are now described.

It is known that melanocytes, but not melanoma cells, express the adhesion receptor designated E-cadherin (Hsu et al., 1996, J. Invest. Dermatol. Symp. Proc. 1:188-194). However, the role of E-cadherin in the failure of keratinocytes to regulate melanoma cells has not previously been appreciated.

In experiments presented in this Example, melanoma cell lines which do not express E-cadherin were transduced with an adenovirus construct which comprised an E-cadherin cDNA (E-cad/Ad5) or a LacZ-encoding DNA (LacZ/Ad5). Cells transduced with E-cad/Ad5 expressed E-cadherin, and neither cell transduced with LacZ/Ad5 nor non-transduced cells expressed E-cadherin. Expression of E-cadherin in cells transduced with E-cad/Ad5 was confirmed by Western blotting using a fluorescent antibody which binds specifically with E-cadherin. The functionality of E-cadherin expressed in these cells was confirmed by observing a 4-fold increase in adhesion of transduced melanoma cells to keratinocytes, relative to cells transduced with LacZ/Ad5 and relative to non-transduced cells. In addition, adherence of E-cad/Ad5-transduced cells was significantly reduced in the presence of an antibody which binds specifically with E-cadherin, as indicated in Figure 1.

Normal (i.e. non-melanoma) melanocytes mixed with normal human keratinocytes at a 1:5 or 1:10 ratio maintained the corresponding ratio over a seven day period of observation, despite proliferation of cells of both types, as indicated in Figure 2. Melanoma cells transduced with E-cad/Ad5 also maintained a substantially constant ratio with co-cultured keratinocytes. Melanoma cells transduced with LacZ/Ad5,
however, did not maintain a constant melanoma cell:keratinocyte ratio, as indicated in Figure 2.

In order to determine whether E-cadherin contributes to the phenotypic plasticity of melanocytes in response to contact exerted by contact of those cells with keratinocytes, cell surface antigen expression was tested in E-cad/Ad5-transduced cells which were cultured in the presence of keratinocytes. Non-transduced and LacZ/Ad5-transduced melanoma cells exhibited no detectable change in expression of melanoma-associated antigens such as the cell-to-cell adhesion molecule designated MelCAM/MUC18 or the β3 subunit of the αvβ3 vitronectin receptor. However, expression of these two antigens could not be detected in melanoma cells transduced with E-cad/Ad5 after seven days of co-culture with keratinocytes. E-cad/Ad5-transduced melanoma cells which were cultured in the absence of keratinocytes exhibited no change in Mel-CAM or β3 subunit antigen expression, relative to non-transduced cells. These results demonstrate that the presence of E-cadherin on the surface of melanocytes is necessary for exertion of contact-mediated control by keratinocytes over the phenotype of melanocytes.

The physiological significance of down-regulation of tumor-associated cell-surface antigens Mel-CAM and β3 on melanoma cell phenotype was demonstrated using a three-dimensional reconstruct model of human skin. Each reconstruct comprised a dermal compartment of fibroblasts which was separated by a basement membrane from an epidermal compartment comprising melanocytes and keratinocytes. Using this skin reconstruct model, it was determined that non-transduced melanoma cells and melanoma cells transduced with LacZ/Ad5 grew deep into the dermal compartment, forming strands of cell nests, as illustrated in Figures 3B and 3D. In contrast, melanoma cells transduced with E-cad/Ad5 remained in the epidermal compartment and the upper portion of the dermal compartment, as illustrated in Figures 3A and 3C. Furthermore, those E-cad/Ad5-transduced cells which were located in the upper portion of the dermal compartment exhibited typical signs of apoptotic cell death, including nuclear condensation and apoptotic body formation. Free 3'-hydroxy ends resulting from DNA fragmentation were detected in these cells using a
commercially available apoptosis detection kit, as indicated in Figure 3E. Invasive melanoma cells in control reconstructions did not exhibit signs of apoptosis, as indicated in Figure 3F.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.
What is claimed is:

1. A method of inhibiting a tumorigenic property of a melanoma cell, said method comprising providing to said cell an isolated polypeptide consisting of at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

2. The method of claim 1, wherein said cell is a human cell.

3. The method of claim 2, wherein said cell is present in the body of a human patient.

4. A method of inhibiting a tumorigenic property of a melanoma cell, said method comprising enhancing expression of E-cadherin in said cell.

5. The method of claim 4, wherein expression of E-cadherin in said cell is enhanced by providing to said cell an expressible vector comprising an isolated polynucleotide encoding at least a portion of E-cadherin.

6. The method of claim 5, wherein the nucleotide sequence of said isolated polynucleotide is at least 90% homologous with SEQ ID NO: 2, and wherein the amino acid sequence of said portion is at least 75% identical to SEQ ID NO: 1.

7. A method of inhibiting a tumorigenic property of a melanoma cell, said method comprising providing to said cell a peptidomimetic of a polypeptide consisting of at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic
helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

8. A method of inhibiting a tumorigenic property of a melanoma cell, said method comprising providing to said cell an isolated polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

9. A method of inhibiting a tumorigenic property of a melanoma cell, said method comprising providing to said cell a peptidomimetic of a polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

10. A method of determining whether a polypeptide or peptidomimetic is effective for inhibiting a tumorigenic property of a melanoma cell, said method comprising

   providing a test polypeptide or peptidomimetic to a test melanoma cell, wherein said test polypeptide or peptidomimetic is derived from a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin,
a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad; and

comparing the tumorigenic property of said test melanoma cell with the same property of a control melanoma cell to which said test polypeptide or peptidomimetic is not provided,

whereby if the tumorigenic property of said test melanoma cell is inhibited relative to the same property of said control melanoma cell, then said test polypeptide or peptidomimetic is effective for inhibiting a tumorigenic property of a melanoma cell.

