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(54) Title: COMPOSITIONS AND METHODS FOR TREATING CHONDROSARCOMAS

JJ012 Human chondrosarcoma cell line

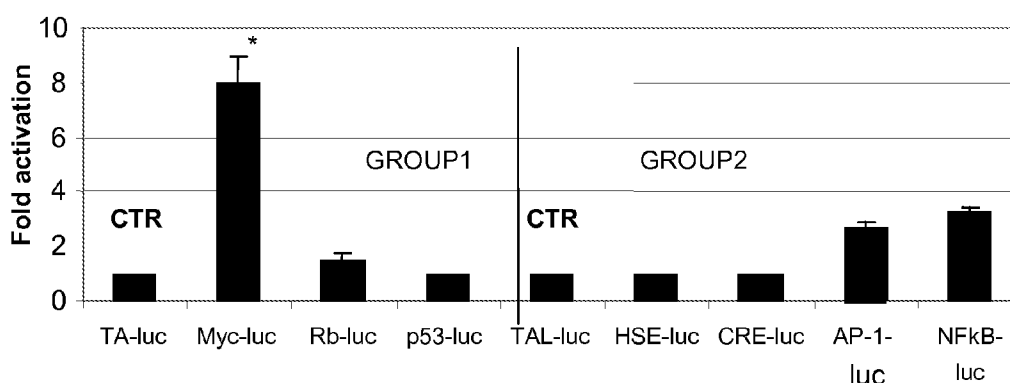


FIG. 1

(57) Abstract: Compositions, kits and methods for inhibiting growth of Chondrosarcoma cells involve compositions including PRP-I or PRP-I analogues in an amount effective to inhibit growth of the cells. A method of inhibiting growth of Chondrosarcoma cells includes providing a composition including PRP-I or a PRP-I analogue in an amount effective to inhibit growth of the Chondrosarcoma cells; and contacting the Chondrosarcoma cells with the composition, wherein contacting the Chondrosarcoma cells with the composition results in inhibition of growth of the Chondrosarcoma cells. A typical adjuvant composition includes PRP-I in an amount effective to inhibit growth of human Chondrosarcoma cells and a pharmaceutically acceptable carrier or diluent. Compositions and methods described herein are based on the discovery that contacting human Chondrosarcoma cells with PRP-I abolished MYC oncogene activity and inhibited growth of the Chondrosarcoma cells.

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COMPOSITIONS AND METHODS FOR TREATING CHONDROSARCOMAS

FIELD OF THE INVENTION

[0001] The invention relates generally to the fields of oncology and medicine. More particularly, the invention relates to compositions and methods for inhibiting growth of cancer cells.

BACKGROUND

[0002] Chondrosarcoma is the second most common malignancy in bone and results from unregulated growth of mesenchymal stem cells; it is a cancer of cartilage. It tends to be locally invasive and then metastatic. One of the biggest problems associated with Chondrosarcoma is that Chondrosarcoma does not appear to respond either to chemotherapy or radiation. In the past several decades, mesenchymal malignancies such as osteosarcoma and Erwing sarcoma have seen a dramatic increase in long term survival. However, other mesenchymal malignancies such as human Chondrosarcoma have a poor prognosis due to the absence of adjuvant therapy. The failure of currently available treatments in offering significant increases in long-term survival suggests an urgent need for the development of new adjuvant therapies for the treatment of Chondrosarcoma.

SUMMARY

[0003] The invention relates to the development of compositions, kits and methods for inhibiting growth of Chondrosarcoma cells. The compositions, kits and methods can be used for treating both primary tumors and metastatic disease. Compositions, kits and methods described herein are based on the discovery that contacting human Chondrosarcoma cells with Proline Rich Polypeptide (PRP-1) abolishes Myc (also referred to herein as MYC) oncogene activity and inhibits growth of the Chondrosarcoma cells. PRP-1 is a neurohormone isolated along with vasopressin and oxytocin from neurosecretory granules of the hypothalamus and neurohypophysis, and identified by biological and chemical methods. Because PRP-1 is released into the general circulation (it has been found in lymphatic nodes, mast cells, etc.), it is classified as a hypothalamic neurohormone. In addition to antitumor properties, PRP-1 possesses cytokine properties, influences the activity of thymocytes, demonstrates potent antiviral and antibacterial properties, and antineurodegenerative properties (e.g., in cases of spinal cord hemisection, trauma, crush syndrome, aluminum toxicosis). Compositions, kits and methods for inhibiting growth of Chondrosarcoma cells

include a composition including PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of Chondrosarcoma cells. Because human Chondrosarcoma cells are not sensitive to radiation or chemotherapy and the only available method for treating Chondrosarcoma is surgical resection, the methods, kits and compositions described herein are particularly valuable.

[0004] Accordingly, described herein is a method of inhibiting growth of Chondrosarcoma cells. The method includes the steps of: providing a composition including PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of the Chondrosarcoma cells; and contacting the Chondrosarcoma cells with the composition, wherein contacting the Chondrosarcoma cells with the composition results in inhibition of growth of the Chondrosarcoma cells. The composition can further include at least one pharmaceutically acceptable diluent. In one embodiment, the composition includes a PRP-1 polypeptide having the amino acid sequence set forth in SEQ ID NO:1 and saline. In one embodiment, the amount effective to inhibit growth of the Chondrosarcoma cells is about 0.5-1 microgram/ml. The composition can include PRP-1 that has been chemically synthesized. Contacting the cells with the composition results in inhibition of MYC activity in the cells.

[0005] Also described herein is an adjuvant composition including PRP-1 in an amount effective to inhibit growth of human Chondrosarcoma cells and a pharmaceutically acceptable diluent. The amount effective to inhibit growth of human Chondrosarcoma cells is about 4 µg/kg to about 10 µg/kg. The PRP-1 can be, for example, in lyophilized powder form or dissolved in saline solution.

[0006] Further described herein is a kit for modulating an immune response. The kit includes an adjuvant composition including PRP-1 in an amount effective to inhibit growth of human Chondrosarcoma cells and a pharmaceutically acceptable diluent, and instructions for use.

[0007] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0008] As used herein, "protein" and "polypeptide" are used synonymously to mean any peptide-linked chain of amino acids, regardless of length or post-translational modification, e.g., glycosylation or phosphorylation. A "purified" polypeptide is one that is substantially separated from other polypeptides in a cell or organism in which the polypeptide naturally occurs (e.g., 30, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 100% free of contaminants).

