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(54) Title: METHODS AND COMPOSITIONS EMPLOYING AN IIP45 TARGETING LIGAND

(57) Abstract: methods and composition for the targeted delivery using targeting conjugates comprising different domains of IIP45 are described. Furthermore, the invention provides targeting conjugates comprising therapeutic agents or diagnostic agents incorporating IIP45 as a targeting ligand for delivery to cells or tissues, such as tumor.



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DESCRIPTION

METHODS AND COMPOSITIONS EMPLOYING AN IIP45 TARGETING LIGAND

The present application claims the priority benefit of United States provisional application number 61/108,385, filed October 24, 2008, the entire contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention relates generally to the field of cell biology, molecular biology, cancer biology, and medicine. More particularly, it concerns targeting conjugates comprising a peptide that incorporates an invasion inhibitory protein 45 (IIP45) sequence or an isoform or homologue thereof as a targeting ligand for targeting cancer cells, and the use of such conjugates in therapy or diagnostics.

2. Description of Related Art

[0002] Therapeutic treatment of many human disease states is limited by the systemic toxicity of the therapeutic agents used. Cancer therapeutic agents in particular exhibit a very low therapeutic index, with rapidly growing normal tissues such as skin and bone marrow affected at concentrations of agent that are not much higher than the concentrations used to kill tumor cells. Treatment of cancer and other organ or tissue confined disease states would be greatly facilitated by the development of compositions and methods for targeted delivery to a desired organ or tissue of a therapeutic agent. Diagnostic imaging would also be facilitated by the targeted delivery of imaging agents to desired organs, tissues or diseased cells.

[0003] The success of any targeted therapy depends on eradicating cells that express specific targets, *i.e.*, proteins that maintain or impact the malignant phenotype. For example, HER-2/neu is a biologically relevant tumor antigen and aberrant signaling via the receptor is an important growth regulator for breast cancers expressing the protein. However, multiple oncogenic pathways are implicated in

cancer progression; thus, additional targets need to be defined to enhance the therapeutic efficacy and impact tumor growth.

[0004] The insulin like growth factor (IGF) pathway is emerging as an important growth regulator in a variety of organs. IGF signaling stimulates proliferation and inhibits apoptosis in cancer cells (Pollak *et al.*, 2004). As part of the insulin-like growth factor (IGF) system, the IGF-binding proteins (IGFBPs) play an important role in the regulation of a multitude of cellular processes (Clemmons 1997, Firth & Baxter 2002). Insulin like growth factor receptor binding protein 2 (IGFBP-2), the second most frequent IGFBP in the human circulation, is involved in many physiological and pathological conditions and processes. IGFBP-2 is also often highly expressed in malignant tumor cells but decreases upon remission (Muller *et al.* 1994, Elmlinger *et al.* 1996, Hoeflich *et al.* 2000, Elmlinger *et al.* 2001, Moore *et al.* 2003, Ranke *et al.* 2003). The elevated expression is reflected by elevated serum concentrations of IGFBP-2 in tumor patients, which is often correlated with tumor malignancy (Boulle *et al.* 2001, Elmlinger *et al.* 2001, Baron-Hay *et al.* 2004). In particular, IGFBP-2 has been shown to be increasingly overexpressed during breast cancer progression (Busund *et al.* 2005). It has been reported that the Iip45 protein may be a selective binding partner for IGFBP-2 (Zhang *et al.*, 2003).

[0005] Due to the limitations and challenges posed by currently available approaches to cancer diagnosis and therapy, there is a need in the art for the development of novel methods and compositions. The present invention fulfills this need, and further provides other related advantages.

SUMMARY OF THE INVENTION

[0006] The present invention solves a long-standing need in the art by providing methods and compositions of preparation and use of targeting conjugates or peptides that are to be targeted to specific cells or tissues. In certain further embodiments, the invention involves a targeting conjugate comprising a targeting peptide that at least an exon 6-encoded sequence (SEQ ID NO:3) of invasion inhibitory protein 45 (Iip45) or an isoform or homologue thereof. Iip45 is encoded by a gene comprised of nine exons (**FIG. 1**; Iip45 is deposited as accession No.

NM_021933 including 10 exons – exon 1 is a non-coding exon and coding regions of exons 2-10 correspond to exons 1-9 disclosed in the present specification and Zhang *et al.*, 2003).

[0007] For example, the targeting peptide may comprise at least an exon 5 through 9-encoded sequence of Iip45 (SEQ ID NO:4), at least an exon 6 through 9-encoded sequence of Iip45 (SEQ ID NO:5), at least an exon 1 through 6-encoded sequence of Iip45 (SEQ ID NO:6), or Iip45 (SEQ ID NO:2). The preferred targeting peptide comprise an exon 5 through 9-encoded sequence of Iip45 (SEQ ID NO:4) because it is the minimum requirement for full binding activity to IGFBP-2 compared with full length Iip45. In a further aspect, the targeting peptide may be encoded by the group consisting of exons 5-9, exons 1-6, exons 6-9, exon 6 or exons 1-9 of Iip45 gene (SEQ ID NO:7 and 8; SEQ ID NO: 9 and 10; SEQ ID NO: 11 and 12; SEQ ID NO:13; SEQ ID NO:14 and 15).

[0008] In still further aspects of the invention, the conjugate may be further defined as a fusion protein or may be chemically conjugated. For example, the fusion protein may include a linker such as G4S, (G4S)₂, the 218 linker, (G4S)₃, enzymatically cleavable linker, pH cleavable linker or any similar linker well known to a person having ordinary skill in this art.

[0009] In various embodiments, the agent may be, but not limited to, a cytotoxic agent, a cytokine, an anti-angiogenic agent, a chemotherapeutic agent, a diagnostic agent, an imaging agent, a radioisotope, a pro-apoptosis agent, an enzyme, a hormone, a growth factor, a peptide, a protein, an antibiotic, an antibody, a Fab fragment of an antibody, an imaging agent, an antigen, a survival factor, an anti-apoptotic agent, a hormone antagonist, a virus, a bacteriophage, a bacterium, a liposome, a microparticle, a magnetic bead, a microdevice, a cell, a nucleic acid or an expression vector.

[0010] In certain embodiments, the agent is a cytotoxic agent. The cytotoxic agent may comprise a peptide, a polypeptide, or a small molecule, or may be selected from the group consisting of gelonin, ricin, abrin, diphtheria toxin, *Pseudomonas* exotoxin, *Clostridium perfringens* enterotoxin, dodecandrin, tricosanthin, tricokirin, bryodin, mirabilis antiviral protein, barley ribosome-inactivating protein (BRIP),

pokeweed antiviral protein (PAPs), saporin, luffin, and momordin. For example, the cytotoxic agent comprises gelonin.

[0011] In other embodiments, the agent is an anti-angiogenic agent selected from the group consisting of thrombospondin, angiostatin, endostatin or pigment epithelium-derived factor, angiotensin, laminin peptides, fibronectin peptides, plasminogen activator inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin 12, platelet factor 4, IP-10, Gro- β , 2-methoxyoestradiol, proliferin-related protein, carboxamidotriazole, CM101, Marimastat, pentosan polysulphate, angiopoietin 2 (Regeneron), interferon- α , herbimycin A, PNU145156E, 16K prolactin fragment, Linomide, thalidomide, pentoxifylline, genistein, TNP-470, paclitaxel, accutin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 and minocycline.

[0012] In another embodiment, the agent is a cytokine selected from the group consisting of interleukin 1 (IL-1), IL-2, IL-5, IL-10, IL-11, IL-12, IL-18, interferon- γ (IF- γ), IF- α , IF- β , tumor necrosis factor- α (TNF- α), or GM-CSF (granulocyte macrophage colony stimulating factor).

[0013] In a further embodiment, the agent may be a pro-apoptosis agent or a signal transduction protein for apoptosis induction. Representative but not limiting examples of such an pro-apoptosis agent include granzyme B, Bax, TNF- α , TNF- β , TNF-like molecule, TGF- β , IL-12, IL-3, IL-24, IL-18, TRAIL, IFN- α , IFN- β , IFN- γ , Bcl-2, Fas ligand and caspases. Specifically, the agent may be granzyme B. More specifically, the targeting conjugate may comprise a fusion protein comprising a targeting peptide that binds to insulin-like growth factor binding protein 2 (IGFBP-2) and containing granzyme B.

[0014] In some further aspects, the conjugate is further defined as being comprised in a pharmaceutically acceptable carrier.

[0015] Additional aspects of the invention concerns methods of targeting delivery to a cell or tissue, in particular aspects a tumor cell or tissue, comprising the step of contacting the cell or tissue with the conjugate. Therefore, the cell or tissue to be targeted may be a tumor cell or tissue. In certain aspects, the conjugate may be

internalized into the tumor cell to exert its effects intracellularly. The tumor cell or tissue may be selected from the group consisting of a melanoma cell or tissue, a lymphoma cell or tissue, a cervical tumor cell or tissue, a breast tumor cell or tissue, a glioblastoma cell or tissue, a colorectal tumor cell or tissue, a prostate tumor cell or tissue, and a bladder tumor cell or tissue. In particular, the tumor cell or tissue may be a melanoma cell or tissue or a breast tumor cell or tissue.

[0016] In some embodiments, the cell or tissue for targeted delivery may be in a subject, such as a human subject. Such a subject may have a tumor selected from the group consisting of melanoma, lymphoma, cervical tumor, breast tumor, leukemia, glioblastoma, colorectal tumor, prostate tumor, breast tumor and bladder tumor. In certain aspects, the conjugate may exert an anti-tumor activity, which may be selected from the group consisting of killing a tumor cell or tissue, inducing apoptosis of a tumor cell or tissue, inhibiting tumor growth, inhibiting metastatic spread, reducing tumor burden and inducing tumor regression.

[0017] The methods may further comprise the step of treating said subject with chemotherapy, radiotherapy, surgery, hormone therapy or gene therapy.

[0018] In still further embodiments of the invention a nucleic acid encoding the conjugate described above is also provided. The nucleic acid could be an isolated nucleic acid.

[0019] Embodiments discussed in the context of methods and/or compositions of the invention may be employed with respect to any other method or composition described herein. Thus, an embodiment pertaining to one method or composition may be applied to other methods and compositions of the invention as well.

[0020] As used herein the terms “encode” or “encoding” with reference to a nucleic acid are used to make the invention readily understandable by the skilled artisan however these terms may be used interchangeably with “comprise” or “comprising” respectively.

[0021] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.

[0022] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0023] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0024] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0026] **FIG. 1:** Iip45 transcript and protein feature. Iip45 transcript includes 9 exons in the coding region, which encode 9 protein domains of this 388 aa protein: amino acid 1-38, 39-154, 155-182, 183-218, 219-238, 239-282, 283-314, 315-350, 351-388.

[0027] **FIG. 2:** Cloning Scheme of Gel/Iip45 (GIP) and Gel/Iip45X5 (GX5).

[0028] **FIG. 3:** Cobalt-IMAC elution (mM imidazole) profile of pET32-Gelonin/IIP45X5 in ArcticExpress(DE3).

[0029] **FIG. 4:** Western Analysis of fusion protein Gelonin/IIP45X5 with anti-IIP45 (45A) and anti-Gelonin antibody.

[0030] **FIG. 5:** Enzymatic assay (RRLA) of Gelonin/IIP45X5. This cell-free assay demonstrates that the protein-synthesis inhibitory activity of the fusion construct is preserved intact and similar to that of unmodified rGelonin toxin.

[0031] **FIG. 6:** Dose Response Curves for Gelonin/IIP45X5 (Batches 1-3) versus rGelonin on A375-M Human Melanoma Cells.

[0032] **FIG. 7:** Minimal contact time for optimal cytotoxic effect of Gelonin/IIP45X5 on A375-M Human Melanoma Cells.

[0033] **FIG. 8:** Cytotoxic effects of GX5 and rGel against 4 different human tumor cell lines.

[0034] **FIG. 9:** Cytotoxicity of Gelonin/IIP45X5 on 13 human tumor cell lines. The Targeting Index is the ratio of IC50 of rGel vs IC50 of the fusion construct.