11. The method of claim 10, wherein said tumorigenic property is selected from the group consisting of cell growth, cell proliferation, and tissue invasiveness.

12. The method of claim 10, wherein said test melanoma cell is maintained in the presence of the test polypeptide or peptidomimetic for a period of at least one hour prior to comparing the tumorigenic property of said test melanoma cell with the same property of said control melanoma cell.

13. A method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said method comprising

providing a test polypeptide or peptidomimetic to a test melanoma cell, wherein said test polypeptide or peptidomimetic is derived from a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic
helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad; comparing the tumorigenic property of said test melanoma cell with the same property of a control melanoma cell to which said test polypeptide or peptidomimetic is not provided; and

if the tumorigenic property of said test melanoma cell is inhibited relative to the same property of said control melanoma cell, combining said test polypeptide or peptidomimetic with a pharmaceutically acceptable carrier to form said pharmaceutical composition.

14. A method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell, said method comprising maintaining a test melanoma cell in the presence of said composition for at least one hour; and comparing Mel-CAM expression in said test melanoma cell with Mel-CAM expression in a control melanoma cell which is not maintained in the presence of said composition,

whereby a lower level of Mel-CAM expression in said test melanoma cell than in said control melanoma cell is an indication that said composition is effective for inhibiting a tumorigenic property of a melanoma cell.

15. The method of claim 14, wherein Mel-CAM expression is assessed by assessing the amount of intracellular mRNA encoding Mel-CAM.

16. The method of claim 14, wherein Mel-CAM expression is assessed by assessing the amount of cellular Mel-CAM protein.

17. The method of claim 14, wherein Mel-CAM expression is assessed by assessing the amount of cell-surface Mel-CAM protein.

18. The method of claim 17, wherein the amount of cell-surface Mel-CAM protein is assessed using a labeled anti-Mel-CAM antibody.

19. A method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell, said method comprising
maintaining a test melanoma cell in the presence of said composition for at least one hour; and

comparing αvβ3 expression in said test melanoma cell with αvβ3 expression in a control melanoma cell which is not maintained in the presence of said composition, whereby a lower level of αvβ3 expression in said test melanoma cell than in said control melanoma cell is an indication that said composition is effective for inhibiting a tumorigenic property of a melanoma cell.

20. The method of claim 19, wherein αvβ3 expression is assessed by assessing the amount of the amount of intracellular mRNA encoding the β3 subunit of αvβ3.

21. The method of claim 19, wherein αvβ3 expression is assessed by assessing the amount of cellular αvβ3 protein.

22. The method of claim 19, wherein αvβ3 expression is assessed by assessing the amount of cell-surface αvβ3 protein.

23. The method of claim 22, wherein the amount of cell-surface αvβ3 protein is assessed using a labeled anti- αvβ3 antibody.

24. A method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell, said method comprising

maintaining a test melanoma cell in the presence of said composition for at least one hour; and

comparing Mel-CAM and αvβ3 expression in said test melanoma cell with Mel-CAM and αvβ3 expression in a control melanoma cell which is not maintained in the presence of said composition,

whereby a lower level of Mel-CAM and αvβ3 expression in said test melanoma cell than in said control melanoma cell is an indication that said composition is effective for inhibiting a tumorigenic property of a melanoma cell.

25. A method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said method comprising

maintaining a test melanoma cell in the presence of a test composition for at least one hour;
comparing Mel-CAM expression in said test melanoma cell with Mel-CAM expression in a control melanoma cell which is not maintained in the presence of said test composition; and

if a lower level of Mel-CAM expression is detected in said test melanoma cell than in said control melanoma cell, then combining said test composition with a pharmaceutically acceptable carrier to form said pharmaceutical composition.

26. A method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said method comprising

maintaining a test melanoma cell in the presence of a test composition for at least one hour;

comparing αvβ3 expression in said test melanoma cell with αvβ3 expression in a control melanoma cell which is not maintained in the presence of said test composition; and

if a lower level of αvβ3 expression is detected in said test melanoma cell than in said control melanoma cell, then combining said test composition with a pharmaceutically acceptable carrier to form said pharmaceutical composition.

27. A method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said method comprising

maintaining a test melanoma cell in the presence of a test composition for at least one hour;

comparing Mel-CAM and αvβ3 expression in said test melanoma cell with Mel-CAM and αvβ3 expression in a control melanoma cell which is not maintained in the presence of said test composition; and

if a lower level of Mel-CAM and αvβ3 expression is detected in said test melanoma cell than in said control melanoma cell, then combining said test composition with a pharmaceutically acceptable carrier to form said pharmaceutical composition.

28. A method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell, said method comprising
maintaining a test melanoma cell in the presence of basal layer keratinocytes and in the presence of said composition for at least one hour; and

comparing gap junction formation between said test melanoma cell and said basal layer keratinocytes with gap junction formation between a control melanoma cell and basal layer keratinocytes maintained in the absence of said composition,

whereby a greater degree of gap junction formation for said test melanoma cell than for said control melanoma cell is an indication that said composition is effective for inhibiting a tumorigenic property of a melanoma cell.