[0009] By the terms "PRP-1 protein," "PRP-1," "PRP-1 polypeptide" and "Galarmin" is meant a 15 amino acid C-terminal peptide fragment of the neurophysin-vasopressin

associated glycoprotein (NVAG) which is a precursor of vasopressin. NVAG within brain neurosecretory granules slowly moves to neurohypophysis by axonal transport via the hypothalamo-neurohypophyseal nervous tract, where the separation of oxytocin, vasopressin, and PRP-1 from NVAG occurs. Analysis of the nucleotide sequence of cloned cDNA that encodes bovine arginine-vasopressin-neurophysin precursor confirmed that the glycopeptide region is at the C-terminus of the precursor (Armen A. Galoyan, Brain Neurosecretory Cytokines, Immune Response and Neuronal Survival. Kluwer Academic, Plenum Publishers, 2004).

[0010] A "functional activity" of a protein is any activity associated with the physiological function of the protein. For example, functional activities of a native PRP-1 protein may include modulating the bioactivity of thymocytes, antiviral activities, antibacterial activities, downregulating (inhibiting) cancerous tumor growth, and downregulating neurodegenerative processes. Because PRP-1 is a hypothalamic neurohormone, PRP-1 functional activities typically include those of hypothalamic neurohormones.

[0011] When referring to a nucleic acid molecule or polypeptide, the term "native" refers to a naturally-occurring (e.g., a "wild-type") nucleic acid or polypeptide. An "analogue" of a native PRP-1 polypeptide is a chemical compound having a structure similar to that of PRP-1 but differing from PRP-1 with respect to at least one structural component. For example, an analogue of PRP-1 is amidated Gx-NH₂ (amidated Galarmin).

[0012] By the term "adjuvant therapy" is meant a treatment that is given to a subject (e.g., human) in addition to a primary (initial) treatment (e.g., surgery). An "adjuvant," as used herein, is any agent that can enhance an immune response.

[0013] By the phrases "therapeutically effective amount," "in an amount effective" and "effective dosage" is meant an amount sufficient to produce a therapeutically (e.g., clinically) desirable result; the exact nature of the result will vary depending on the nature of the disorder being treated. For example, where the disorder to be treated is Chondrosarcoma, the result can be inhibition of growth of Chondrosarcoma cells. The compositions described herein can be administered from one or more times per day to one or more times per week. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compositions of the invention can include a single treatment or a series of treatments. In

terms of an adjuvant, an effective amount is one sufficient to enhance the immune response, for example, after a surgery has been performed. The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art.

[0014] As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent described herein, or identified by a method described herein, to a patient, or application or administration of the therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease, or the predisposition toward disease.

[0015] The terms "patient" "subject" and "individual" are used interchangeably herein, and mean a mammalian subject to be treated, with human patients being preferred. In some cases, the methods of the invention find use in experimental animals, in veterinary applications, and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters, as well as primates.

[0016] As used herein, the term "Chondrosarcoma" means a malignant neoplasm derived from cartilage tissue.

[0017] Although compositions, kits and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable compositions, kits and methods are described below. All publications, patent applications, and patents mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. The particular embodiments discussed below are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1 is a graph showing transactivation of response elements in JJ012 human Chondrosarcoma cells.

[0019] FIG. 2 is a graph showing downregulation of Myc-luc construct transactivation in transiently transfected JJ012 human Chondrosarcoma cultured cells under influence of hypothalamic PRP-1

[0020] FIG. 3 is a micrograph of a C-28 human chondrocyte culture (24 hour incubation) treated with 0.5 μ g PRP-1.

[0021] FIG. 4 is a micrograph of confluent Chondrosarcoma cells actively dividing after 24 hours of incubation (Control).

[0022] FIG. 5 is a micrograph showing that PRP-1 (0.5 μ g) impedes cell proliferation significantly in comparison with the control (24 hours incubation).

[0023] FIG. 6 is a micrograph of human Chondrosarcoma JJ012 cells treated with 1 μ g of PRP-1 (24 hours of incubation).

[0024] FIG. 7 is a micrograph of human Chondrosarcoma cells treated with 10 μ g of PRP-1 (24 hours of incubation).

[0025] FIG. 8 is a micrograph of human Chondrosarcoma cells treated with 20 μ g of PRP-1 (24 hours of incubation).

[0026] FIG. 9 is a micrograph showing that a low concentration of PRP-1 inhibited human Chondrosarcoma cell proliferation 24 hours incubation ($P < 0.0001$, $N = 6$).

DETAILED DESCRIPTION

[0027] The invention provides compositions, kits and methods for inhibiting growth of Chondrosarcoma cells. The below described preferred embodiments illustrate adaptations of these compositions and methods. Nonetheless, from the description of these embodiments, other aspects of the invention can be made and/or practiced based on the description provided below.

Biological Methods

[0028] Methods involving conventional molecular biology techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises such as *Molecular Cloning: A Laboratory Manual*, 3rd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and *Current Protocols in Molecular Biology*, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Cell culture methods are also generally known in the art. Cells can be grown in suspension or adherent cultures. In the Examples below, cells were grown as adherent cultures. Adherent cells require a surface, such as tissue culture plastic, which may be coated with extracellular matrix components to increase adhesion properties and provide other signals needed for growth and differentiation. Most cells derived from solid tissues are adherent. Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO₂ for mammalian cells) in a cell incubator, in flasks. Culture conditions vary widely for each cell type.

[0029] In the Examples below, culture conditions for maintaining a stable phenotype of chondrosarcoma cells are described. Human chondrosarcoma cell lines JJ012 were gained

from tumors obtained at resection and were established as described previously (J.A. Block, S.E. Inerot, S. Gitelis and J.H. Kimura, Synthesis of chondrocytic keratan sulphate-containing proteoglycans by human chondrosarcoma cells in long-term cell cultures, J. Bone Joint Surg. 73 (1991), pp. 647–658).

Adjuvant Compositions and Adjuvant Therapy

[0030] PRP-1 and compositions containing PRP-1 may find use as adjuvants in adjuvant therapy for Chondrosarcoma. Adjuvant therapy for cancer usually refers to surgery (primary treatment) followed by chemo- or radiotherapy to help decrease the risk of the cancer recurring. However, because Chondrosarcoma does not respond to chemotherapy or radiation, adjuvant therapy for Chondrosarcoma as described herein generally includes surgery as the initial treatment followed by treatment with a composition including PRP-1. This method can also be referred to as immunotherapy, which is a treatment to stimulate or restore the ability of the immune system to fight infection or disease. PRP-1 is a cytokine and a stimulator of immune cells, myelopoiesis, T cell differentiation and hematopoiesis. PRP-1 is a potent antibacterial and antiviral agent and is responsible for an increase in macrophage and T cell activity, stimulation of antigen presenting functions of macrophages and antibody formation, etc. The immunostimulatory properties of PRP-1 (a neurohormone cytokine) inhibit growth of Chondrosarcoma cells.