[0035] **FIG. 10:** Tumor invasion of brain tumor cell lines transfected with IGFBP2 gene(C 17 and C18) or vector alone(V1 and V2). Doses of 1 nM of GX5 had no effect on cell invasion, but doses of 4 nM showed a dramatic impact on tumor invasion on transfected cells only(C17 and C18).

[0036] **FIG. 11:** Comparison of the effects of GX5 vs rGel toxin. Lower series of pictures shows the reduction of tumor cells migrating into matrigel only in the transfected cells treated with GX5.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. The Present Invention

[0037] A number of groups have examined molecular profiles of various tumors in an attempt to identify novel genes, gene profiles or unique markers which may be useful in understanding disease biology or therapeutic intervention. This is an essential first step in the development of targeted therapeutic approaches which employ specific differences between tumor and normal cells.

[0038] The instant invention meets the current needs for novel targeted delivery by providing methods and compositions concerning Iip45 containing targeting conjugates. These methods could target cells or tissues for therapy or imaging and useful for treating tumor cell or tissue. Further embodiments and advantages of the invention are described below.

II. IIP45

[0039] A gene, invasion inhibitory protein 45 (Iip45), whose protein product bound to IGFBP-2 through the thyroglobulin-RGD region of the C terminus of IGFBP-2, has been identified (Song, *et al.*, 2003). The Iip45 gene is located on chromosome 1p36 and has nine exons.

[0040] The Iip45 protein has three SEG (segment of low compositional complexity) domains and an integrin-binding RGD motif. The Iip45 protein is not expressed in some glioblastoma multiforme (GBM), the highest grade of glioma. Functional studies showed that Iip45 inhibited GBM cell invasion both in vitro and in xenograft model. Gene expression profiling studies showed that Iip45 consistently inhibited the expression of cell invasion-associated genes, such as the transcriptional NFκB, and its downstream target gene, intercellular adhesion molecule 1. In addition, Iip45 was shown to antagonize IGFBP-2 stimulation of glioma cell invasion by binding IGFBP-2 (Song *et al.*, 2003).

[0041] Recent results suggest that Iip45 is inactivated in gliomas through a tumor-specific alternative splicing mechanism, which is unrelated to the frequent deletion of 1p36 in tumors. Iip45S isoform results from exclusion of Iip45 exon 7 and encodes a variant protein that carries a COOH terminus different from that of Iip45 due to a frame-shift mutation. Therefore, the tumor-specific alternative splicing of Iip45 generates a mutant isoform encoding a highly unstable protein product that is recognized and degraded by the ubiquitin-proteasome machinery. This study supports the notion that alternative splicing is the predominant mechanism for inactivation of a group of tumor suppressor genes that include p73 and Iip45.

[0042] In the present invention, various truncated constructs of Iip45 with binding activity to IGFBP-2 are contemplated as comprised in a targeting conjugate for targeted delivery. The peptides comprising at least exon 6-encoded domain, such

as that encoded by exons 4-9, 3-9, 2-9, 5-9, 6-9, 1-6, 2-6, 3-6, 4-6, 5-6, 6 or full length may be used to improve specificity and efficacy of targeting conjugate used in therapy and diagnostics. "Iip45 peptides" refers generally to these Iip45 domains retaining binding activity to IGFBP-2

[0043] The cell structure that is targeted by the Iip45 targeting ligands of the present invention is not entirely clear. Earlier scientific publications taught that Iip45 can bind tightly to IGFBP-2 (Zhang et al., 2003), and, indeed, the present inventors have found that IGFBP-2 expressing tumors can be selectively targeted by Iip45 targeting constructs of the present invention. However, the inventors have more recently, and somewhat surprisingly, determined that Iip45 constructs can also selectively target tumors that do not express, or express only very low levels of, IGFBP-2 (essentially undetectable, *e.g.* by visual inspection, by Western analysis using an anti-IGFBP-2 antibody). Recently, it has been demonstrated that Iip45 ligand can also interact with histone deacetylase 6 (HDAC6). HDAC6 belongs to a subtype of the HDAC family, a class II histone deacetylase specifically for deacetylation of alpha tubulin, the most abundant microtubule component, rather than histone. HDAC6 also deacetylate cortactin, a protein that promotes polymerization and rearrangement of the actin cytoskeleton and is important in promoting lamellipodia formation, invadopodia formation, and cell migration. A number of studies have shown that HDAC6 regulates microtubule dynamics/cytoskeleton structure and increases cell migration by reduction of alpha tubulin and cortactin acetylation. Given these findings, it is possible that IIP45 could bind to HDAC family members or similar structural motifs present on the surface of some tumor cells.

III. Conjugates

[0044] Compositions and methods of the present invention involve IGFBP-2 targeting conjugates for targeted delivery are provided. The conjugates may be chemically conjugated, crosslinked, or fused at the protein level using conventional methods.

A. Linkers

[0045] Bifunctional cross-linking reagents have been extensively used for a variety of purposes including preparation of affinity matrices, modification and

stabilization of diverse structures, identification of ligand and receptor binding sites, and structural studies.

[0046] Homobifunctional reagents that carry two identical functional groups proved to be highly efficient in inducing cross-linking between identical and different macromolecules or subunits of a macromolecule, and linking of polypeptide ligands to their specific binding sites. Heterobifunctional reagents contain two different functional groups. By taking advantage of the differential reactivities of the two different functional groups, cross-linking can be controlled both selectively and sequentially. The bifunctional cross-linking reagents can be divided according to the specificity of their functional groups, *e.g.*, amino, sulfhydryl, guanidino, indole, carboxyl specific groups. Of these, reagents directed to free amino groups have become especially popular because of their commercial availability, ease of synthesis and the mild reaction conditions under which they can be applied.

[0047] A majority of heterobifunctional cross-linking reagents contains a primary amine-reactive group and a thiol-reactive group. In another example, heterobifunctional cross-linking reagents and methods of using the cross-linking reagents are described (U.S. Pat. No. 5,889,155, specifically incorporated herein by reference in its entirety). The cross-linking reagents combine a nucleophilic hydrazide residue with an electrophilic maleimide residue, allowing coupling in one example, of aldehydes to free thiols. The cross-linking reagent can be modified to cross-link various functional groups.

[0048] If desired, dimers or multimers of IIp45 peptides, or an IIp45 peptide and a non-self, non-IIp45 peptide or adjuvant may be joined via a biologically-releasable bond, such as a selectively-cleavable linker or amino acid sequence. For example, peptide linkers that include a cleavage site for an enzyme preferentially located or active within a tumor environment are contemplated. Exemplary forms of such peptide linkers are those that are cleaved by urokinase, plasmin, thrombin, Factor IXa, Factor Xa, or a metalloproteinase, such as collagenase, gelatinase, or stromelysin.

[0049] Amino acids such as selectively-cleavable linkers, synthetic linkers, or other amino acid sequences may be used to separate an IIP45 peptide from another peptide, adjuvant or a therapeutic compound.

[0050] Additionally, while numerous types of disulfide-bond containing linkers are known that can successfully be employed to conjugate the toxin moiety with the targeting agent, certain linkers will generally be preferred over other linkers, based on differing pharmacologic characteristics and capabilities. For example, linkers that contain a disulfide bond that is sterically "hindered" are to be preferred, due to their greater stability in vivo, thus preventing release of the toxin moiety prior to binding at the site of action. Furthermore, while certain advantages in accordance with the invention will be realized through the use of any of a number of toxin moieties, the inventors have found that the use of ricin A chain, and even more preferably deglycosylated A chain, will provide particular benefits. i) Biochemical cross-linkers It can be considered as a general guideline that any biochemical cross-linker that is appropriate for use in an immunotoxin will also be of use in the present context, and additional linkers may also be considered.

[0051] Exemplary methods for cross-linking ligands to liposomes are described in U.S. Pat. No. 5,603,872 and U.S. Pat. No. 5,401,511, each specifically incorporated herein by reference in its entirety). Various ligands can be covalently bound to liposomal surfaces through the cross-linking of amine residues. Liposomes, in particular, multilamellar vesicles (MLV) or unilamellar vesicles such as microemulsified liposomes (MEL) and large unilamellar liposomes (LUVET), each containing phosphatidylethanolamine (PE), have been prepared by established procedures. The inclusion of PE in the liposome provides an active functional residue, a primary amine, on the liposomal surface for cross-linking purposes. Ligands such as epidermal growth factor (EGF) have been successfully linked with PE-liposomes. Ligands are bound covalently to discrete sites on the liposome surfaces. The number and surface density of these sites are dictated by the liposome formulation and the liposome type. The liposomal surfaces may also have sites for non-covalent association. To form covalent conjugates of ligands and liposomes, cross-linking reagents have been studied for effectiveness and biocompatibility. Cross-linking reagents include glutaraldehyde (GAD), bifunctional oxirane (OXR), ethylene glycol

diglycidyl ether (EGDE), and a water soluble carbodiimide, preferably 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Through the complex chemistry of cross-linking, linkage of the amine residues of the recognizing substance and liposomes is established.

[0052] Once conjugated, the peptide generally will be purified to separate the conjugate from unconjugated targeting agents or coagulants and from other contaminants. A large a number of purification techniques are available for use in providing conjugates of a sufficient degree of purity to render them clinically useful.

[0053] Purification methods based upon size separation, such as gel filtration, gel permeation or high performance liquid chromatography, will generally be of most use. Other chromatographic techniques, such as Blue-Sepharose separation, may also be used.

[0054] In addition to chemical conjugation, an Ilp45 peptide may be modified at the protein level. Included within the scope of the invention are protein fragments or other derivatives or analogs that are differentially modified during or after translation, for example by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, and proteolytic cleavage. Any number of chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄, acetylation, formylation, farnesylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin.

B. Fusion Proteins

[0055] Other embodiments of the present invention concern fusion proteins. These molecules generally have all or a substantial portion of a targeting Ilp45 peptide, linked at the N- or C-terminus, to all or a portion of a second polypeptide or protein. For example, fusions may employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion includes the addition of an immunologically active domain, such as an antibody epitope, to facilitate purification of the fusion protein. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other useful fusions include linking of functional

domains, such as active sites from enzymes, glycosylation domains, cellular targeting signals or transmembrane regions. In preferred embodiments, the fusion proteins of the instant invention comprise a targeting peptide linked to a therapeutic protein or peptide.

[0056] Examples of proteins or peptides that may be incorporated into a fusion protein include cytostatic proteins, cytotoxic proteins, pro-apoptosis agents, anti-angiogenic agents, hormones, cytokines, growth factors, peptide drugs, antibodies, Fab fragments antibodies, antigens, receptor proteins, enzymes, lectins, MHC proteins, cell adhesion proteins and binding proteins. These examples are not meant to be limiting and it is contemplated that within the scope of the present invention virtually any protein or peptide could be incorporated into a fusion protein comprising a targeting peptide.

[0057] Methods of generating fusion proteins are well known to those of skill in the art. Such proteins can be produced, for example, by chemical attachment using bifunctional cross-linking reagents, by de novo synthesis of the complete fusion protein, or by attachment of a DNA sequence encoding the targeting peptide to a DNA sequence encoding the second peptide or protein, followed by expression of the intact fusion protein.

IV. Therapeutic or Diagnostic Agents

[0058] In certain embodiments, it may be desirable to couple specific bioactive agents to one or more targeting peptides (particularly Iip45 peptides) for targeted delivery to an organ, tissue or cell type. Such agents include, but are not limited to, cytotoxic molecules, cytokines, chemokines, pro-apoptosis factors and anti-angiogenic factors as well as imaging agents.

A. Cytokines and Chemokines

[0059] The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators.