29. A method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said method comprising

maintaining a test melanoma cell in the presence of basal layer keratinocytes and in the presence of a test composition for at least one hour;

comparing gap junction formation between said test melanoma cell and said basal layer keratinocytes with gap junction formation between a control melanoma cell and basal layer keratinocytes maintained in the absence of said test composition; and

if a greater degree of gap junction formation is detected for said test melanoma cell than for said control melanoma cell, then combining said test composition with a pharmaceutically acceptable carrier to form said pharmaceutical composition.

30. A pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said composition comprising an active agent admixed with a pharmaceutically acceptable carrier, wherein said active agent is an expressible vector comprising an isolated polynucleotide encoding at least a portion of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.
31. A pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said composition comprising an active agent admixed with a pharmaceutically acceptable carrier, wherein said active agent is an isolated polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.

32. A pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell, said composition comprising an active agent admixed with a pharmaceutically acceptable carrier, wherein said active agent is a peptidomimetic of a polypeptide having an amino acid sequence which is at least 75% identical with at least four consecutive amino acid residues of a protein selected from the group consisting of E-cadherin, α-catenin, β-catenin, plakoglobin, p120 (ctn), an axin, a glycogen synthase kinase, APC, PKB/Akt, an Lef/TCF protein, a POU domain containing protein, an Ets protein, an E-box binding protein, a protein of the myc family, a protein having one or more leucine zipper motifs, a homeobox protein, a protein having one or more homeobox motifs, Shc, a helix-loop-helix protein, a basic helix-loop-helix leucine zipper-containing protein, a protein of the dishevelled and frizzled family of proteins, a protein which modulates apoptosis, Bcl-2, Bax, and Bad.
Fig. 1
Fig. 2
Fig. 4B
<table>
<thead>
<tr>
<th>MGPWS</th>
<th>RSLSA</th>
<th>LLLLL</th>
<th>QVSSW</th>
<th>LÇQEP</th>
<th>EPCHP</th>
<th>GFDAE</th>
<th>SYTFT</th>
<th>VPRRH</th>
<th>LERGR</th>
<th>VLGRV</th>
<th>55</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFEDC</td>
<td>TGRQR</td>
<td>TAYFS</td>
<td>LDTRF</td>
<td>KVGTD</td>
<td>GVITV</td>
<td>KRPLR</td>
<td>FHNPQ</td>
<td>IHFLV</td>
<td>YAWDS</td>
<td>TURKF</td>
<td>110</td>
</tr>
<tr>
<td>STKVVT</td>
<td>LNTVG</td>
<td>HHHRP</td>
<td>PPHQA</td>
<td>SVSGI</td>
<td>QAEEL</td>
<td>TFPNS</td>
<td>SPGLR</td>
<td>RQKRD</td>
<td>WVIPP</td>
<td>ISCPE</td>
<td>165</td>
</tr>
<tr>
<td>NEKGP</td>
<td>FPKNL</td>
<td>VQIKS</td>
<td>NDKKE</td>
<td>GKVFY</td>
<td>STITQ</td>
<td>GADTP</td>
<td>PVGVF</td>
<td>IIBRE</td>
<td>TGWLK</td>
<td>VTEPL</td>
<td>220</td>
</tr>
<tr>
<td>DRERI</td>
<td>ATYTL</td>
<td>FSHAV</td>
<td>SSNGN</td>
<td>AVEDP</td>
<td>MEILI</td>
<td>TVTDQ</td>
<td>NDNKP</td>
<td>EFTQE</td>
<td>VFKGS</td>
<td>VMEGA</td>
<td>275</td>
</tr>
<tr>
<td>LPGTS</td>
<td>VMEVT</td>
<td>ATDAD</td>
<td>DDVNT</td>
<td>YNAAI</td>
<td>AYTIL</td>
<td>SQDPE</td>
<td>LPDKN</td>
<td>MFTIN</td>
<td>RNTGV</td>
<td>ISVVT</td>
<td>330</td>
</tr>
<tr>
<td>TGLDR</td>
<td>ESFPT</td>
<td>YTLVV</td>
<td>QAADL</td>
<td>QGEGL</td>
<td>STTAT</td>
<td>AVITV</td>
<td>TDTND</td>
<td>NPPIF</td>
<td>NPTTI</td>
<td>KQVVP</td>
<td>385</td>
</tr>
<tr>
<td>ENEAN</td>
<td>VVITT</td>
<td>LKVTD</td>
<td>ADAPN</td>
<td>TPAWE</td>
<td>AVYTI</td>
<td>LNDDG</td>
<td>QGFVV</td>
<td>TTNPV</td>
<td>NNDGI</td>
<td>LKTAK</td>
<td>440</td>
</tr>
<tr>
<td>GLDFE</td>
<td>AKQYQ</td>
<td>ILHVA</td>
<td>VTNVV</td>
<td>PEFSV</td>
<td>LTTST</td>
<td>ATVTV</td>
<td>DVLDV</td>
<td>NEAPI</td>
<td>FVPPE</td>
<td>KRVEV</td>
<td>495</td>
</tr>
<tr>
<td>SEDFG</td>
<td>VGQEI</td>
<td>TSYTA</td>
<td>QEPTD</td>
<td>FMEQK</td>
<td>ITYRI</td>
<td>WRDTA</td>
<td>NWLEI</td>
<td>NPDTG</td>
<td>AISTR</td>
<td>AELDR</td>
<td>550</td>
</tr>
<tr>
<td>EDFEH</td>
<td>VKNST</td>
<td>YTALI</td>
<td>IATDN</td>
<td>GSPVA</td>
<td>TGTGT</td>
<td>LLLIL</td>
<td>SDVND</td>
<td>NAPIP</td>
<td>EPREI</td>
<td>FFCER</td>
<td>605</td>
</tr>
<tr>
<td>NPKPO</td>
<td>VINII</td>
<td>DADLP</td>
<td>PNTSP</td>
<td>FTAEL</td>
<td>THGAS</td>
<td>ANWTVI</td>
<td>QYNDP</td>
<td>TQESTI</td>
<td>ILKPK</td>
<td>MALEV</td>
<td>660</td>
</tr>
<tr>
<td>GDYKI</td>
<td>NLKLM</td>
<td>DNQNK</td>
<td>DQVTT</td>
<td>LEVSV</td>
<td>CDCG</td>
<td>AAGVC</td>
<td>RKAQP</td>
<td>VEAGL</td>
<td>QIPAI</td>
<td>LGILG</td>
<td>715</td>
</tr>
<tr>
<td>GILAL</td>
<td>LILLI</td>
<td>LLLLIF</td>
<td>LRRAA</td>
<td>VKEP</td>
<td>LLPPE</td>
<td>DDTRD</td>
<td>NVYY</td>
<td>DEEGG</td>
<td>GEEDQ</td>
<td>DFOLS</td>
<td>770</td>
</tr>
<tr>
<td>QLHRG</td>
<td>LDAPR</td>
<td>EVTRN</td>
<td>DVAFT</td>
<td>LMSVP</td>
<td>RYLP</td>
<td>PANPD</td>
<td>EIGNF</td>
<td>IDENL</td>
<td>KAADT</td>
<td>DPTAP</td>
<td>825</td>
</tr>
<tr>
<td>PYDSL</td>
<td>LVFDY</td>
<td>EGSGS</td>
<td>EAASL</td>
<td>SSLNS</td>
<td>SESDK</td>
<td>DQYD</td>
<td>YLNEW</td>
<td>GNRFK</td>
<td>KLADM</td>
<td>YGGGE</td>
<td>880</td>
</tr>
</tbody>
</table>