[0031] In one embodiment, an adjuvant composition as described herein includes PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of Chondrosarcoma cells and a pharmaceutically acceptable diluent. A typical diluent is saline solution (e.g., 0.9% NaCl). In one embodiment of an adjuvant composition, PRP-1 is dissolved in a saline diluent (0.9% NaCl) and is administered in that form. PRP-1 is stable as a saline solution for three to four years. In another embodiment of an adjuvant composition, PRP-1 is lyophilized, and the lyophilized powder is packaged in an ampoule. In this embodiment, the lyophilized powder can be dissolved in diluent (e.g., saline) prior to administration to a subject (e.g., human). In a variation of this embodiment, the lyophilized powder form of PRP-1 may be administered to a subject.

PRP-1 Proteins

[0032] An example of a PRP-1 protein is a chemically synthesized native PRP-1 protein that has the amino acid sequence of SEQ ID NO:1. Variants of native PRP-1 proteins such as fragments, analogs and derivatives of native PRP-1 proteins are also within the invention.

Any suitable methods of producing PRP-1 proteins can be used. In the Examples below, the PRP-1 sequence was chemically synthesized based on its 15 amino acid residue structure.

Inhibiting Growth of Chondrosarcoma Cells

[0033] Described herein are compositions and methods for inhibiting growth of Chondrosarcoma cells. A composition for inhibiting growth of Chondrosarcoma cells includes PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of the Chondrosarcoma cells. Any suitable form of PRP-1 or a PRP-1 analogue can be used. A composition can further include at least one pharmaceutically acceptable carrier or diluent. Typically, an amount effective of PRP-1 or an analogue thereof to inhibit growth of Chondrosarcoma cells *in vivo* is about 4 µg/kg to about 10 µg/kg. In the experiments described below, growth of Chondrosarcoma cells was inhibited using compositions as described herein. However, a variety of cancers can be treated using the compositions, kits and methods described herein.

[0034] A typical method of inhibiting growth of Chondrosarcoma cells includes providing a composition including PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of the Chondrosarcoma cells, and contacting the Chondrosarcoma cells with the composition. In this method, contacting the cells with the composition results in inhibition of growth of the Chondrosarcoma cells. Contacting the cells with the composition also inhibits Myc activity in the cells. In one example of a method, the composition includes a PRP-1 polypeptide having the amino acid sequence of SEQ ID NO:1 and saline. The PRP-1 can be, for example, in lyophilized powder form or dissolved in a suitable solvent or diluent (e.g., saline). Generally, a composition further includes at least one pharmaceutically acceptable carrier or diluent. Using a typical method, a variety of cancers can be treated using the compositions described herein.

Treating Chondrosarcoma In a Mammal

[0035] In the Examples below, PRP-1 inhibited growth of Chondrosarcoma cells *in vitro*. Also described herein are methods of treating Chondrosarcoma in a mammal (e.g., rodent, human). A typical method of treating Chondrosarcoma in a mammal includes providing a composition including PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of Chondrosarcoma cells, and administering the composition to the mammal having a Chondrosarcoma, wherein administering the composition to the mammal results in inhibition of growth of the Chondrosarcoma cells in the mammal. Typically, the composition includes

a pharmaceutically acceptable carrier or diluent (e.g., physiological saline or buffer) and is administered intramuscularly, intravenously, or subcutaneously.

[0036] In some embodiments of treating Chondrosarcoma in a mammal, the mammal first undergoes surgery for removal of the Chondrosarcoma, and subsequently receives treatment with PRP-1 (e.g., administration of a composition including PRP-1 or a PRP-1 analogue in an amount effective to inhibit growth of Chondrosarcoma cells). In this immunotherapy method, the immunostimulatory properties of PRP-1 (a neurohormone cytokine) inhibit growth of the Chondrosarcoma.

Administration of Compositions

[0037] The compositions described herein may be administered to mammals (e.g., rodents, humans) in any suitable formulation. For example, PRP-1 or analogues thereof or compositions thereof may be formulated in pharmaceutically acceptable carriers or diluents such as physiological saline or a buffered salt solution. Suitable carriers and diluents can be selected on the basis of mode and route of administration and standard pharmaceutical practice. A description of exemplary pharmaceutically acceptable carriers and diluents, as well as pharmaceutical formulations, can be found in Remington's Pharmaceutical Sciences, a standard text in this field, and in USP/NF. Other substances may be added to the compositions to stabilize and/or preserve the compositions.

[0038] The compositions of the invention may be administered to mammals by any conventional technique. Typically, such administration will be parenteral (e.g., intravenous, subcutaneous, intratumoral, intramuscular, intraperitoneal, or intrathecal introduction). The compositions may also be administered directly to a target site by, for example, surgical delivery to an internal or external target site, or by catheter to a site accessible by a blood vessel. The compositions may be administered in a single bolus, multiple injections, or by continuous infusion (e.g., intravenously, by peritoneal dialysis, pump infusion). For parenteral administration, the compositions are preferably formulated in a sterilized pyrogen-free form.

Effective Doses

[0039] The compositions described herein are preferably administered to a mammal (e.g., a rat, human) in an effective amount, that is, an amount capable of producing a desirable result in a treated mammal (e.g., inhibiting growth of Chondrosarcoma cells). Such a therapeutically effective amount can be determined as described below.

[0040] Toxicity and therapeutic efficacy of the compositions utilized in methods of the invention can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD₅₀ (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Those compositions that exhibit large therapeutic indices are preferred. While those that exhibit toxic side effects may be used, care should be taken to design a delivery system that minimizes the potential damage of such side effects. The dosage of preferred compositions lies preferably within a range that includes an ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Generally, a dose for treating a mammal is about 4 to about 10 µg/kg.

[0041] As is well known in the medical and veterinary arts, dosage for any one subject depends on many factors, including the subject's size, body surface area, age, the particular composition to be administered, time and route of administration, general health, and other drugs being administered concurrently.

Kits

[0042] Described herein are kits including a composition as described herein and instructions for use of the composition. A "kit," as used herein, typically defines a package or an assembly including one or more of the compositions of the invention, and/or other compositions associated with the invention. As described herein, a typical kit includes an adjuvant composition including PRP-1 in an amount effective to inhibit growth of human Chondrosarcoma cells and a pharmaceutically acceptable diluent, and instructions for use. Each of the compositions of the kit may be provided in liquid form (e.g., in solution), or in solid form (e.g., a dried powder). When the composition provided is a dry powder, the composition may be reconstituted by the addition of a suitable solvent, which may also be provided. In embodiments where liquid forms of the composition are used, the liquid form may be concentrated or ready to use.