[0060] Examples of such cytokines are lymphokines, monokines, growth factors and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth

hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; prostaglandin, fibroblast growth factor; prolactin; placental lactogen, OB protein; tumor necrosis factor-.alpha. and -.beta.; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-.beta.; platelet-growth factor; transforming growth factors (TGFs) such as TGF-.alpha. and TGF-.beta.; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-.alpha., -.beta., and -.gamma.; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1.alpha., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, LIF, G-CSF, GM-CSF, M-CSF, EPO, kit-ligand or FLT-3, angiostatin, thrombospondin, endostatin, tumor necrosis factor and LT. As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

[0061] Chemokines generally act as chemoattractants to recruit immune effector cells to the site of chemokine expression. It may be advantageous to express a particular chemokine gene in combination with, for example, a cytokine gene, to enhance the recruitment of other immune system components to the site of treatment. Chemokines include, but are not limited to, RANTES, MCAF, MIP1-alpha, MIP1-Beta, and IP-10. The skilled artisan will recognize that certain cytokines are also known to have chemoattractant effects and could also be classified under the term chemokines.

B. Cytotoxic Agents

[0062] Chemotherapeutic (cytotoxic) agents of potential use include, but are not limited to, 5-fluorouracil, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin (CDDP), cyclophosphamide, dactinomycin, daunorubicin, doxorubicin, estrogen receptor binding agents, etoposide (VP16), farnesyl-protein transferase inhibitors, gemcitabine, ifosfamide, mechlorethamine, melphalan,

mitomycin, navelbine, nitrosurea, plicomycin, procarbazine, raioxifene, tamoxifen, taxol, temazolomide (an aqueous form of DTIC), transplatinum, vinblastine and methotrexate, vincristine, or any analog or derivative variant of the foregoing. Most chemotherapeutic agents fall into the categories of alkylating agents, antimetabolites, antitumor antibiotics, corticosteroid hormones, mitotic inhibitors, and nitrosoureas, hormone agents, miscellaneous agents, and any analog or derivative variant thereof.

[0063] In addition, there are a variety of protein toxins (cytotoxic proteins), which include a number of different classes, such as those that inhibit protein synthesis: ribosome-inactivating proteins of plant origin, such as ricin, abrin, gelonin, and a number of others, and bacterial toxins such as pseudomonas exotoxin and diphtheria toxin.

[0064] Chemotherapeutic agents and methods of administration, dosages, *etc.* are well known to those of skill in the art (see for example, the "Physicians Desk Reference", Goodman & Gilman's "The Pharmacological Basis of Therapeutics" and in "Remington's Pharmaceutical Sciences" 15^{sup}.th ed., pp 1035-1038 and 1570-1580, incorporated herein by reference in relevant parts), and may be combined with the invention in light of the disclosures herein. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Examples of specific chemotherapeutic agents and dose regimes are also described herein. Of course, all of these dosages and agents described herein are exemplary rather than limiting, and other doses or agents may be used by a skilled artisan for a specific patient or application. Any dosage in-between these points, or range derivable therein is also expected to be of use in the invention.

C. Regulators of Programmed Cell Death

[0065] Apoptosis, or programmed cell death, is an essential process for normal embryonic development, maintaining homeostasis in adult tissues, and suppressing carcinogenesis (Kerr *et al.*, 1972). The Bcl-2 family of proteins and ICE-like proteases have been demonstrated to be important regulators and effectors of apoptosis in other systems. The Bcl-2 protein, discovered in association with follicular lymphoma, plays a prominent role in controlling apoptosis and enhancing cell survival in response to diverse apoptotic stimuli (Bakhshi *et al.*, 1985; Cleary and

Sklar, 1985; Cleary *et al.*, 1986; Tsujimoto *et al.*, 1985; Tsujimoto and Croce, 1986). The evolutionarily conserved Bcl-2 protein now is recognized to be a member of a family of related proteins, which can be categorized as death agonists or death antagonists.

[0066] Subsequent to its discovery, it was shown that Bcl-2 acts to suppress cell death triggered by a variety of stimuli. Also, it now is apparent that there is a family of Bcl-2 cell death regulatory proteins that share in common structural and sequence homologies. These different family members have been shown to either possess similar functions to Bcl-2 (*e.g.*, Bcl_{XL}, Bcl_w, Bcl_s, Mcl-1, A1, Bfl-1) or counteract Bcl-2 function and promote cell death (*e.g.*, Bax, Bak, Bik, Bim, Bid, Bad, Harakiri).

[0067] Non-limiting examples of pro-apoptosis agents contemplated within the scope of the present invention include granzyme B, Bax, TNF- α , TNF- β , TNF-like molecule, TGF- β , IL-12, IL-3, IL-24, IL-18, TRAIL, IFN- α , IFN- β , IFN- γ , Bcl-2, Fas ligand, caspases, gramicidin, magainin, mellitin, defensin, cecropin, (KLAKLAK)₂ (SEQ ID NO:16), (KLAKKLA)₂ (SEQ ID NO:17), (KAAKKAA)₂ (SEQ ID NO:18) or (KLGKKLG)₃ (SEQ ID NO:19). Specifically, the agent may be granzyme B. More specifically, the targeting conjugate may comprise a fusion protein comprising a targeting peptide that binds to insulin-like growth factor binding protein 2 (IGFBP-2) and granzyme B. It is understood in the art that granzyme can be conjugated to a targeting peptide as fusion proteins as in certain embodiments of the present invention (U.S. Pat. Nos. 7,101,977 and 7,371,723, incorporated herein by reference).

D. Angiogenic Inhibitors

[0068] In certain embodiments the present invention may concern administration of targeting peptides attached to anti-angiogenic agents, such as angiotensin, laminin peptides, fibronectin peptides, plasminogen activator inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin 12, platelet factor 4, IP-10, Gro- β , thrombospondin, 2-methoxyestradiol, proliferin-related protein, carboxamidotriazole, CM101, Marimastat, pentosan polysulphate, angiopoietin 2 (Regeneron), interferon- α , herbimycin A, PNU145156E, 16K prolactin fragment,

Linomide, thalidomide, pentoxifylline, genistein, TNP470, endostatin, paclitaxel, accutin, angiostatin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 or minocycline.

[0069] Proliferation of tumors cells relies heavily on extensive tumor vascularization, which accompanies cancer progression. Thus, inhibition of new blood vessel formation with anti-angiogenic agents and targeted destruction of existing blood vessels have been introduced as an effective and relatively non-toxic approach to tumor treatment. (Arap *et al.*, 1998; Arap *et al.*, 1998; Ellerby *et al.*, 1999). A variety of anti-angiogenic agents and/or blood vessel inhibitors are known. (*e.g.*, Folkman, 1997; Eliceiri and Cheresch, 2001).

E. Imaging Agents and Radioisotopes

[0070] In certain embodiments, the claimed peptides or proteins of the present invention may be attached to imaging agents of use for imaging and diagnosis of various diseased organs, tissues or cell types. Many appropriate imaging agents are known in the art, as are methods for their attachment to proteins or peptides (see, *e.g.*, U.S. Pat. Nos. 5,021,236 and 4,472,509, both incorporated herein by reference). Certain attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a DTPA attached to the protein or peptide (U.S. Pat. No. 4,472,509). Proteins or peptides also may be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate.

[0071] Non-limiting examples of paramagnetic ions of potential use as imaging agents include chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and erbium (III), with gadolinium being particularly preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

[0072] Radioisotopes of potential use as imaging or therapeutic agents include astatine²¹¹, ¹⁴carbon, ⁵¹chromium, ³⁶chlorine, ⁵⁷cobalt, ⁵⁸cobalt, copper⁶⁷, ¹⁵²Eu,

gallium⁶⁷, ³hydrogen, iodine¹²³, iodine¹²⁵, iodine¹³¹, indium¹¹¹, ⁵⁹iron, ³²phosphorus, rhenium¹⁸⁶, rhenium¹⁸⁸, ⁷⁵selenium, ³⁵sulphur, technetium^{99m} and yttrium⁹⁰. ¹²⁵I is often being preferred for use in certain embodiments, and technetium^{99m} and indium¹¹¹ are also often preferred due to their low energy and suitability for long range detection.

[0073] Radioactively labeled proteins or peptides of the present invention may be produced according to well-known methods in the art. For instance, they can be iodinated by contact with sodium or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Proteins or peptides according to the invention may be labeled with technetium-^{99m} by ligand exchange process, for example, by reducing pertechnetate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the peptide to this column or by direct labeling techniques, *e.g.*, by incubating pertechnetate, a reducing agent such as SnCl_2 , a buffer solution such as sodium-potassium phthalate solution, and the peptide. Intermediary functional groups that are often used to bind radioisotopes that exist as metallic ions to peptides are diethylenetriaminepenta-acetic acid (DTPA) and ethylene diaminetetra-acetic acid (EDTA). Also contemplated for use are fluorescent labels, including rhodamine, fluorescein isothiocyanate and renographin.

[0074] In certain embodiments, the claimed proteins or peptides may be linked to a secondary binding ligand or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase and glucose oxidase. Preferred secondary binding ligands are biotin and avidin or streptavidin compounds. The use of such labels is well known to those of skill in the art in light and is described, for example, in U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241; each incorporated herein by reference.

F. Alkylating Agents

[0075] Alkylating agents are drugs that directly interact with genomic DNA to prevent cells from proliferating. This category of chemotherapeutic drugs represents agents that affect all phases of the cell cycle, that is, they are not phase-specific. An

alkylating agent, may include, but is not limited to, a nitrogen mustard, an ethylenimine, a methylmelamine, an alkyl sulfonate, a nitrosourea or a triazines. They include but are not limited to: busulfan, chlorambucil, cisplatin, cyclophosphamide (cytoxan), dacarbazine, ifosfamide, mechlorethamine (mustargen), and melphalan.

G. Antimetabolites

[0076] Antimetabolites disrupt DNA and RNA synthesis. Unlike alkylating agents, they specifically influence the cell cycle during S phase. Antimetabolites can be differentiated into various categories, such as folic acid analogs, pyrimidine analogs and purine analogs and related inhibitory compounds. Antimetabolites include but are not limited to, 5-fluorouracil (5-FU), cytarabine (Ara-C), fludarabine, gemcitabine, and methotrexate.

H. Natural Products

[0077] Natural products generally refer to compounds originally isolated from a natural source, and identified as having a pharmacological activity. Such compounds, analogs and derivatives thereof may be, isolated from a natural source, chemically synthesized or recombinantly produced by any technique known to those of skill in the art. Natural products include such categories as mitotic inhibitors, antitumor antibiotics, enzymes and biological response modifiers.

[0078] Mitotic inhibitors include plant alkaloids and other natural agents that can inhibit either protein synthesis required for cell division or mitosis. They operate during a specific phase during the cell cycle. Mitotic inhibitors include, for example, docetaxel, etoposide (VP16), teniposide, paclitaxel, taxol, vinblastine, vincristine, and vinorelbine.

[0079] Taxoids are a class of related compounds isolated from the bark of the ash tree, *Taxus brevifolia*. Taxoids include but are not limited to compounds such as docetaxel and paclitaxel. Paclitaxel binds to tubulin (at a site distinct from that used by the vinca alkaloids) and promotes the assembly of microtubules.

[0080] Vinca alkaloids are a type of plant alkaloid identified to have pharmaceutical activity. They include such compounds as vinblastine (VLB) and vincristine.

I. Antibiotics

[0081] Certain antibiotics have both antimicrobial and cytotoxic activity. These drugs also interfere with DNA by chemically inhibiting enzymes and mitosis or altering cellular membranes. These agents are not phase specific so they work in all phases of the cell cycle. Examples of cytotoxic antibiotics include, but are not limited to, bleomycin, dactinomycin, daunorubicin, doxorubicin (Adriamycin), plicamycin (mithramycin) and idarubicin.

J. Miscellaneous Agents

[0082] Miscellaneous cytotoxic agents that do not fall into the previous categories include, but are not limited to, platinum coordination complexes, anthracenediones, substituted ureas, methyl hydrazine derivatives, amsacrine, L-asparaginase, and tretinoin. Platinum coordination complexes include such compounds as carboplatin and cisplatin (cis-DDP). An exemplary anthracenedione is mitoxantrone. An exemplary substituted urea is hydroxyurea. An exemplary methyl hydrazine derivative is procarbazine (N-methylhydrazine, MIH). These examples are not limiting and it is contemplated that any known cytotoxic, cytostatic or cytocidal agent may be attached to targeting peptides and administered to a targeted organ, tissue or cell type within the scope of the invention.