**Fig. 5**
<110> THE WISTAR INSTITUTE

<120> INHIBITION OF TUMORIGENIC PROPERTIES OF MELANOMA CELLS

<130> 9924-20WO [Herlyn PCT]

<140> NOT YET ASSIGNED

<141> 2000-10-14

<150> U.S. 60/159,353

<151> 1999-10-14

<160> 2

<170> PatentIn Ver. 2.1

<210> 1

<211> 882

<212> PRT

<213> Homo sapiens

<400> 1

Met Gly Pro Trp Ser Arg Ser Leu Ser Ala Leu Leu Leu Leu Leu Gln

1  5  10  15

Val Ser Ser Trp Leu Cys Gln Glu Pro Glu Pro Cys His Pro Gly Phe

20  25  30

Asp Ala Glu Ser Tyr Thr Phe Thr Val Pro Arg Arg His Leu Glu Arg

35  40  45

Gly Arg Val Leu Gly Arg Val Asn Phe Glu Asp Cys Thr Gly Arg Gln

50  55  60

Arg Thr Ala Tyr Phe Ser Leu Asp Thr Arg Phe Lys Val Gly Thr Asp

65  70  75  80

Gly Val Ile Thr Val Lys Arg Pro Leu Arg Phe His Asn Pro Gln Ile

85  90  95

His Phe Leu Val Tyr Ala Trp Asp Ser Thr Tyr Arg Lys Phe Ser Thr

100  105  110

Lys Val Thr Leu Asn Thr Val Gly His His Arg Pro Pro Pro His

115  120  125
Gln Ala Ser Val Ser Gly Ile Gln Ala Glu Leu Leu Thr Phe Pro Asn
130 135 140
Ser Ser Pro Gly Leu Arg Arg Gln Lys Arg Asp Trp Val Ile Pro Pro
145 150 155 160
Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro Phe Pro Lys Asn Leu Val
165 170 175
Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly Lys Val Phe Tyr Ser Ile
180 185 190
Thr Gly Gln Gly Ala Asp Thr Pro Pro Val Gly Val Phe Ile Ile Glu
195 200 205
Arg Glu Thr Gly Trp Leu Lys Val Thr Glu Pro Leu Asp Arg Glu Arg
210 215 220
Ile Ala Thr Tyr Thr Leu Phe Ser His Ala Val Ser Ser Asn Gly Asn
225 230 235 240
Ala Val Glu Asp Pro Met Glu Ile Leu Ile Thr Val Thr Asp Gln Asn
245 250 255
Asp Asn Lys Pro Glu Phe Thr Gln Glu Val Phe Lys Gly Ser Val Met
260 265 270
Glu Gly Ala Leu Pro Gly Thr Ser Val Met Glu Val Thr Ala Thr Asp
275 280 285
Ala Asp Asp Asp Val Asn Thr Tyr Asn Ala Ala Ile Ala Tyr Thr Ile
290 295 300
Leu Ser Gln Asp Pro Glu Leu Pro Asp Lys Asn Met Phe Thr Ile Asn
305 310 315 320
Arg Asn Thr Gly Val Ile Ser Val Val Thr Gly Leu Asp Arg Glu
325 330 335
Ser Phe Pro Thr Tyr Thr Leu Val Val Gln Ala Ala Asp Leu Gln Gly
340 345 350
Glu Gly Leu Ser Thr Thr Ala Thr Ala Val Ile Thr Val Thr Asp Thr
355 360 365
Asn Asp Asn Pro Pro Ile Phe Asn Pro Thr Thr Tyr Lys Gly Gln Val
370 375 380
Pro Glu Asn Glu Ala Asn Val Val Ile Thr Thr Leu Lys Val Thr Asp 385 390 395 400