EXAMPLES

[0043] The present invention is further illustrated by the following specific examples. The examples are provided for illustration only and should not be construed as limiting the scope of the invention in any way.

Example 1 – Myc-Oncogene inactivating effect by PRP-1 in Chondrosarcoma JJ012 cells

[0044] The discovery of cytokine secretion by hypothalamic magnocellular nuclei as well as biosynthesis of a new class of cytokines (PRPs) illustrate the capability of the brain's neuroendocrine cells to synthesize cytokines along with vasopressin, oxytocin and organotropic (cardioactive) neurohormones. Strong antibacterial (*in vivo*) and antiviral (*in vitro*) properties of hypothalamic proline rich peptides (Galoyan et al., Neurochem Research, 2006) as well as antineurodegenerative and immunotropic properties of PRP family cytokines provide evidence for the existence of brain protective mechanisms against infection, neurodegeneration, immunodefficiencies, tumors, etc. (Galoian et al., Neurochem Research July 9, 2008).

[0045] PRP-1 contains 15 amino acid residues (Ala- Gly-Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln-Pro-Gly-Val-Tyr (SEQ ID NO:1)) and is a unique regulator of hematopoiesis, a stimulator of bone-marrow hematogenesis in a normal physiological state and during lymphocytopenia induced by cyclophosphamide (Galoyan and Aprikyan, Neurochem Research 27(4):305-312, 2002). PRP-1 and a new amidated peptide (Gx-NH₂) discovered recently (Galoyan A.A. Brain Neurosecretory cytokines: Immune Response and Neuronal Survival Kluwer Academic/Plenum Publishers, p.188, 2004) are powerful drugs for the treatment of anthrax-induced by Bacillus anthracis. PRP-1 has been shown to have a direct effect on T-cell proliferation, confirmed by the inhibition of proliferation of human T-cell line Jurkat under PRP-1 influence (*in vitro*) (Galoyan A.A. Brain Neurosecretory cytokines: Immune Response and Neuronal Survival Kluwer Academic/Plenum Publishers, p.188, 2004). In the experiments described below, the effect of PRP-1 on Chondrosarcoma cells (human JJ012 Chondrosarcoma cells) was examined.

Materials and Methods

[0046] The Chondrosarcoma cells JJ012 were cultured in monolayer until semiconfluent. Media consisted of Dulbecco's modified Eagle's medium (DMEM)/MEM supplemented with F12, 10% fetal bovine serum, 25 µg/ml ascorbic acid, 100 ng/ml insulin, 100 nM hydrocortisone, and 1% penicillin/streptomycin

[0047] FIG. 1 shows transactivation of response elements in JJ012 human Chondrosarcoma cells. Cells were seeded at a density of 2×10^5 cells/ml in 6-well plates 24 hrs prior to transfection and incubated at 37°C for 24 hrs. Transfection was performed using Lipofectamine 2000 reagent from Invitrogen (Carlsbad, CA) according to the manufacturer's instructions, as described previously (Galoian et al., Exp. Biol. Med. 232(4):515-522, 2007).

[0048] PRP-1 (1ug) was added to the wells (except the control) 10 min prior to reporter plasmids (500ng/ul) to the transfection complex. Six hours posttransfection, the lipofectin complex was removed and substituted with fresh growth media. Cells were harvested for luciferase assay (Promega, Madison, WI) 24 hrs after transfection, and luciferase activity was quantified using a TD20/20 luminometer (Turner BioSystems, Sunnyvale, CA). All values were normalized to protein content or to renilla luciferase activity. The Pathway Profiling Systems (Clontech) provides a means to assess the activation of signal transduction pathways *in vivo*. These systems consist of sets of vectors that each contain a distinct *cis*-acting enhancer element upstream of a reporter gene. Several of the vectors are also available separately. A single transfection experiment provides a profile of the transcription factors that are affected by a given stimulus, drug candidate, or gene product of interest. Most of the *cis*-acting enhancer elements included in transcription factor systems are regulated by more than one transcription factor, so multiple signal transduction pathways can be monitored, and direct measurement of the corresponding pathway is possible upon the binding of transcription elements to corresponding DNA consensus sites (AP1, CRE, MYC etc). Expression of the reporter means that a transactivator present in the cell has bound the enhancer element, indicating that a particular signaling pathway or pathways has been activated. Transcription factor binding to the enhancer element initiates Luciferase reporter gene induction (transcription), which can be measured from cell lysates using a standard assay. All experiments were performed in triplicate, and $P < 0.05$ was considered significant. Data analysis was performed using one-way analysis of variance (ANOVA) and unpaired *t*-test (GraphPad Prism; GraphPad Software, Inc., San Diego, CA).

Experimental Results

[0049] Cells were seeded at a density of 2×10^5 cells/ml in 6-well plates 24 hrs prior to transfection and incubated at 37°C for 24 hrs. Transfection was performed using Lipofectamine 2000 reagent from Invitrogen (Carlsbad, CA) according to the manufacturer's instructions. Plasmids (500ng/ul) were added to the transfection complex. Six hours posttransfection, the lipofectin complex was removed and substituted with fresh growth media. Cells were harvested for a luciferase assay (Promega, Madison, WI) 24 hrs after transfection, and luciferase activity was quantified using a TD20/20 luminometer (Turner BioSystems, Sunnyvale, CA). All values were normalized to protein content. Results demonstrated an 8-fold transactivation of pMyc-luc in comparison with control Group 1 and NFkB and AP-1luc transactivation 2-fold in comparison with control Group 2.

[0050] Group 1 vectors contain a TATA box (TA Promoter) from the Herpes Simplex Virus Thymidine kinase (HSV-TK) promoter and the pTA-luc vector serves as a negative control for this group; it doesn't contain any enhancer element. Group 2 vectors contain TATA -like region (TAL) promoter from HSV-TK promoter and pTAL-luc serves as a negative control for Group 2 correspondingly; it doesn't contain any enhancer elements. The following cis-acting enhancer elements attached to luciferase reporter genes were obtained from Clontech. *pMyc-luc* was used for the activation of cMyc and cMyc mediated signal transduction pathways. Overexpression of the Myc protein causes cell transformation by activation of genes required for cell growth. The Myc protein forms a tetradimer complex with the Max protein, which binds to the E box DNA binding element and initiates transcription of genes responsible for cellular proliferation. The Myc-Ta-luc vector contains six tandem copies of the Ebox consensus sequence located upstream of the minimal TA promoter. *pRb-luc* was used for the activation of Rb (retinoblastoma response element). *p53-luc* was used for the activation of p53 mediated signal transduction pathway. *HSE-luc* - (Heat shock element) was used for the activation of heat shock factor (HSF) and heat shock mediated signal transduction pathways. *CRE-luc* was used for the activation of cAMP binding protein (CREB) and cAMP mediated signal transduction pathways. *AP-1-luc* was used for the induction of activator protein (AP-1) and the stress activated protein kinase/Jun N-terminal kinase (SAPK/JNK) signal transduction pathway. *NFkB-luc*-(eukaryotic nucleic factor kB) was used for the activation of NFkB signal transduction pathways.