K. Dosages

[0083] The skilled artisan is directed to "Remington's Pharmaceutical Sciences" 15th Edition, chapter 33, and in particular to pages 624-652. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and general safety and purity standards as required by the FDA Office of Biologics standards.

V. Proteins and Peptides

[0084] In certain embodiments, the present invention concerns novel compositions comprising at least one protein or peptide, such as an IIP45 peptide.

These peptides may be comprised in a fusion protein or conjugated to an agent as described *supra*.

A. Proteins and Peptides

[0085] As used herein, a protein or peptide generally refers, but is not limited to, a protein of greater than about 200 amino acids, up to a full length sequence translated from a gene; a polypeptide of greater than about 100 amino acids; and/or a peptide of from about 3 to about 100 amino acids. For convenience, the terms "protein," "polypeptide" and "peptide" are used interchangeably herein.

[0086] In certain embodiments the size of at least one protein or peptide may comprise, but is not limited to, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, about 110, about 120, about 130, about 140, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 525, about 550, about 575, about 600, about 625, about 650, about 675, about 700, about 725, about 750, about 775, about 800, about 825, about 850, about 875, about 900, about 925, about 950, about 975, about 1000, about 1100, about 1200, about 1300, about 1400, about 1500, about 1750, about 2000, about 2250, about 2500 or greater amino acid residues.

[0087] As used herein, an "amino acid residue" refers to any naturally occurring amino acid, any amino acid derivative or any amino acid mimic known in the art. In certain embodiments, the residues of the protein or peptide are sequential, without any non-amino acid interrupting the sequence of amino acid residues. In other embodiments, the sequence may comprise one or more non-amino acid moieties. In particular embodiments, the sequence of residues of the protein or peptide may be interrupted by one or more non-amino acid moieties.

[0088] Accordingly, the term "protein or peptide" encompasses amino acid sequences comprising at least one of the 20 common amino acids found in naturally

occurring proteins, or at least one modified or unusual amino acid, including but not limited to those shown on Table 1 below.

TABLE 1			
Modified and Unusual Amino Acids			
Abbr.	Amino Acid	Abbr.	Amino Acid
Aad	2-Aminoadipic acid	EtAsn	N-Ethylasparagine
Baad	3- Aminoadipic acid	Hyl	Hydroxylysine
Bala	β -alanine, β -Amino-propionic acid	AHyl	allo-Hydroxylysine
Abu	2-Aminobutyric acid	3Hyp	3-Hydroxyproline
4Abu	4- Aminobutyric acid, piperidinic acid	4Hyp	4-Hydroxyproline
Acp	6-Aminocaproic acid	Idc	Isodesmosine
Ahe	2-Aminoheptanoic acid	Alle	allo-Isoleucine
Aib	2-Aminoisobutyric acid	MeGly	N-Methylglycine, sarcosine
Baib	3-Aminoisobutyric acid	Melle	N-Methylisoleucine
Apm	2-Aminopimelic acid	MeLys	6-N-Methyllysine
Dbu	2,4-Diaminobutyric acid	MeVal	N-Methylvaline
Des	Desmosine	Nva	Norvaline
Dpm	2,2'-Diaminopimelic acid	Nle	Norleucine
Dpr	2,3-Diaminopropionic acid	Orn	Ornithine
EtGly	N-Ethylglycine		

[0089] Proteins or peptides may be made by any technique known to those of skill in the art, including the expression of proteins, polypeptides or peptides through standard molecular biological techniques, the isolation of proteins or peptides from natural sources, or the chemical synthesis of proteins or peptides. The nucleotide and protein, polypeptide and peptide sequences corresponding to various genes have been previously disclosed, and may be found at computerized databases known to those of ordinary skill in the art. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (<http://www.ncbi.nlm.nih.gov/>). The coding regions for known genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

Alternatively, various commercial preparations of proteins, polypeptides and peptides are known to those of skill in the art.

B. Protein Purification

[0090] In certain embodiments a protein or peptide may be isolated or purified. Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the homogenization and crude fractionation of the cells, tissue or organ to polypeptide and non-polypeptide fractions. The protein or polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, gel exclusion chromatography, polyacrylamide gel electrophoresis, affinity chromatography, immunoaffinity chromatography and isoelectric focusing. An example of receptor protein purification by affinity chromatography is disclosed in U.S. Pat. No. 5,206,347, the entire text of which is incorporated herein by reference. A particularly efficient method of purifying peptides is fast performance liquid chromatography (FPLC) or even high performance liquid chromatography (HPLC).

[0091] A purified protein or peptide is intended to refer to a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally-obtainable state. An isolated or purified protein or peptide, therefore, also refers to a protein or peptide free from the environment in which it may naturally occur. Generally, "purified" will refer to a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more of the proteins in the composition.

[0092] Various methods for quantifying the degree of purification of the protein or peptide are known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE

analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity therein, assessed by a "-fold purification number." The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification, and whether or not the expressed protein or peptide exhibits a detectable activity.

[0093] Various techniques suitable for use in protein purification are well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies and the like, or by heat denaturation, followed by: centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of these and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

[0094] There is no general requirement that the protein or peptide always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater "-fold" purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

[0095] Affinity chromatography is a chromatographic procedure that relies on the specific affinity between a substance to be isolated and a molecule to which it can specifically bind. This is a receptor-ligand type of interaction. The column material is synthesized by covalently coupling one of the binding partners to an insoluble matrix. The column material is then able to specifically adsorb the substance from the solution. Elution occurs by changing the conditions to those in which binding will not

occur (*e.g.*, altered pH, ionic strength, temperature, *etc.*). The matrix should be a substance that itself does not adsorb molecules to any significant extent and that has a broad range of chemical, physical and thermal stability. The ligand should be coupled in such a way as to not affect its binding properties. The ligand should also provide relatively tight binding. And it should be possible to elute the substance without destroying the sample or the ligand.

VI. Nucleic Acids

[0096] Nucleic acids according to the present invention may encode a targeting peptide, a fusion protein or other protein or peptide. The nucleic acid may be derived from genomic DNA, complementary DNA (cDNA) or synthetic DNA. Where incorporation into an expression vector is desired, the nucleic acid may also comprise a natural intron or an intron derived from another gene. Such engineered molecules are sometime referred to as "mini-genes."

[0097] A "nucleic acid" as used herein includes single-stranded and double-stranded molecules, as well as DNA, RNA, chemically modified nucleic acids and nucleic acid analogs. It is contemplated that a nucleic acid within the scope of the present invention may be of almost any size, determined in part by the length of the encoded protein or peptide.

[0098] It is contemplated that targeting peptides, fusion proteins and receptors may be encoded by any nucleic acid sequence that encodes the appropriate amino acid sequence. The design and production of nucleic acids encoding a desired amino acid sequence is well known to those of skill in the art, using standardized codon tables. In preferred embodiments, the codons selected for encoding each amino acid may be modified to optimize expression of the nucleic acid in the host cell of interest. Codon preferences for various species of host cell are well known in the art.

[0099] In addition to nucleic acids encoding the desired peptide or protein, the present invention encompasses complementary nucleic acids that hybridize under high stringency conditions with such coding nucleic acid sequences. High stringency conditions for nucleic acid hybridization are well known in the art. For example, conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50 degree to

about 70 degree. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleotide content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence or concentration of formamide, tetramethylammonium chloride or other solvent(s) in a hybridization mixture.

VII. Combination Treatments

[00100] In order to increase the effectiveness of a targeted delivery of therapeutic agents to IGFBP-2 expressing cell or tissue in a subject, it may be desirable to combine these targeting compositions with other agents effective in the treatment of a IGFBP-2 related disease, such as anti-cancer agents.

[00101] An “anti-cancer” agent is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. More generally, these other compositions would be provided in a combined amount effective to kill or inhibit proliferation of the cell. This process may involve contacting the cells with the expression construct and the agent(s) or multiple factor(s) at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations, at the same time, wherein one composition includes the expression construct and the other includes the second agent(s).

[00102] In the context of the present invention, it is contemplated that IGFBP-2 targeted therapy could be used in conjunction with chemotherapeutic, radiotherapeutic, immunotherapeutic intervention, or other pro-apoptotic or cell cycle regulating agents.

[00103] Alternatively, the targeted therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and expression construct are applied separately to the cell, one

would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and expression construct would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one may contact the cell with both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several d (2, 3, 4, 5, 6 or 7) to several wk (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[00104] Various combinations may be employed, IGFBP-2 targeted therapy is “A” and the secondary agent, such as radio- or chemotherapy, is “B”:

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A

B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[00105] Administration of the therapeutic targeting conjugates of the present invention to a patient will follow general protocols for the administration of chemotherapeutics, taking into account the toxicity, if any, of the targeting conjugates. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the described targeted cell therapy.

A. Chemotherapy

[00106] Cancer therapies also include a variety of combination therapies with both chemical and radiation based treatments. Combination chemotherapies include, for example, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase

inhibitors, transplatin, 5-fluorouracil, vincristin, vinblastin and methotrexate, or any analog or derivative variant of the foregoing.

B. Radiotherapy

[00107] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors effect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

[00108] The terms “contacted” and “exposed,” when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with the target cell. To achieve cell killing or stasis, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

C. Immunotherapy

[00109] Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually effect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, *etc.*) and serve merely as a targeting agent to serve as a second targeting conjugate. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

[00110] In certain aspects, the IGFBP-2 targeting conjugate may comprise an antibody or fragment thereof for immunotherapy. Alternatively, Immunotherapy could be used as part of a combined therapy, in conjunction with IGFBP-2 targeted therapy. The general approach for combined therapy is discussed below. Generally, the tumor cell must bear some additional marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present invention. Common tumor markers include carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, *erb B* and p155.

D. Gene Therapy

[00111] In yet another embodiment, the secondary treatment is a gene therapy in which a therapeutic polynucleotide is administered before, after, or at the same time the targeting conjugate is delivered. Delivery of a targeting conjugate in conjunction with a vector encoding one of the following gene products will have a combined anti-hyperproliferative effect on target tissues. A variety of proteins are encompassed within the invention, some of which are described below.

[00112] The tumor suppressor oncogenes function to inhibit excessive cellular proliferation. The inactivation of these genes destroys their inhibitory activity, resulting in unregulated proliferation. For example, the tumor suppressors p53, p16 and C-CAM may be used.

[00113] Another inhibitor of cellular proliferation is p16. The major transitions of the eukaryotic cell cycle are triggered by cyclin-dependent kinases, or CDK's. p16^{INK4} belongs to a newly described class of CDK-inhibitory proteins that also includes p16^B, p19, p21^{WAF1}, and p27^{KIP1}. Restoration of wild-type p16^{INK4} function by transfection with a plasmid expression vector reduced colony formation by some human cancer cell lines (Okamoto, 1994; Arap, 1995).

[00114] Other genes that may be employed according to the present invention include Rb, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, *zac1*, p73, VHL, MMAC1 / PTEN, DBCCR-1, FCC, *rsk-3*, p27, p27/p16 fusions, p21/p27

fusions, anti-thrombotic genes (*e.g.*, COX-1, TFPI), PGS, Dp, E2F, *ras*, *myc*, *neu*, *raf*, *erb*, *fms*, *trk*, *ret*, *gsp*, *hst*, *abl*, E1A, p300, genes involved in angiogenesis (*e.g.*, VEGF, FGF, thrombospondin, BAI-1, GDAIF, or their receptors) and MCC.