Ala Asp Ala Pro Asn Thr Pro Ala Trp Glu Ala Val Tyr Thr Ile Leu 405 410 415
Asn Asp Asp Gly Gly Gln Phe Val Val Thr Thr Asn Pro Val Asn Asn 420 425 430 435
Asp Gly Ile Leu Lys Thr Ala Lys Gly Leu Asp Phe Glu Ala Lys Gln 440 445
Gln Tyr Ile Leu His Val Ala Val Thr Asn Val Val Pro Phe Glu Val 450 455 460
Ser Leu Thr Thr Ser Thr Ala Thr Val Thr Val Asp Val Leu Asp Val 465 470 475 480
Asn Glu Ala Pro Ile Phe Val Pro Pro Glu Lys Arg Val Glu Val Ser 485 490 495
Glu Asp Phe Gly Val Gly Gln Glu Ile Thr Ser Tyr Thr Ala Gln Glu 500 505 510
Pro Asp Thr Phe Met Glu Gln Ile Thr Tyr Arg Ile Trp Arg Asp 515 520 525
Thr Ala Asn Trp Leu Glu Ile Asn Pro Asp Thr Gly Ala Ile Ser Thr 530 535 540
Arg Ala Glu Leu Asp Arg Glu Phe Glu His Val Lys Asn Ser Thr 545 550 555 560
Tyr Thr Ala Leu Ile Ile Ala Thr Asp Gly Ser Pro Val Ala Thr 565 570 575
Gly Thr Gly Thr Leu Leu Ile Leu Ser Asp Val Asn Asp Asn Ala 580 585 590
Pro Ile Pro Glu Pro Arg Thr Ile Phe Phe Cys Glu Arg Asn Pro Lys 595 600 605
Pro Gln Val Ile Asn Ile Ile Asp Ala Asp Leu Pro Pro Asn Thr Ser 610 615 620
Pro Phe Thr Ala Glu Leu Thr His Gly Ala Ser Ala Asn Trp Thr Ile 625 630 635 640
Gln Tyr Asn Asp Pro Thr Gln Glu Ser Ile Ile Leu Lys Pro Lys Met
645 650 655
Ala Leu Glu Val Gly Asp Tyr Lys Ile Asn Leu Lys Leu Met Asp Asn
660 665 670
Gln Asn Lys Asp Gln Val Thr Leu Glu Val Ser Val Cys Asp Cys
675 680 685
Glu Gly Ala Ala Gly Val Cys Arg Lys Ala Gln Pro Val Glu Ala Gly
690 695 700
Leu Gln Ile Pro Ala Ile Leu Gly Ile Leu Gly Gly Ile Leu Ala Leu
705 710 715 720
Leu Ile Leu Ile Leu Leu Leu Leu Leu Phe Leu Arg Arg Arg Ala Val
725 730 735
Val Lys Glu Pro Leu Leu Pro Pro Glu Asp Asp Thr Arg Asp Asn Val
740 745 750
Tyr Tyr Tyr Asp Glu Gly Gly Gly Gly Glu Asp Gln Asp Phe Asp
755 760 765
Leu Ser Gln Leu His Arg Gly Leu Asp Ala Arg Pro Glu Val Thr Arg
770 775 780
Asn Asp Val Ala Pro Thr Leu Met Ser Val Pro Arg Tyr Leu Pro Arg
785 790 795 800
Pro Ala Asn Pro Asp Glu Ile Gly Asn Phe Ile Asp Glu Asn Leu Lys
805 810 815
Ala Ala Asp Thr Asp Pro Thr Ala Pro Pro Tyr Asp Ser Leu Leu Val
820 825 830
Phe Asp Tyr Glu Gly Ser Gly Ser Glu Ala Ala Ser Leu Ser Ser Leu
835 840 845
Asn Ser Ser Glu Ser Asp Lys Asp Gln Asp Tyr Asp Tyr Leu Asn Glu
850 855 860
Trp Gly Asn Arg Phe Lys Leu Ala Asp Met Tyr Gly Gly Gly Glu
865 870 875 880
Asp Asp
<210> 2
<211> 4778
<212> DNA
<213> Homo sapiens