[0051] As shown in FIG. 2, Myc-luc construct transactivation was downregulated in transiently transfected JJ012 human Chondrosarcoma cultured cells under the influence of hypothalamic PRP-1. The seeding and transfection was performed exactly the same way described in FIG. 1. PRP-1 (1ug) was added to the wells (except the control) 10 min prior to reporter plasmids (500ng/ul) to the transfection complex. Six hours posttransfection, the lipofectin complex was removed and substituted with fresh growth media. Cells were harvested for a luciferase assay (Promega, Madison, WI) 24 hrs after transfection, and luciferase activity was quantified using a TD20/20 luminometer (Turner BioSystems, Sunnyvale, CA). All values were normalized to protein content. It appeared that PRP-1 is a powerful downregulator of transcription for promoters containing MYC E boxes.

[0052] Upon MYC inactivation, tumors variously undergo proliferative arrest, cellular differentiation, and apoptosis and in some cases, apparently permanently revoking tumorigenesis. However, whereas oncogene inactivation can push cancer to the brink of normalcy, some cells retain the latent capacity to turn cancerous again, arguing that they may

exist in a state of tumor dormancy. To date, only a few drugs have been identified that have been successfully shown to have clinical efficacy through the inactivation of a specific oncogene phenotype. However, recent work in experimental transgenic model systems suggests that the targeted inactivation of a single oncogene may be generally effective in the treatment of some cancers (Felsher DW. Nat Rev Cancer 3:375-80, 2003).

[0053] To explain how oncogene inactivation might allow tumor cells to uncover latent stem cell-like properties, one possibility is that tumors, even if they seem morphologically or histologically homogenous, consist of different cell populations with discrete differences at the molecular level. This is consistent with the evidence from other groups indicating that cancer might originate from the transformation of a cancer stem cell. In leukemia and breast cancer, a specific subpopulation of the tumor is able to propagate the tumorigenic properties of the cancer. If liver cancer induced by MYC activation arises specifically from stem cells, then upon MYC inactivation one might predict that these stem cells would regain the ability to resume their physiologic program and to undergo differentiation into progenitor cells as well as more mature cell lineages. However, because of their stem cell roots, these differentiated cells might be capable of maintaining a population of cells that can self-renew at a slower rate. In these cells, which retain some stem cell features, reactivation of the MYC oncogene could result in the restoration of the neoplastic program. Others will undergo initial regression but remain present in an occult dormant state that reemerges upon cessation of therapy, leading to a rapid reoccurrence. The experimental results described herein of PRP-1 action on Human Chondrosarcoma JJ012 cells demonstrated inactivation, abolishment of Myc oncogene activity usually upregulated in Chondrosarcoma cells almost 8-fold (sometimes almost 20-fold) in comparison with control. This data indicates that PRP-1 has powerful antioncogenic action, and may be used as a therapeutic tool for Chondrosarcoma treatment.

Example 2 – Antitumorigenic Effect of brain PRP-1 in human Chondrosarcoma

[0054] Taking into consideration our preliminary data on antitumor and unique diverse biological properties of PRP-1 previously described by (Galoian et al., Neurochem Research July 9, 2008) we proceeded with investigation of the PRP-1 effect on chondrosarcoma, opening up possibilities to consider PRP-1 as a potential therapeutic tool for chondrosarcoma treatment.

Materials and Methods

[0055] The Chondrosarcoma cells JJ012 were cultured as described in Example 1. Transfection was performed using Lipofectamine 2000 reagent from Invitrogen (Carlsbad, CA) according to the manufacturer's instructions, as described previously (Galoian et al., *Neurochem Res.* Jul 9, 2008). PRP-1 (1 μ g) was added to the wells (except the control) 10 min prior to reporter plasmids (500 ng/ μ l) to the transfection complex. Six hours posttransfection the lipofectin complex was removed and substituted with fresh growth media. Cells were harvested for luciferase assay (Promega, Madison, WI) 24 h after transfection, and luciferase activity was quantified using a TD20/20 luminometer (Turner BioSystems, Sunnyvale, CA). All values were normalized to protein content or to renilla luciferase activity. The pathway profiling systems (Clontech) provides means to assess the activation of signal transduction pathways in vivo. These systems consist of sets of vectors that each contain a distinct cis-acting enhancer element upstream of a reporter gene. Several of the vectors are also available separately. A single transfection experiment provides a profile of the transcription factors that are affected by a given stimulus, drug candidate, or gene product of interest. Most of the cis-acting enhancer elements included in transcription factor systems are regulated by more than one transcription factor, so multiple signal transduction pathways can be monitored, direct measurement of the corresponding pathway is possible upon the binding of transcription elements to corresponding DNA consensus sites (AP1, CRE, MYC etc.). Expression of the reporter means that a transactivator present in the cell has bound the enhancer element, indicating that a particular signaling pathway or pathways has been activated. Transcription factor binding to the enhancer element initiates Luciferase reporter gene induction (transcription), which can be measured from cell lysates using a standard assay. Imaging experiments were performed using Zeiss (Thornwood, NY) Axiovert 200M inverted motorized microscope with TempControl 37-2 Digital warming plate. A plan-neofluar 10x/0.3 NA (0.63 μ m/pixel) or plan-neofluar 20x/0.5 NA (0.315 μ m/pixel) objective lens was used with a Hamamatsu ORCA-ER digital CCD camera. Timelapse images were acquired using Zeiss Axiovision software, and AVI movie files made in Axiovision or MetaMorph 5.07 (Molecular Devices/Universal Imaging, Downingtown, PA) software

Results

[0056] PRP-1 action in C28 normal human chondrocyte culture was examined as a control. These cells were treated with 0.5 μ g of PRP-1 (FIG. 3). No changes in C-28 treated cells with different concentrations of PRP-1 incubated 24-72 hours range were observed in

comparison with non-treated cells. No changes were observed with other concentrations of PRP-1 (10,20 $\mu\text{g/ml}$). The control JJ012 human Chondrosarcoma cell line is shown in FIG. 4 (24 hours incubation) and FIG. 5 (48 hours of incubation). FIG. 5 illustrates still actively dividing cells captured by Live Time Lapse Video Microscopy. Unlike in the chondrocyte culture, an interesting dose-related effect of PRP-1 was observed in the human Chondrosarcoma JJ012 cell line. Low concentrations of PRP-1 0.5 μg (FIG. 5) and 1 μg (FIG. 6) exerted its antiproliferative effect in this cell line after 24 hours of incubation.