[00115] Regulators of programmed cell death may also be used in the present invention for a combined therapy. Apoptosis, or programmed cell death, is an essential process for normal embryonic development, maintaining homeostasis in adult tissues, and suppressing carcinogenesis (Kerr *et al.*, 1972). The Bcl-2 family of proteins and ICE-like proteases have been demonstrated to be important regulators and effectors of apoptosis in other systems. Subsequent to its discovery, it was shown that Bcl-2 acts to suppress cell death triggered by a variety of stimuli. Also, it now is apparent that there is a family of Bcl-2 cell death regulatory proteins which share in common structural and sequence homologies. These different family members have been shown to either possess similar functions to Bcl-2 (*e.g.*, Bcl_{XL}, Bcl_W, Bcl_S, Mcl-1, A1, Bfl-1) or counteract Bcl-2 function and promote cell death (*e.g.*, Bax, Bak, Bik, Bim, Bid, Bad, Harakiri).

E. Surgery

[00116] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative and palliative surgery. Curative surgery is a cancer treatment that may be used in conjunction with other therapies, such as the treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy and/or alternative therapies.

[00117] Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically controlled surgery (Mohs' surgery). It is further contemplated that the present invention may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

[00118] Upon excision of part of all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion,

direct injection or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

F. Other agents

[00119] It is contemplated that other agents may be used in combination with the present invention to improve the therapeutic efficacy of treatment. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers. Immunomodulatory agents include tumor necrosis factor; interferon alpha, beta, and gamma; IL-2 and other cytokines; F42K and other cytokine analogs; or MIP-1, MIP-1beta, MCP-1, RANTES, and other chemokines. It is further contemplated that the upregulation of cell surface receptors or their ligands such as Fas / Fas ligand, DR4 or DR5 / TRAIL would potentiate the apoptotic inducing abilities of the present invention by establishment of an autocrine or paracrine effect on hyperproliferative cells. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with the present invention to improve the treatment efficacy.

[00120] Hormonal therapy may also be used in conjunction with the present invention or in combination with any other cancer therapy previously described. The use of hormones may be employed in the treatment of certain cancers such as breast, prostate, ovarian, or cervical cancer to lower the level or block the effects of certain hormones such as testosterone or estrogen. This treatment is often

used in combination with at least one other cancer therapy as a treatment option or to reduce the risk of metastases.

VIII. Pharmaceutical Compositions

[00121] Where clinical applications are contemplated, it may be necessary to prepare pharmaceutical compositions--expression vectors, virus stocks, proteins, antibodies and drugs--in a form appropriate for the intended application. Generally, this will entail preparing IGFBP-2 targeting conjugate compositions that are essentially free of impurities that could be harmful to humans or animals.

[00122] One generally will desire to employ appropriate salts and buffers to render delivery vectors stable and allow for uptake by target cells. Buffers also are employed when recombinant cells are introduced into a patient. Aqueous compositions of the present invention may comprise an effective amount of a protein, peptide, fusion protein, recombinant phage and/or expression vector, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase "pharmaceutically or pharmacologically acceptable" refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the proteins or peptides of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[00123] The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention are via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intraarterial or intravenous injection. Such compositions normally would be administered as pharmaceutically acceptable compositions, described supra.

[00124] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it is preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00125] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

IX. Examples

[00126] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques

discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Construction and Expression of rGelonin/IIP45 (GIP) and rGelonin/IIP45X5 (GX5)

[00127] PCR construction of rGelonin/IIP45 (GIP) and rGelonin/IIP45X5 (GX5) has been illustrated in **FIG. 2**. These fusion constructs was constructed in the expression vector pET32a from Novagen. The fusion rGelonin/IIP45X5 molecule(also called GX5) was then expressed in ArcticExpress™ (DE3) bacteria cells and purified by cobalt-IMAC (Immobilized Metal Affinity Chromatography) with increasing imidazole concentration (15 -150 mM) (**FIG. 3**). Followed by thrombin cleavage of hexahistidine tags on the fusion protein and a round of sepharose purification, high mounts of purified fusion protein rGelonin/IIP45X5 was confirmed by western blotting using anti-IIP45 antibodies and anti-rGelonin antibodies (**FIG. 4**).

Example 2

RRLA of Gelonin/IIP45X5

[00128] Cell-free protein synthesis inhibitory activity of the rGelonin/IIP45X5 (GX5) was compared with rGelonin by testing the rGelonin enzymatic activity in RRLA (Rabbit Reticulocyte Lysate Assays) (**FIG. 5**). This cell-free assay demonstrates that the protein-synthesis inhibitory activity of the fusion construct is preserved intact and similar to that of unmodified rGelonin toxin.

Example 3

Cytotoxicity of Gelonin/IIP45X5

[00129] Cytotoxicity of different batches of Gelonin/IIP45X5 (GX5) and rGelonin against log-phase human melanoma cells (A375-M) in culture were compared in the form of dose response curves (**FIG. 6**). Minimal contact time (the

shortest time necessary for cytotoxicity) for optimal cytotoxic effect of Gelonin/IIP45X5 and rGelonin on A375-M cells was measured (**FIG. 7**); this test suggests how rapidly the agent internalizes into cells. The minimal contact time appears to be between 6 and 24 hrs. Cytotoxic effects of GX5 and rGel against 4 different human tumor cell lines were also tested (**FIG. 8**). These results demonstrates that Gelonin/IIP45X5 fusion protein kill certain tumor cells at least 100 fold more active than rGelonin itself.

[00130] Cytotoxicity of Gelonin/IIP45X5 and rGelonin on 13 human tumor cell lines (**FIG. 9**) was also been tested: the Targeting Index is the ratio of IC50 of rGelonin vs IC50 of the fusion construct. Gelonin/IIP45X5 appears to be superior to Gelonin in a majority of cell lines tested. For melanoma, the fusion construct was 87 times more active than rGelonin.

Example 4

Effects of Gelonin/IIP45X5 on Tumor Cells

[00131] Tumor invasion of brain tumor cell lines transfected with IGFBP2 gene (C 17 and C18) or vector alone (V1 and V2) was tested under different doses of Gelonin/IIP45X5 compared with Gelonin. Doses of 1 nM of Gelonin/IIP45X5 (GX5) had no effect on cell invasion, but doses of 4 nM showed a dramatic impact on tumor invasion on transfected cells only (C17 and C18) (**FIG. 10**). The effects of Gelonin/IIP45X5 (GX5) and rGelonin toxin on numbers of breast tumor cells were also compared. Lower series of pictures shows the reduction of tumor cells into matrigel only in the transfected cells treated with GX5 (**FIG. 11**).

* * *

[00132] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically

and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A targeting conjugate comprising a targeting peptide that comprises at least an exon 6-encoded sequence (SEQ ID NO:3) of invasion inhibitory protein 45 (Iip45) or an isoform or homologue thereof attached to an agent to be targeted to a IGFBP-2-expressing cell, wherein said peptide.
2. The conjugate of claim 1, wherein said conjugate is further defined as a fusion protein.
3. The conjugate of claim 1, wherein said agent and targeting peptide is chemically conjugated.
4. The conjugate of claim 1, wherein the targeting peptide comprises at least an exon 5 through 9-encoded sequence of Iip45 (SEQ ID NO:4).
5. The conjugate of claim 1, wherein the targeting peptide comprises at least an exon 6 through 9-encoded sequence of Iip45 (SEQ ID NO:5).
6. The conjugate of claim 1, wherein the targeting peptide comprises at least an exon 1 through 6-encoded sequence of Iip45 (SEQ ID NO:6).
7. The conjugate of claim 1, wherein the targeting peptide comprises Iip45 (SEQ ID NO:2).
8. The conjugate of claim 1, wherein said targeting peptide is encoded by the group consisting of exons 5-9, exons 1-6, exons 6-9, exon 6 or exons 1-9 of Iip45 gene (SEQ ID NO:7 and 8; SEQ ID NO: 9 and 10; SEQ ID NO: 11 and 12; SEQ ID NO:13; SEQ ID NO:14 and 15).
9. The conjugate of claim 1, wherein said agent is a cytotoxic agent, a cytokine, an anti-angiogenic agent, a chemotherapeutic agent, a diagnostic agent, an imaging agent, a radioisotope, a pro-apoptosis agent, an enzyme, a hormone, a growth factor, a peptide, a protein, an antibiotic, an antibody, a Fab fragment of an antibody, an imaging agent, an antigen, a survival factor, an anti-apoptotic agent, a hormone antagonist, a virus, a bacteriophage, a bacterium, a liposome, a microparticle, a magnetic bead, a microdevice, a cell, a nucleic acid or an expression vector.

10. The conjugate of claim 1, wherein said agent is a cytotoxic agent.
11. The conjugate of claim 10, wherein said cytotoxic agent comprises a peptide, a polypeptide, or a small molecule.
12. The conjugate of claim 10, wherein said cytotoxic agent is selected from the group consisting of gelonin, ricin, abrin, diphtheria toxin, *Pseudomonas* exotoxin, *Clostridium perfringens* enterotoxin, dodecandrin, tricosanthin, tricokirin, bryodin, mirabilis antiviral protein, barley ribosome-inactivating protein (BRIP), pokeweed antiviral protein (PAPs), saporin, luffin, and momordin.
13. The conjugate of claim 10, wherein said cytotoxic agent comprises gelonin.
14. The conjugate of claim 9, wherein said agent is an anti-angiogenic agent selected from the group consisting of thrombospondin, angiostatin, endostatin or pigment epithelium-derived factor, angiotensin, laminin peptides, fibronectin peptides, plasminogen activator inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin 12, platelet factor 4, IP-10, Gro-.beta., 2-methoxyoestradiol, proliferin-related protein, carboxiamidotriazole, CM101, Marimastat, pentosan polysulphate, angiopoietin 2 (Regeneron), interferon-alpha, herbimycin A, PNU145156E, 16K prolactin fragment, Linomide, thalidomide, pentoxifylline, genistein, TNP-470, paclitaxel, accutin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 or minocycline.
15. The conjugate of claim 9, wherein said agent is a cytokine selected from the group consisting of interleukin 1 (IL-1), IL-2, IL-5, IL-10, IL-11, IL-12, IL-18, interferon- γ (IF- γ), IF- α , IF- β , tumor necrosis factor- α (TNF- α), or GM-CSF (granulocyte macrophage colony stimulating factor).
16. The conjugate of claim 9, wherein said agent is a pro-apoptosis agent selected from the group consisting of granzyme B, Bax, TNF-a, TNF-b, TNF-like molecule, TGF-b, small interfering RNA, IL-12, IL-3, IL-24, IL-18, TRAIL, IFN-a, IFN-b, IFN-g, Bcl-2, Fas ligand and caspases.
17. The conjugate of claim 9, wherein said agent is granzyme B.

18. The conjugate of claim 1, further defined as being comprised in a pharmaceutically acceptable carrier.
19. A method of targeting delivery to a cell or tissue, comprising the step of contacting said cell or tissue with the conjugate of claim 1.
20. The method of claim 19, wherein said cell or tissue is a tumor cell or tissue.
21. The method of claim 19, wherein said conjugate is internalized into said tumor cell.
22. The method of claim 20, wherein said tumor cell or tissue is selected from the group consisting of a melanoma cell or tissue, a lymphoma cell or tissue, a cervical tumor cell or tissue, a breast tumor cell or tissue, a glioblastoma cell or tissue, a colorectal tumor cell or tissue, a prostate tumor cell or tissue, and a bladder tumor cell or tissue.
23. The method of claim 22, wherein said tumor cell or tissue is a melanoma cell or tissue.
24. The method of claim 22, wherein said tumor cell or tissue is a breast tumor cell or tissue.
25. The method of claim 19, wherein said tumor cell or tissue has a loss of PTEN expression or activity.
26. The method of claim 19, wherein said cell or tissue is in a subject.
27. The method of claim 26, wherein said subjects is a human subject.
28. The method of claim 26, wherein said subject has a tumor selected from the group consisting of melanoma, lymphoma, cervical tumor, breast tumor, leukemia, glioblastoma, colorectal tumor, prostate tumor, breast tumor and bladder tumor.
29. The method of claim 19, wherein said conjugate exerts an anti-tumor activity.
30. The method of claim 29, wherein said anti-tumor activity is selected from the group consisting of killing a tumor cell or tissue, inducing apoptosis of a tumor cell or

tissue, inhibiting tumor growth, inhibiting metastatic spread, reducing tumor burden and inducing tumor regression .