<400> 2

gcttgccgaa gtcagttcag actccagccc gctccagccc ggcccagacc gaccgcaacc 60
ggcgcctgcc ctgcgcctgc gcctccccgcg acgcatgggc cttggagacc gcacgccttc 120
ggcgtgtgct gctgattctgc agggctcttg cttggtcttc caggacgcca agcccttgca 180
cctctgcttt gcagccgaga gctacaagtct caaggtgctcc cggccgccac cggagagag 240
cggcgctctg ggccagaggt attttgagttg cggccacgc gggagagttt 300
ttcccctgcac accccgattcag aagttgggac acagtgtcttg attacagctca aagggcctct 360
acggttttcat aaccacagag tccattctttt ggtctacgcc tgggactcacc ctacagaca 420
gtttccccac aaggtccagc tggagtacag gggccacacc cacgccaccc gcggccatca 480
ggcctccgttt ctctggaatcc aacgcaagtt gctcacatttt cccacactct ctacctgcctc 540
cagaagagac aggagagact gggttattccc tccctacagc tacggccagaa atgaaagaag 600
cccatttcttc aaaaaacctgg ttcagatccaa atccaaacaa gcaagaagac gcgtggtttt 660
cacacagtc aagtggccagac tgggtgacacct aacctcgcttg ggtgctcttt tcattgtaag 720
agaacacagaga tgctgtaagag tgcagagcct tcttgataga gaacgcattgc ccacatcaac 780
tctctctcttg cacagctgtgat ctcaccaacag gattgcttgatt gaggtgctca aagagatttt 840
gatcacagtgta atgcgcaagct gccggagattt cccacagagg tctcttaagg 900
tgtctgcatgg gaggagctgtt gctcagccag cttgagttgt gaggctacag cccacagagc 960
gcacagtcag atgctaacacht accatcggcatt catcgtctcttta accatcctcagg gcacagatcc 1020
tgagtctctct tgcacaaata tgcttctccat caacaggaac cacagaggtaca tgtctgcgg 1080
cacacctgaggg ctggacagag agaggttttcc tacgttaacc ctgtgctggtta aagctgctgta 1140
cctctaaact gagggtgatt gccatcacagc aacagctcatt atccacgtaa ctcacccaga 1200
cgtaactcctg ccagatcctta atccccacacag gtacacagttt caggtgctgct aagacagagg 1260	taacgctgtaga atccaccacac gtaagaagtgac tgaagtgctat gctggccaatgccctcagtgct 1320
ggagaggtgtct ctcgaggtata accgccagcct cggagtgctag cctcccagatc cacagagggc 1380
agtgaacacagt gaggccatttt tgaacactgac aagggcttgag gatttttagg ccagacagca 1440
gtactatttct taccacctttc ctggagctagtg gttacattttt cgggtctcttc tacacccatttct 1500
cacagccgcc gttccagctttt gttcagttgac tcgtgtaagga ggcccgccatttt gttgctcctg 1560
tgaaagaagtg gttgggagtttg cggagctgctg caggacatat ccacatcactc cagagactcc 1620
tgcacagagag gcacacagattt ttaagagagcc gaaaaataca tataagttttt cggagacagc 1680
tgcacactgct ctggagattt aacgacagcct cgggtgtcatt ttcacccagct cttgaggtga 1740
caggaggtgt tggagccagct tgaagaaagag caagctcacata gcctcaatac tagctacagc 1800
caatgtttctt ccaagtttcag ctggacagcg gacacattcctg cttgatcctgtt ggcagaaataag 1860
tgcaacagcct ccacatcagc aaccttcgac atatgtccttct tgtgtagaggc atccacagccc 1920
tcaggcaatac acactgatttt tccctcccact atcctcccttc tcaacagcga 1980	actaacaacc ggccccggagtc gggagattgt ctgcagttgcac ggcggacacatt caacatcaca 2040	tatctatattt gtcgaggttgc gtttttggagtgct cccatttaaag cccaaaaatc aatcagcttgca 2100
catgctaaaact aacccaggtac cacccagtcac gctctttttg tctgcttact gaggctcttttt gtcctg 2160
agggcccccg gccggtcttgta ggaaggcccc gcgcgcttcct ggggtcgtgctc gacagtcattgac 2220
ctatgtggtg acatcttttc tttgctaaatt ctgatccact gcgtcttccctgctgctgctgctgctgctgct 2280
cttttttttg agggcagcctt gtcgggtcagc tggctgtccagcc cgacccagctt acagcctttc 2340
ggcaacagtttt cattatcattag atgagaaaggg ccggccgagaag gacggccagctt actctgactt 2400
gggcaagcattga cccagccagcag gctcggacag ctcgcttccag cgctcagagct gcctgtaagtg 2460
aaacctcatgt agtgcctccc ggtatctccc ccgccctgcc aatcoccagt aattggaaga 2520
tttatgtgat gaaatactgta aacgctgctga tactgacccc acagccccgg ctttatgattc 2580
ttcgctctgg tttgactatt aggaagccgg ttcggagcct gctagctgta gctccctgaa 2640
tctcccaagag tcacaccaag accagagacta tgcactacttg aacgaatgta gcacactgtt 2700
caaagaagct gcgtcacatgt aagagagccgg caggagacag taggggactc gagaagcgcc 2760
ggccccacag ccattgcttg gccagagagt aaatcaagctt acctgctgat ttctcagtcct 2820
cctcgccttga gatgagtttc tgtgggaaaaa aaagaagactg gttgaagtgtc cagttagtat 2880
ggctttatca tcccctcaact tttatagcttc aataagtttg tgttgaagaa gttttgcatct 2940