[0057] As described in Example 1, 1 μg of PRP-1 corresponds to Myc oncogene inactivating activity. Higher concentrations of PRP-1 didn't demonstrate cell cycle proliferation arrest in comparison with the control line 10 μg and 20 μg of PRP-1 (FIG. 7 and FIG. 8) correspondingly captured by Live Time Lapse Video Microscopy.

[0058] The viable cell count of Control (non treated) and human Chondrosarcoma JJ012 cells treated with different PRP-1 concentration is shown in Table 1. A growth inhibitory effect was observed with 0.5-1 μg of PRP-1 in PRP-1-treated cells. Higher concentrations didn't have a growth inhibitory effect (FIG. 9 and Table 1).

Table 1. Viable Cell concentration in Human JJ012 Chondrosarcoma cell line treated with PRP-1.(n=6) 9 fold difference in viable cell number was observed when compared to control.

Treatment	Control	PRP-1	PRP-1	PRP-1	PRP-1
Concentration ($\mu\text{g/ml}$)	0	0.5	1	10	20
Mean (cells/ml)	108.83	12.41	15.25	60.33	117
STDV	9.49	3.52	6.53	8.06	14.8

[0059] A caspase activation assay was conducted to prove that apoptosis-related changes are not observed under PRP-1 influence. A Western blot of a Caspase 3 cleavage experiment with 24 hour incubated Human Chondrosarcoma JJ012 cells demonstrated that caspase 3 was not activated under different PRP-1 dose influence in comparison with the nontreated control. This result demonstrates that inhibition of tumor growth is not through caspase activation, and that inhibition of Chondrosarcoma cells by PRP-1 at low concentrations is not via programmed cell death. The Western blot was performed on control JJ012 cells that were not treated with PRP-1, JJ012 cells that were treated with 0.5, 1.0, and 10 μg of PRP-1, and a cleaved caspase control.

[0060] Reduced viable cell numbers were observed in comparison with control cells (97% growth inhibition) in the human Chondrosarcoma JJ012 line incubated with low

concentrations of PRP-1(0.5-1 μ g). No apoptosis was observed in JJ012 cells treated with PRP-1. Without wishing to be bound to theory, it is possible that PRP-1 in low concentrations impedes cell cycle progression in human Chondrosarcoma cells via MYC oncogene inactivation.

Other Embodiments

[0061] Any improvement may be made in the compositions and kits, and part or all of the method steps. All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended to illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. For example, although the experiments described herein involved Chondrosarcoma, the compositions and methods described herein can find use in a number of other diseases and disorders, including other metastatic diseases such as hematologic malignancies. Any statement herein as to the nature or benefits of the invention or of the preferred embodiments is not intended to be limiting, and the appended claims should not be deemed to be limited by such statements. More generally, no language in the specification should be construed as indicating any non-claimed element as being essential to the practice of the invention. This invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contraindicated by context.

CLAIMS

What is claimed is:

1. A method of inhibiting growth of Chondrosarcoma cells, the method comprising the steps of:
providing a composition comprising proline rich polypeptide (PRP-1) or a PRP-1 analogue in an amount effective to inhibit growth of the Chondrosarcoma cells; and
contacting the Chondrosarcoma cells with the composition,
wherein contacting the Chondrosarcoma cells with the composition results in inhibition of growth of the Chondrosarcoma cells.
2. The method of claim 1, wherein the composition consists of a PRP-1 polypeptide having the amino acid sequence set forth in SEQ ID NO:1 and saline.
3. The method of claim 1, wherein the composition further comprises at least one pharmaceutically acceptable diluent.
4. The method of claim 1, wherein the amount effective to inhibit growth of the Chondrosarcoma cells is about 0.5-1 microgram/ml.
5. The method of claim 1, wherein the composition comprises PRP-1 that has been chemically synthesized.
6. The method of claim 1, wherein contacting the cells with the composition results in inhibition of Myc activity in the cells.
7. An adjuvant composition comprising PRP-1 in an amount effective to inhibit growth of human Chondrosarcoma cells and a pharmaceutically acceptable diluent.
8. The adjuvant composition of claim 7, wherein the amount effective to inhibit growth of human Chondrosarcoma cells is about 4 µg/kg to about 10 µg/kg.

9. The adjuvant composition of claim 7, wherein the PRP-1 is in lyophilized powder form.
10. The adjuvant composition of claim 7, wherein the PRP-1 is dissolved in saline solution.
11. A kit for modulating an immune response, the kit comprising an adjuvant composition comprising PRP-1 in an amount effective to inhibit growth of human Chondrosarcoma cells and a pharmaceutically acceptable diluent, and instructions for use.