31. The method of claim 26, further comprising the step of treating said subject with chemotherapy, radiotherapy, surgery, hormone therapy or gene therapy.

32. A nucleic acid encoding the conjugate of any one of claims 2 or 4 through 15.

Ilp45 transcript and protein feature

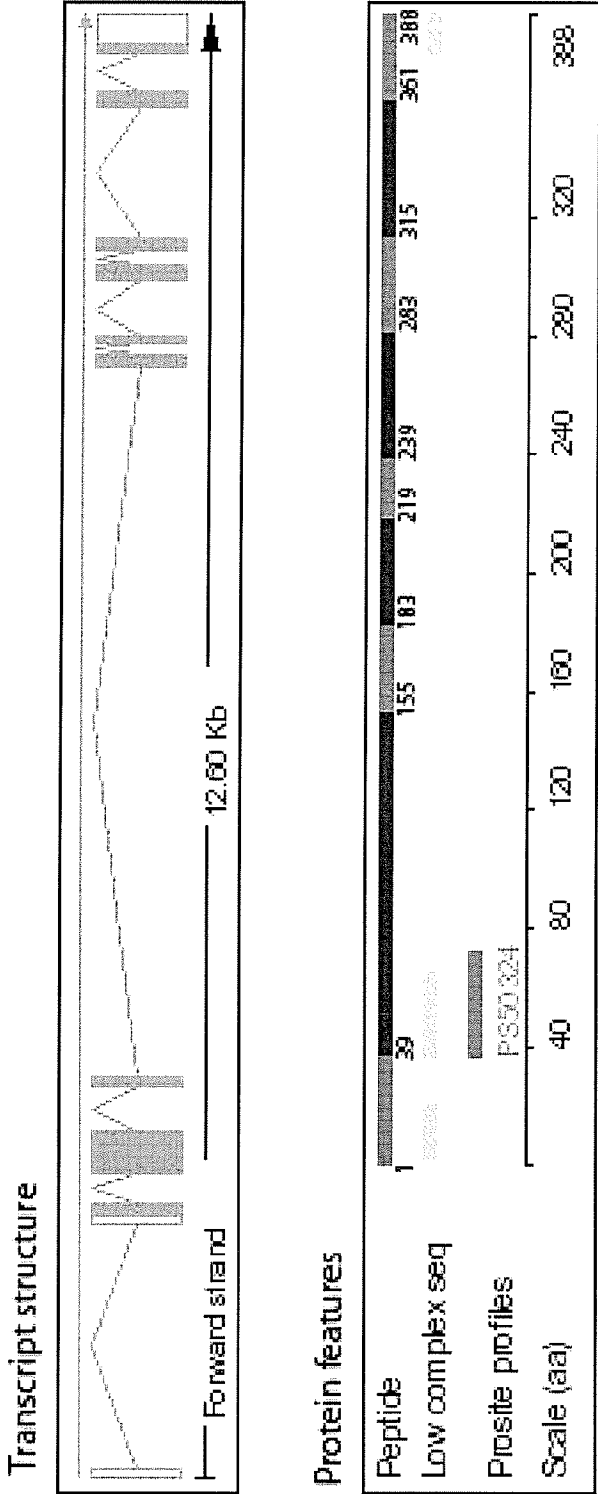
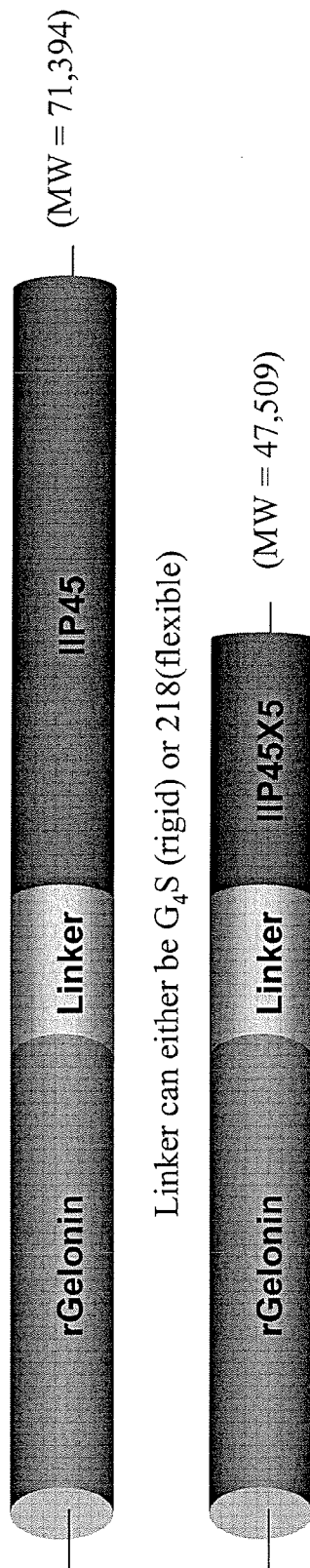


FIG. 1



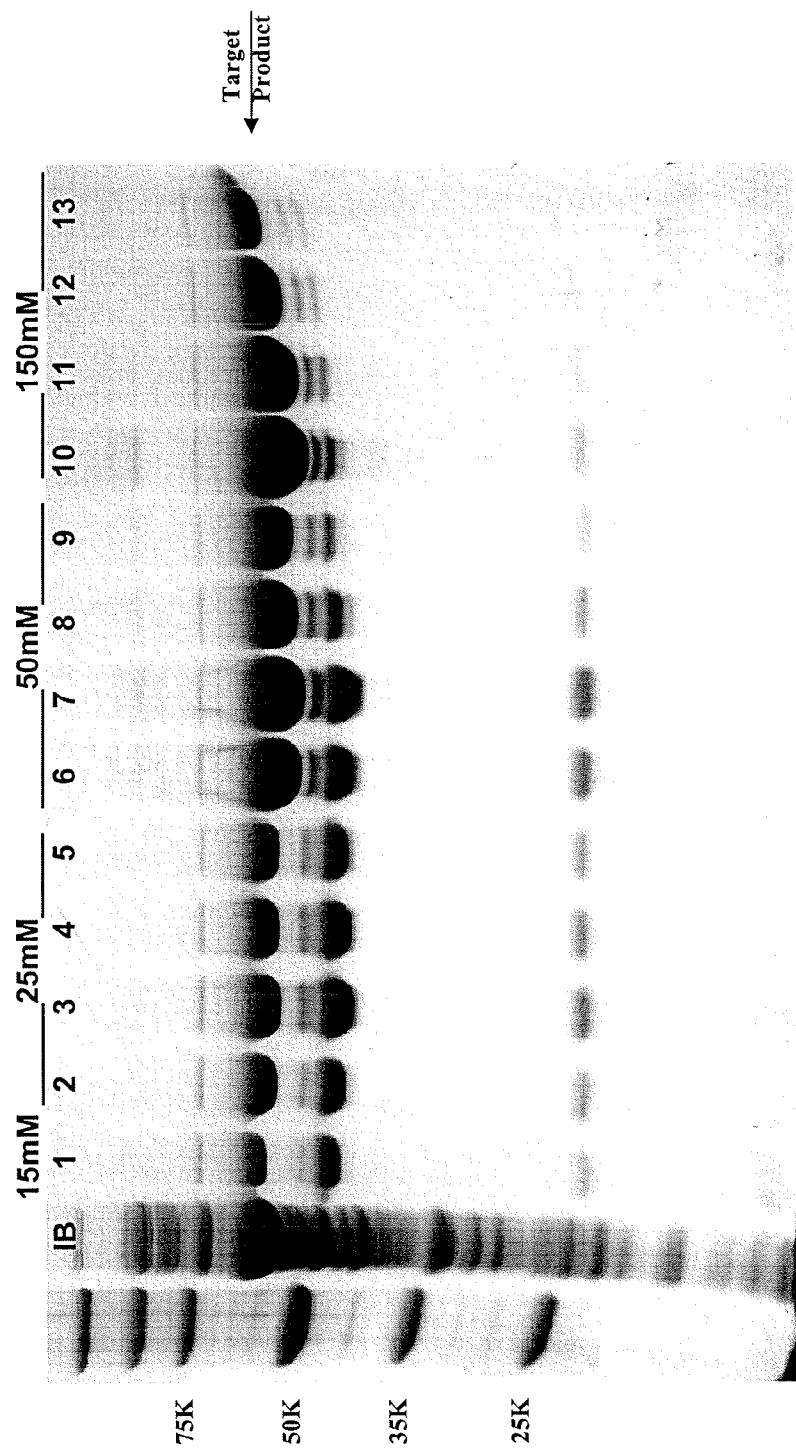


FIG. 3

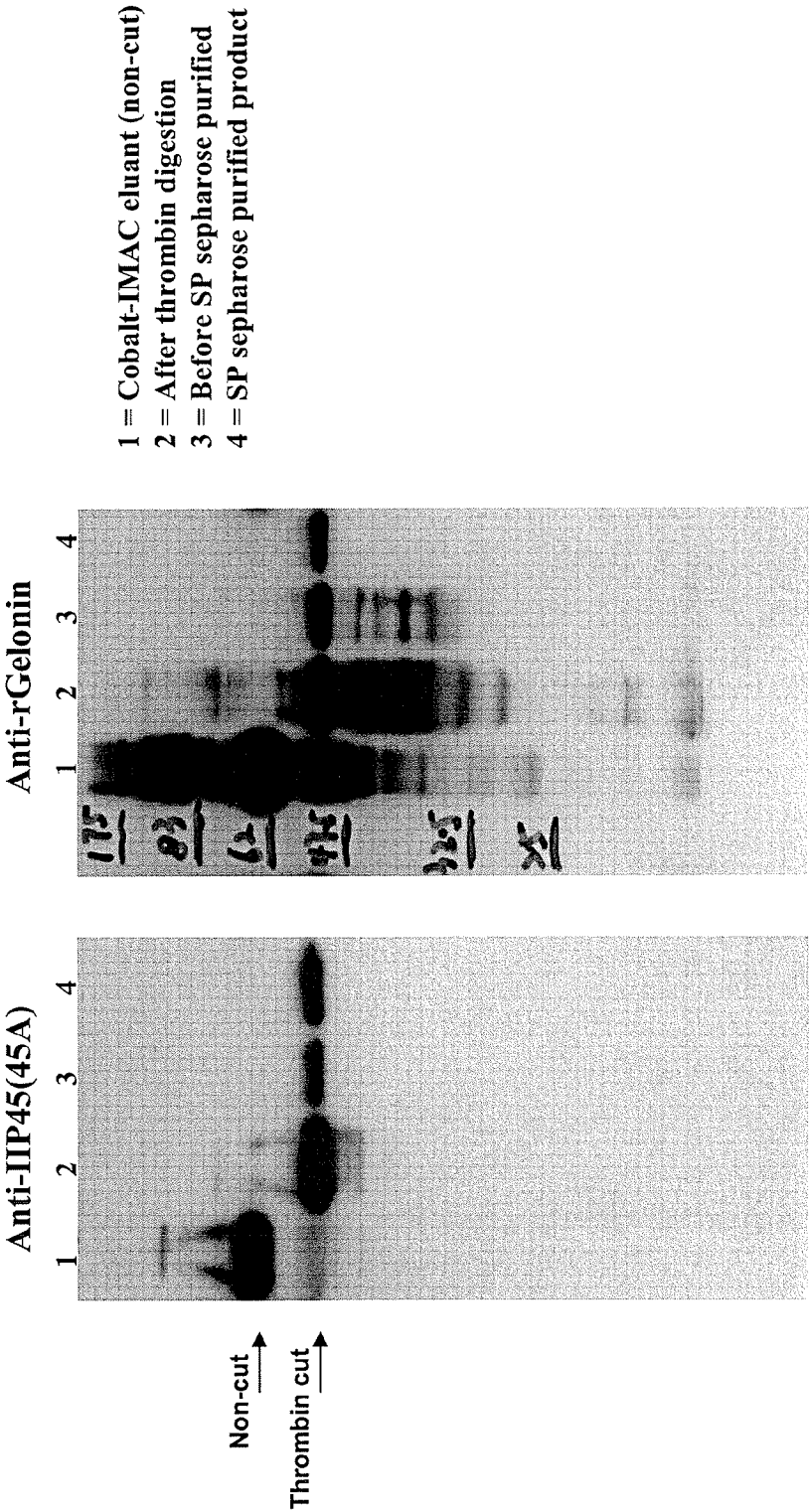


FIG. 4

RRLA of Gelonin/IIP45X5 (GX5)