attctctaaa gcttttttttt tttttttctct actcctttatc tgggtgtgtg cttcacaaga 3000	taaccaaatatt caaatatttcc aagagacaaat ctttaagcata aaggtgtcca cccagcccc 3060
tgccagatatt cttaaaggaat ttgtctctac ttttaaataa aagggagaa ggctcatctcatt 3120
cagcctgtgc tttttttttttt ttgtgtgtgct tctgtctcatc 3180
actacacctgg tggctcccttc tcgctctttta ttttaatatta agacaggtgct tcatttcatc 3240
ggccagccct ctgctcagctt gataactgcag gatctcaatc agcttggctc gcctgccctt 3300
agalactcacc actcactcacc accttgacag ccagaggtgct gctgctgttc cttttttctc 3360
agtactatatt tttttttatttt tttgtgtgcgc ttgctgctgct gctgctgttc ctttttttttt 3420
acccacctgtc ctaccctgatt acctttacct gcttcttccac 3480
acgccagcct ctctgtgcag cttcttctct cctttgcaaat agctttgatgct cgtggcatct 3540
ccactccctca ggcttgaggg ctgctgcgtg cagattagct ctctggcaact gccacacttc 3600
ggctctacac actccctctca cccacccacg ctttttttttt tcagcagatg ggtgtcttgtc 3660
agtgtgccac aagctctctct ctaacactgta ccttttctctc ctttttttttt ttttttttct 3720
cagaagctgct gcagagaggg ctgctcagag ctgctgcgtc gtttttttttt ttgctgcgttc 3780
ttcctcatct gaaattcagtt gctttgtgcca agatatgaggt tctcttgctgc gcagtatcct 3840
gggctcttttt aaggttaagaa gttgtgtttct tttgttgtgccc acatctttgac taggtatttt 3900
tctctccgaga gacckttttaac gcttttttctc ttttctctct cagattagct ctttttttttt 3960
ggcagctctact cagctctcttg cttcttctct ctttttttttt tcagtcagtc tttttttttt 4020
agcaagagtt atatactgca ggatctggaa tagtttgtcaag aagctctcagc cccaagagca 4080
ggccagaact ttgaaagttg gaggcttgag ctttgtagatg ggcagagacag cttgctcaac 4140	tttagctgcc tcgaggtctac gattggattg ctggtgtcttc actctctctc aaattttttttt 4200
aaggaatggg gcaggtctcag ctagtttccc tgcacacaaga tccgctgtgtt gtctgcataa 4260
cccagaacct ccaagtgtcct gccttttgtag atgctctcag aaaaagccgg cttgctgcgg cttctctct 4320
ccacttggcc caattctcagg tgtcgacaga aaacgagaga tatcctaaat tccaaaatttt 4380	ttcttcagag gaggaaagaaa atgttgcccc caagagggtt aagtcgggag tagttggtttag 4440	tgaggatttt cttttttttttt tttatttttattaaagttgctttt ctttaccatttg cagacatcca 4500
agaaaaagact tttggtggaga tagctttcact gtttctctcaag ttggtggtgg agaaaaaatta 4560
acccctgcaat caaatctttgg aatgtgctctg attttcttggc agttcagact atatccataa 4620	tagtctctttg tagacaatgt ctctgtaatttt ggtagtacct gcacgtggcg ggcctggataa 4680	tcttggatttt ttttttgggag ttgagaaaaa aaaaatactta agctgagaa agttttctctca 4740
aagatgcatt tttataaatt ttattaaacaa attttttttttt 4778
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPCT(7) : A61K 31/00, 38/00, 38/02; C07H 21/00; C07K 14/00, C12N 15/09, 15/12, 15/63
US CL : 435/320.1; 514/2, 44; 530/350; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S.: 435/320.1; 514/2, 44; 530/350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 5,539,096 A (BABAI et al) 23 July 1996 (23.07.1996), abstract and column 1, lines 22-30.</td>
<td>1-3, 8</td>
</tr>
<tr>
<td>X</td>
<td>FRISCH, S.M., Tumor suppression of the adenovirus E1A gene; tumor suppressor ffr induction of epithelium phenotype and apoptosis, for use in cancer gene therapy (conference abstract), 1994, Cancer Gene Therapy, vol. 1, no. 4, pages 330-331 (abstract only).</td>
<td>1-3, 8</td>
</tr>
<tr>
<td>X,P</td>
<td>WO 92/2976,837 A (JACOBS, et al) 02 November 1999 (02.11.1999), column 23, lines 19-26 and column 36, lines 1-42.</td>
<td>1-3, 8</td>
</tr>
<tr>
<td>Y</td>
<td>HUANG, Y.W., et al, Adhesion molecules as targets for cancer therapy, April 1997, Histology and Histopathology, vol. 12, no. 9, pages 467-477 (abstract only).</td>
<td>1-3, 8</td>
</tr>
<tr>
<td>X</td>
<td>WO 00/54839 A2 (INTRIGEN THERAPEUTICS, INC.) 21 September 2000 (21.09.00), abstract, page 5, line 27 - page 6, line 2, page 7, lines 4-7.,P</td>
<td>1-3, 8</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search
05 January 2001 (05.01.2001)