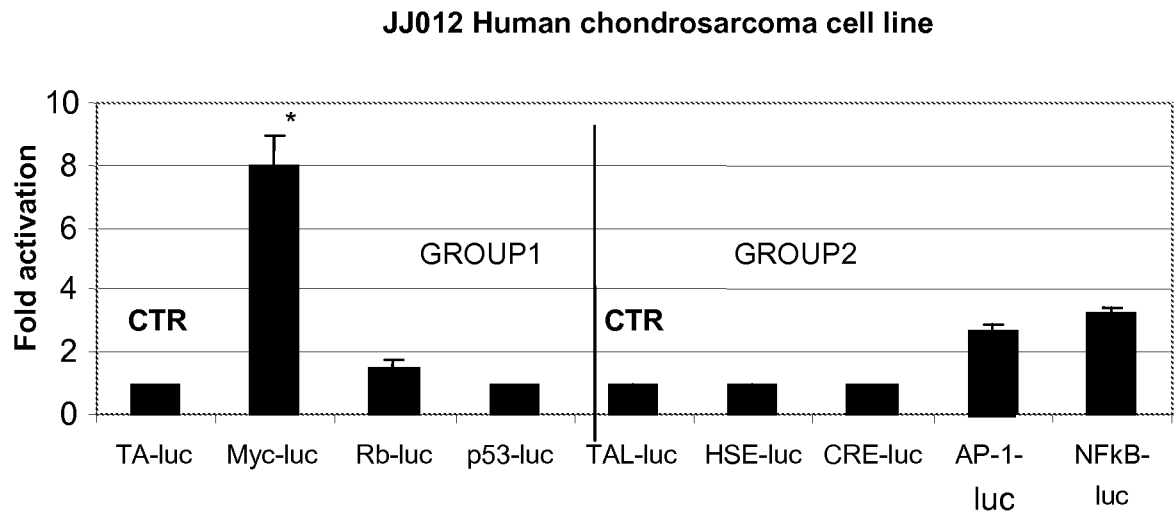


FIG. 1

PRP (1ug) effect in Human Chondrosarcoma cell line

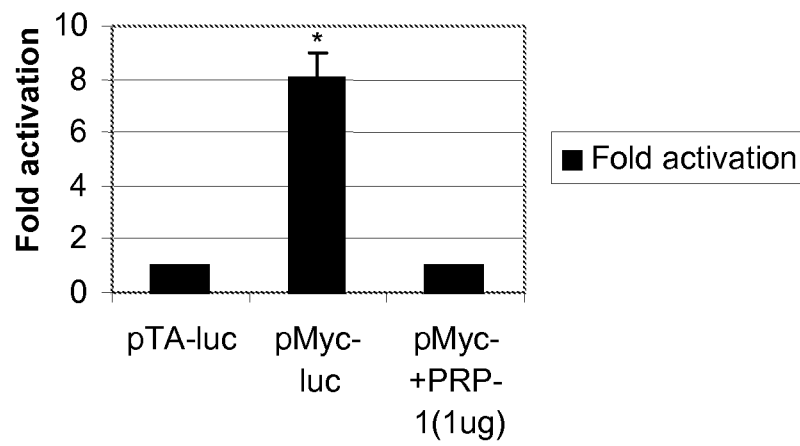


FIG. 2

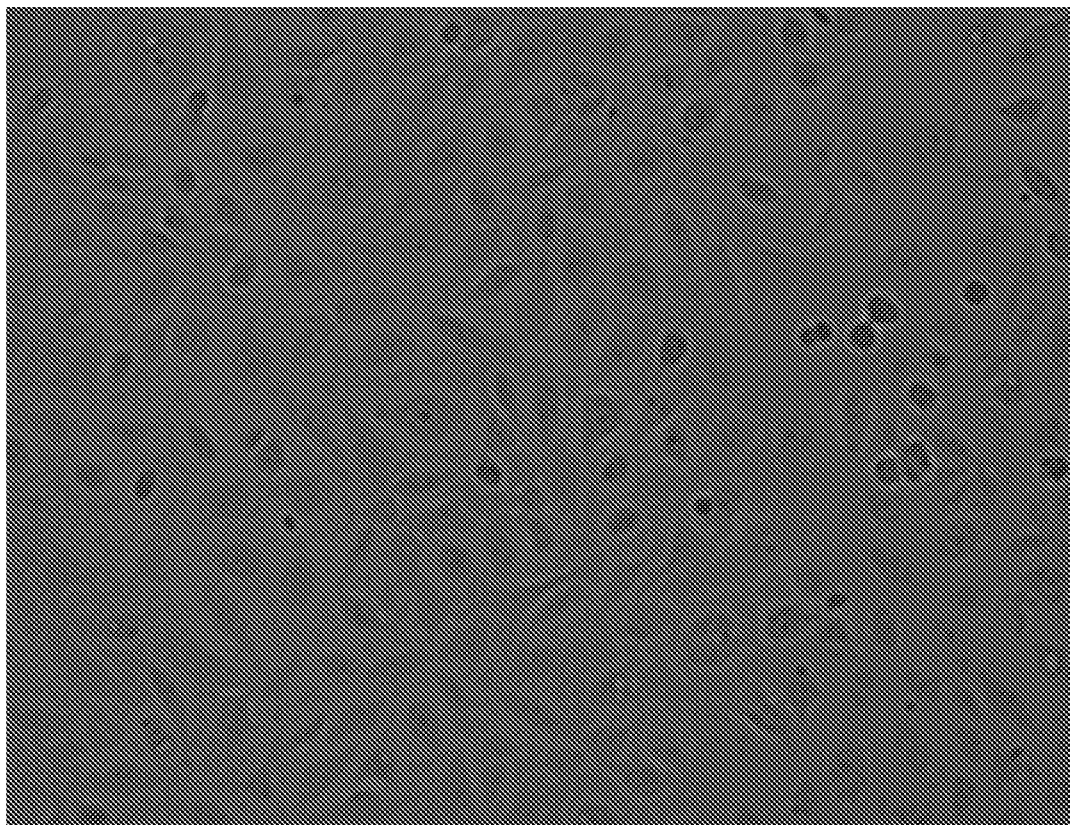