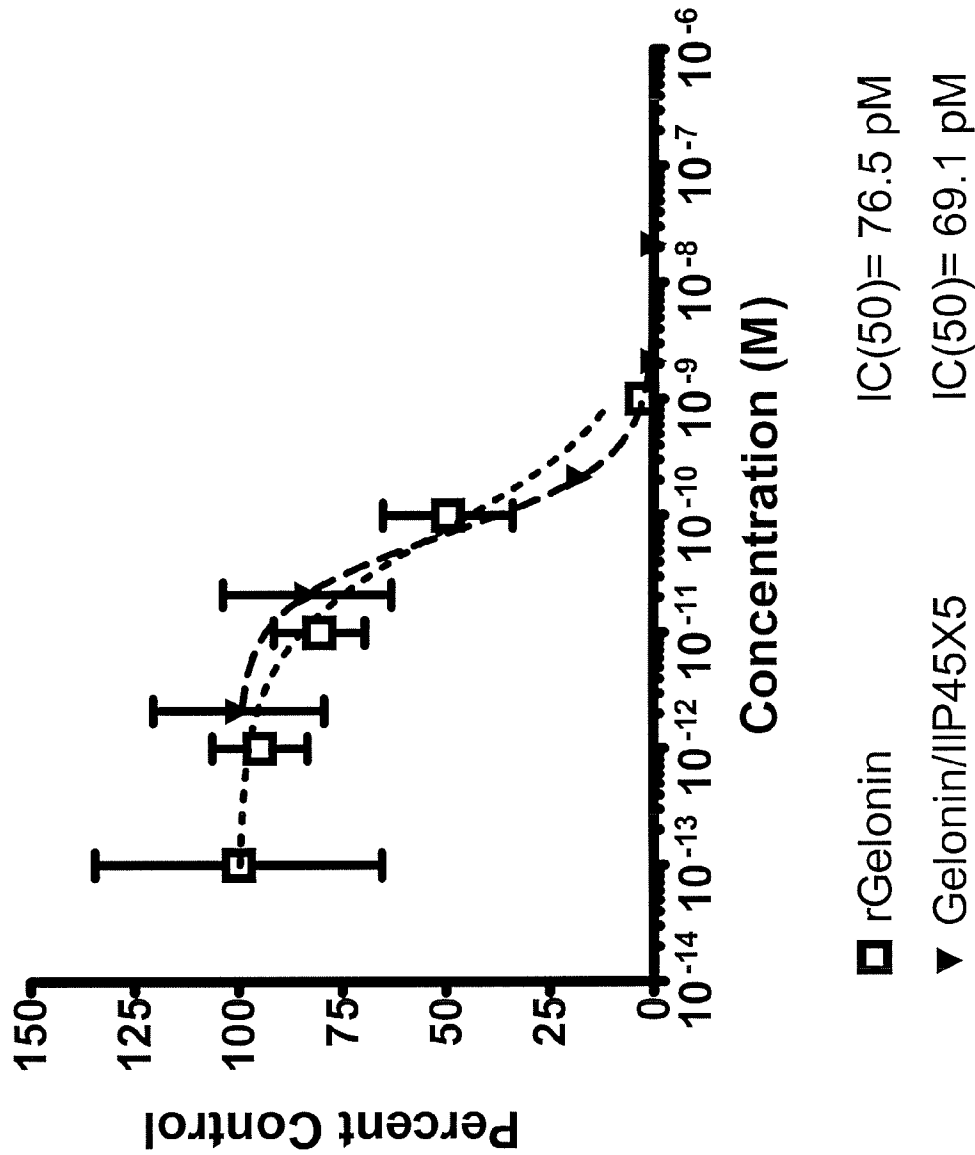


FIG. 5

Dose Response Curves for Gelonin/IIP45X5(Batches 1-3) versus rGelonin on A375-M Human Melanoma Cells