Date of mailing of the international search report
31 JAN 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703)305-3230

Authorized officer
Susan N. Ungar, Ph.D.
Telephone No. (703) 305-2481

Form PCT/ISA/210 (second sheet) (July 1998)
### INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/US00/27953

**C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 5,976,838 A (JACOBS et al) 02 November 1999 (02.11.1999), column 24, line 62 - column 25, line 3 and column 37, line 50 - column 38, line 46.</td>
<td>1-3, 8</td>
</tr>
<tr>
<td>X</td>
<td>WO 98/40508 A1 (SOSNOWSKI, et al) 17 September 1998 (17.09.98), abstract, page 1 (lines 4-8), page 68 (line 25) - page 69 (line 3), and page 81 (lines 2-5).</td>
<td>1-3, 8</td>
</tr>
</tbody>
</table>

Form PCT/ISA/210 (continuation of second sheet) (July 1998)
**INTERNATIONAL SEARCH REPORT**

**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
   - because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3, 8, as drawn to E-cadherin

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)
BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups 1-23, claim(s) 1-3 and 8, drawn to a method of inhibiting a tumorigenic property of a melanoma cell comprising providing to said cell any one of twenty-three (23) different isolated polypeptides having an amino acid sequence that is at least 75% identical to the amino acid sequence of either (a) E-cadherin, (b) alpha-catenin, (c) beta-catenin, (d) plakoglobin, (e) p120(ctn), (f) an axin, (g) a glycogen synthase kinase, (h) APC, (i) PKB/Akt, (j) an Lef/TCF protein, (k) a POU domain containing protein, (l) an Ets protein, (m) an E-box binding protein, (n) a protein of the myc family, (o) a protein having one or more leucine zipper motifs, (p) a homeobox protein, (q) a protein having one or more homeobox motifs, (r) Shc, (s) a helix-loop-helix protein, (t) a basic helix-loop-helix leucine zipper-containing protein, (u) a protein of the dishevelled family, (v) a protein of the frizzled family, (w) a protein that modulates apoptosis, respectively.

Note: Applicant is required to identify and elect a single group as specifically drawn to one of 23 different proteins listed (a-w). Dependent claims 2-3 will be examined as they are drawn to the elected group.

Group 24, claim(s) 4-6, drawn to a method of inhibiting a tumorigenic property of a melanoma cell comprising enhancing the expression of E-cadherin in said cell.

Groups 25-50, claim(s) 7 and 9, drawn to a method of inhibiting a tumorigenic property of a melanoma cell comprising providing to said cell a peptidomimetic of any one of twenty-three (23) different polypeptides, each polypeptide having at least 75% identity to the amino acid sequence of either (a) E-cadherin, (b) alpha-catenin, (c) beta-catenin, (d) plakoglobin, (e) p120 (ctn), (f) an axin, (g) a glycogen synthase kinase, (h) APC, (i) PKB/Akt, (j) an Lef/TCF protein, (k) a POU domain containing protein, (l) an Ets protein, (m) an E-box binding protein, (n) a protein of the myc family, (o) a protein having one or more leucine zipper motifs, (p) a homeobox protein, (q) a protein having one or more homeobox motifs, (r) Shc, (s) a helix-loop-helix protein, (t) a basic helix-loop-helix leucine zipper-containing protein, (u) a protein of the dishevelled family, (v) a protein of the frizzled family, (w) a protein that modulates apoptosis, respectively.

Note: Applicant is required to identify and elect a single group as specifically drawn to one of 23 different proteins listed (a-w).

Groups 51-99, claim(s) 10-12, drawn to a method of determining whether a polypeptide or a peptidomimetic is effective for inhibiting a tumorigenic property of a melanoma cell, wherein the polypeptide or the peptidomimetic is derived from any one of twenty-three (23) different proteins consisting of either (a) E-cadherin, (b) alpha-catenin, (c) beta-catenin, (d) plakoglobin, (e) p120(ctn), (f) an axin, (g) a glycogen synthase kinase, (h) APC, (i) PKB/Akt, (j) an Lef/TCF protein, (k) a POU domain containing protein, (l) an Ets protein, (m) an E-box binding protein, (n) a protein of the myc family, (o) a protein having one or more leucine zipper motifs, (p) a homeobox protein, (q) a protein having one or more homeobox motifs, (r) Shc, (s) a helix-loop-helix protein, (t) a basic helix-loop-helix leucine zipper-containing protein, (u) a protein of the dishevelled family, (v) a protein of the frizzled family, (w) a protein that modulates apoptosis, respectively.

Note: Applicant is required to identify and elect a single group as specifically drawn to either a single polypeptide or a single peptidomimetic derived from any one of 23 different proteins listed (a-w). Dependent claims 11-12 will be examined as they are drawn to the elected group.

Groups 100-151, claim(s) 13, drawn to a method of making a pharmaceutical composition for inhibiting a tumorigenic property of a melanoma cell comprising either a polypeptide or a peptidomimetic that is derived from any one of twenty-three (23) different proteins consisting of either (a) E-cadherin, (b) alpha-catenin, (c) beta-catenin, (d) plakoglobin, (e) p120(ctn), (f) an axin, (g) a glycogen synthase kinase, (h) APC, (i) PKB/Akt, (j) an Lef/TCF protein, (k) a POU domain containing protein, (l) an Ets protein, (m) an E-box binding protein, (n) a protein of the myc family, (o) a protein having one or more leucine zipper motifs, (p) a homeobox protein, (q) a protein having one or more homeobox motifs, (r) Shc, (s) a helix-loop-helix protein, (t) a basic helix-loop-helix leucine zipper-containing protein, (u) a protein of the dishevelled family, (v) a protein of the frizzled family, (w) a protein that modulates apoptosis, respectively.

Note: Applicant is required to identify and elect a single group as specifically drawn to either a single polypeptide or a single peptidomimetic derived from any one of 23 different proteins listed (a-w).

Group 152, claim(s) 14-18, drawn to a method of determining whether a composition is effective for inhibiting a tumorigenic property of a melanoma cell comprising assessing Mel-CAM expression in said cell.

Form PCT/ISA/210 (extra sheet) (July 1998)