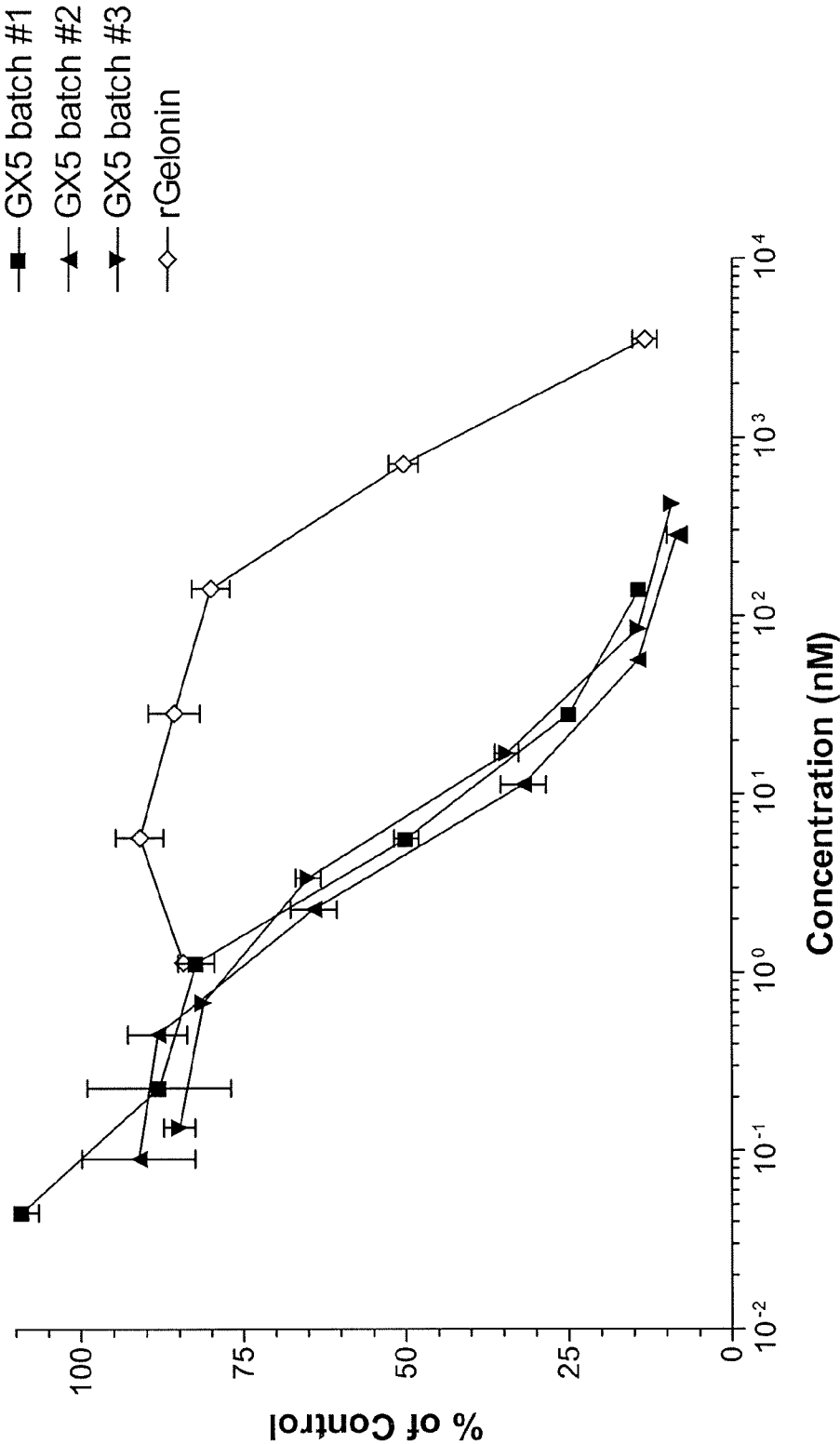


FIG. 6

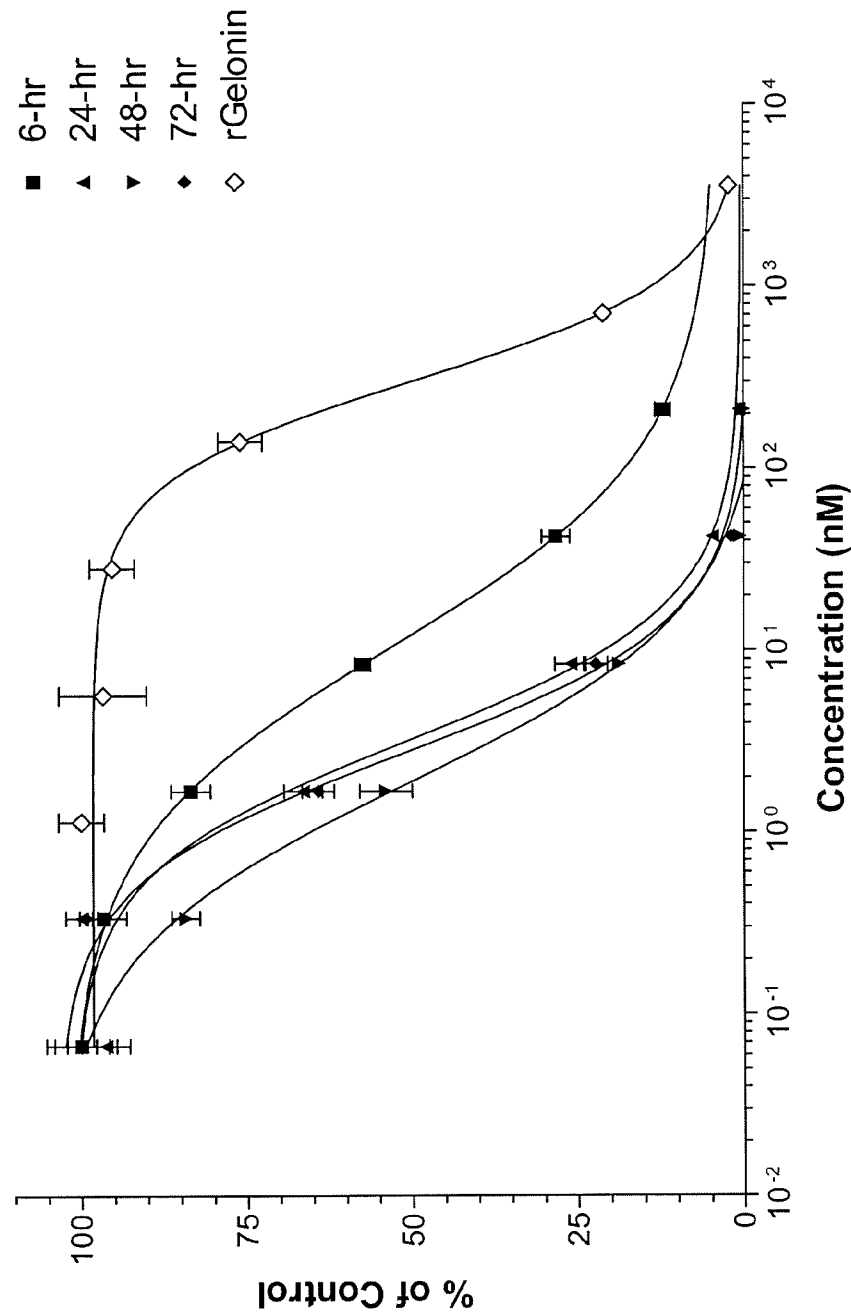


FIG. 7

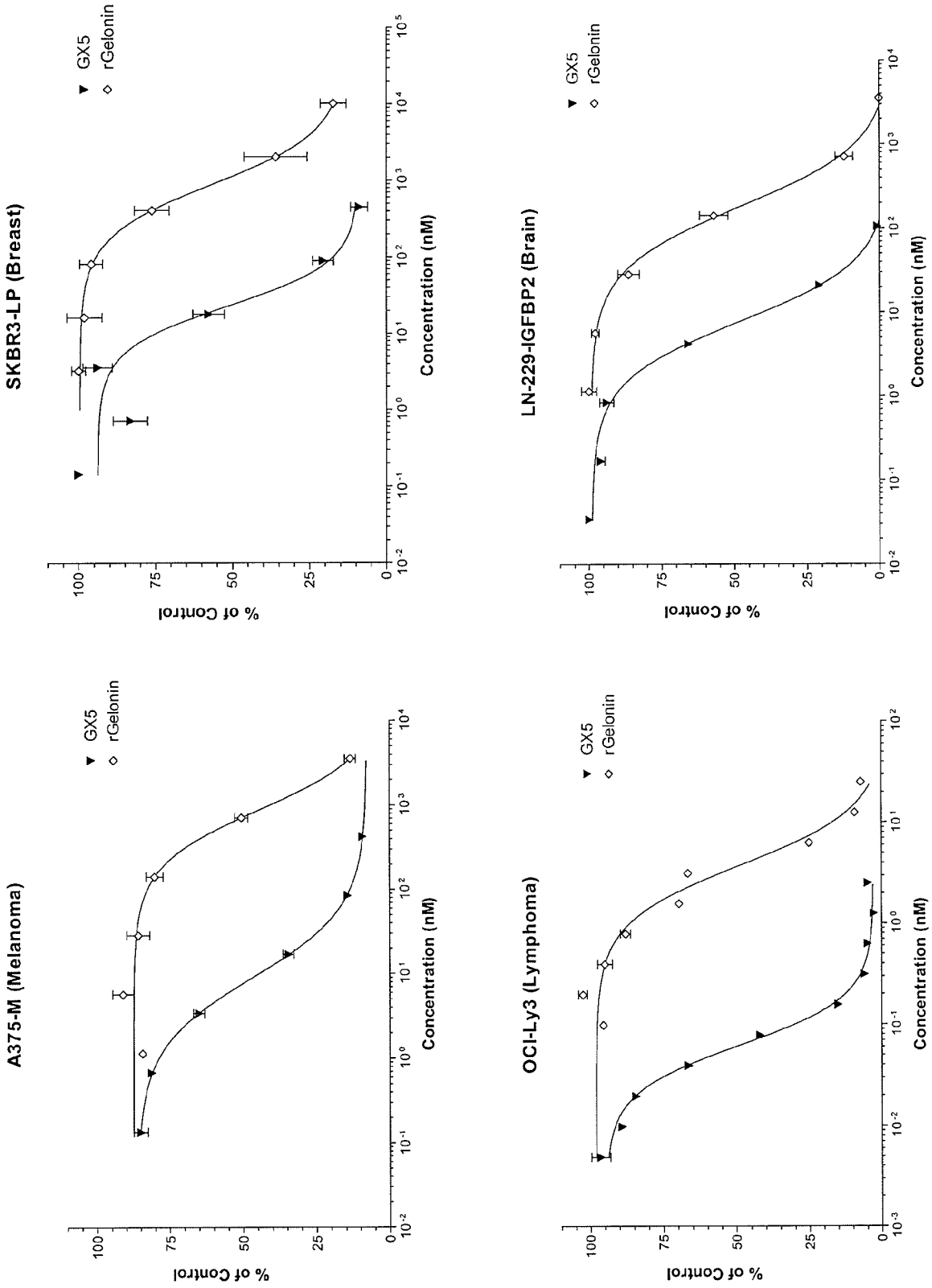


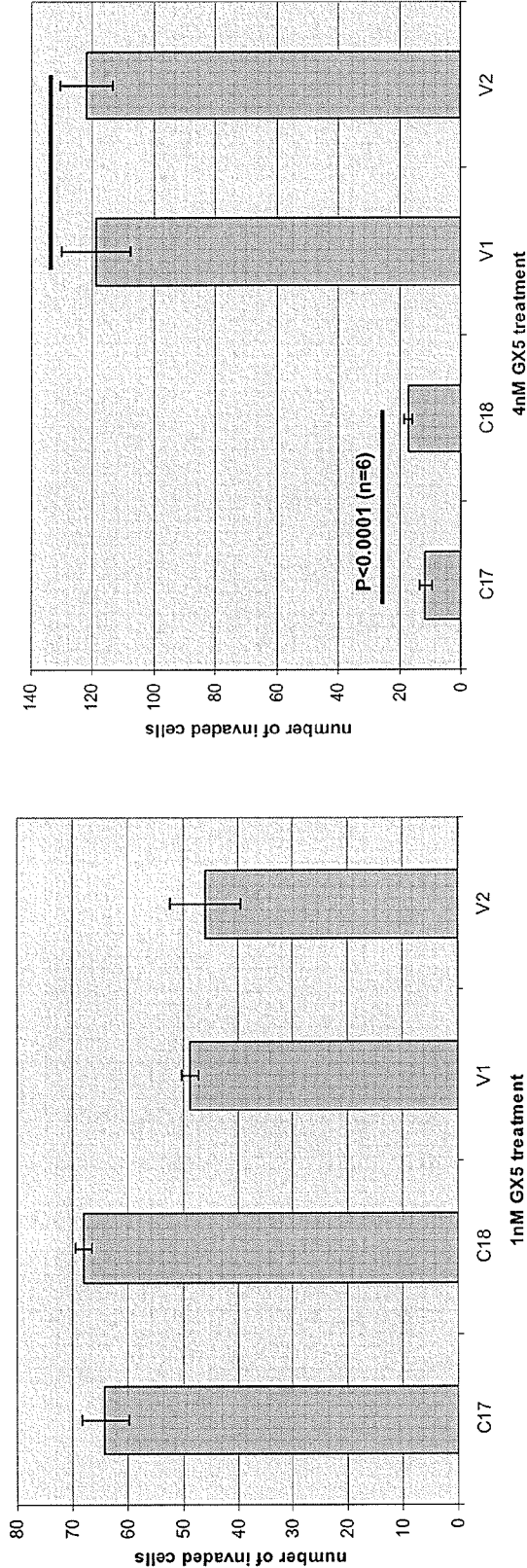
FIG. 8

Cell Line	Date	Sample	IC ₅₀ , nM	Targeting Index
A375m (melanoma)	4/17/2008	GX5	8.03	87.3
		rGeloinin	700.8	
OCI-Ly3 (lymphoma)	5/27/2008	GX5	0.058	56.9
		rGeloinin	3.3	
SK-BR-3-LP (breast)	4/15/2008	GX5	29.18	43.56
		rGeloinin	1271	
	5/10/2008	GX5	10.11	35.51
		rGeloinin	359	
MDA MB468 (breast)	5/10/2008	GX5	12.9	29.83
		rGeloinin	384.8	
eB-1 (breast)	5/10/2008	GX5	20.14	23.8
		rGeloinin	479.4	
KM12-SM (melanoma)	4/17/2008	GX5	46.21	21.64
		rGeloinin	1000	
SK-BR-3-HP (breast)	5/10/2008	GX5	16.74	15.13
		rGeloinin	235.3	
BT474 (breast)	5/10/2008	GX5	237	4.93
		rGeloinin	1168	
MDA MB231 (breast)	5/10/2008	GX5	75.48	4.18
		rGeloinin	315.5	
OCI-Ly10 (lymphoma)	5/13/2008	GX5	4.8	3.65
		rGeloinin	17.5	
Me180 (cervical)	4/15/2008	GX5	21.42	3.55
		rGeloinin	76.05	
MCF-7 (breast)	5/10/2008	GX5	108.8	1.89
		rGeloinin	205.3	
SKOV3 (ovarian)	4/15/2008	GX5	184.9	0.49
		rGeloinin	91.09	

FIG. 9

Treatment with IIP45X5/rGel will suppress Matrigel Invasion

P<0.0001 (n=6)



LN229 cell lines treated with 4nM GX5 and 1nM GX5, respectively, for 24h and in vitro invasion assay was performed.

FIG. 10

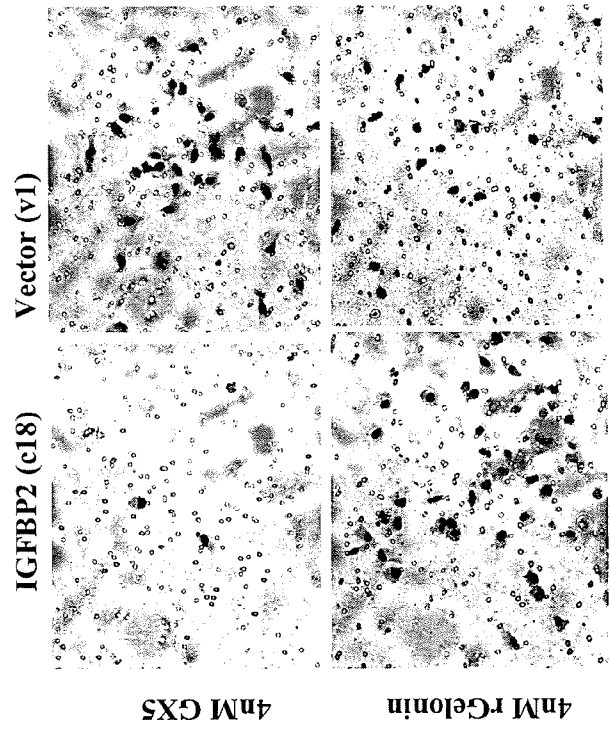
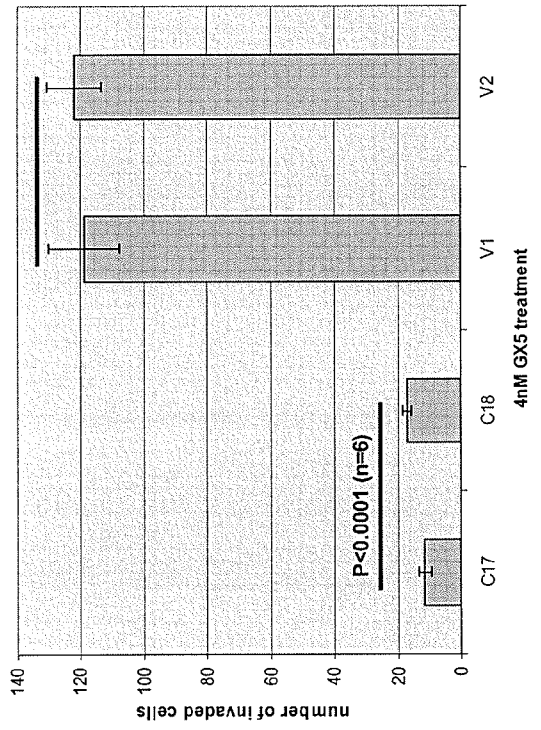
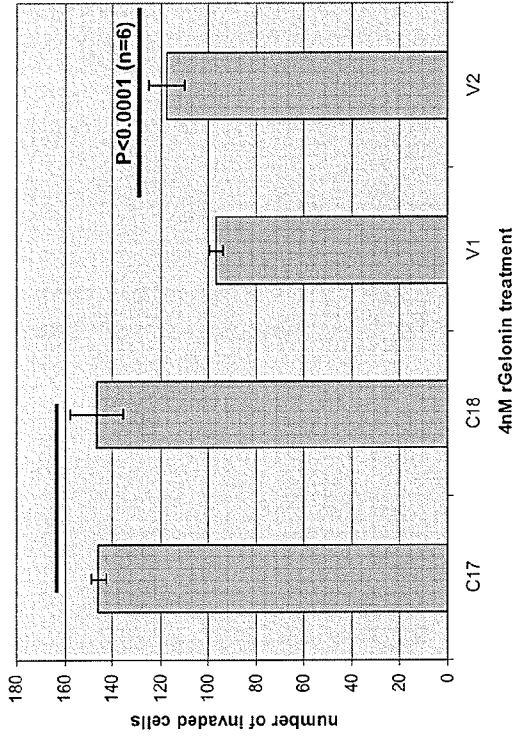


FIG. 11