FIG. 3

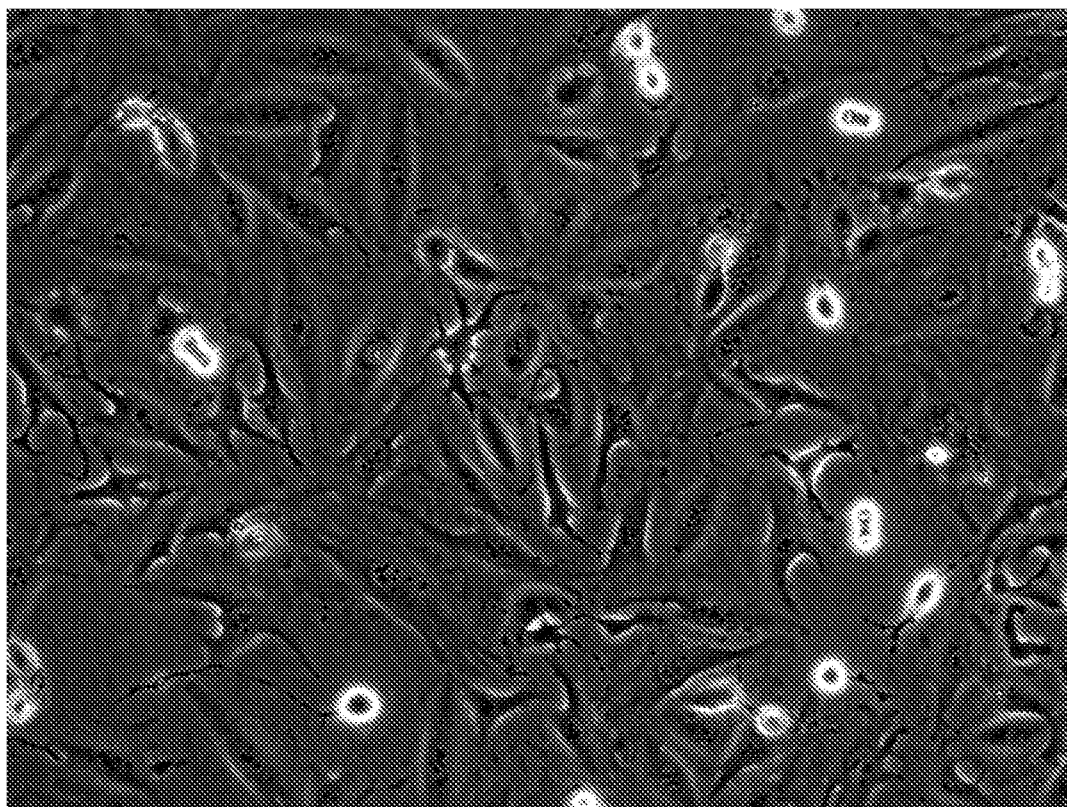


FIG. 4

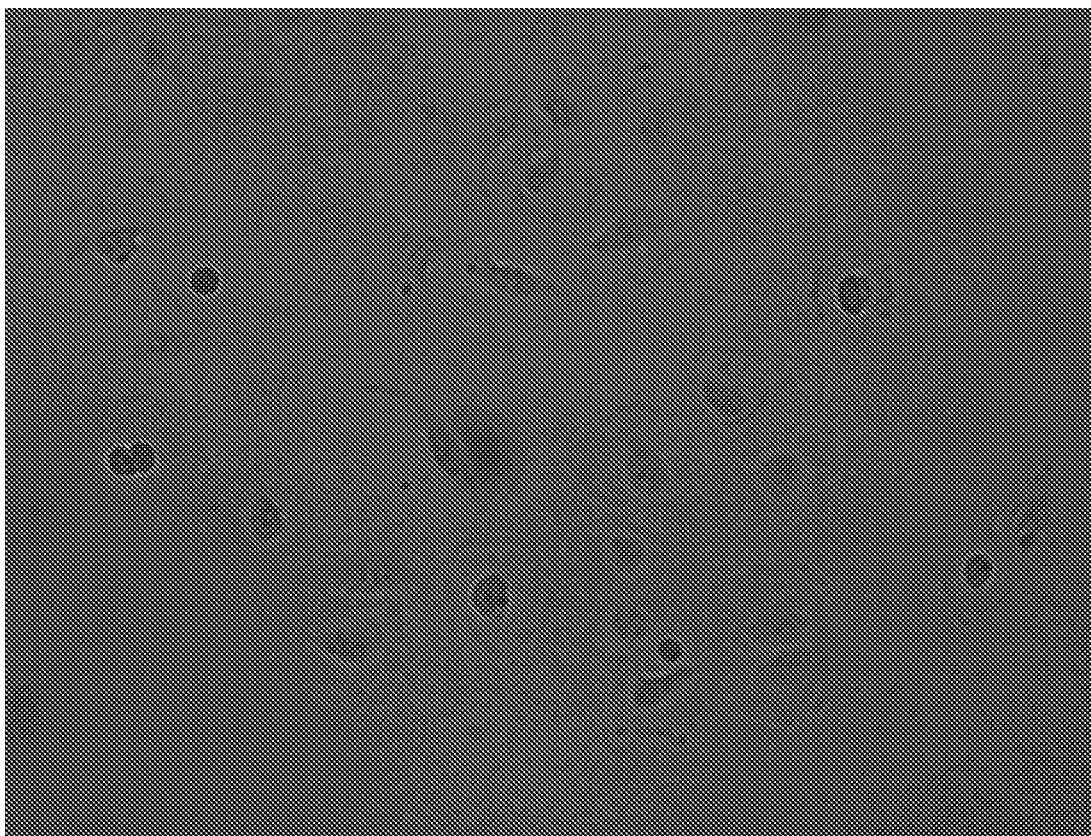


FIG. 5

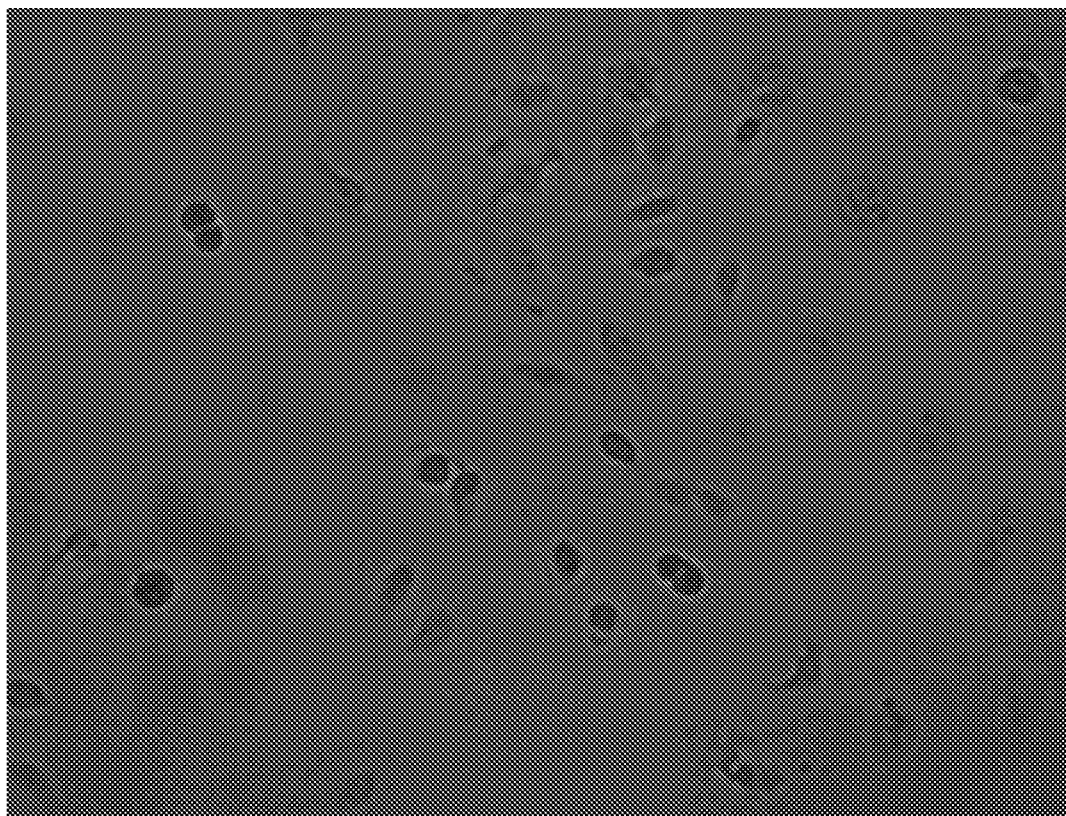


FIG. 6

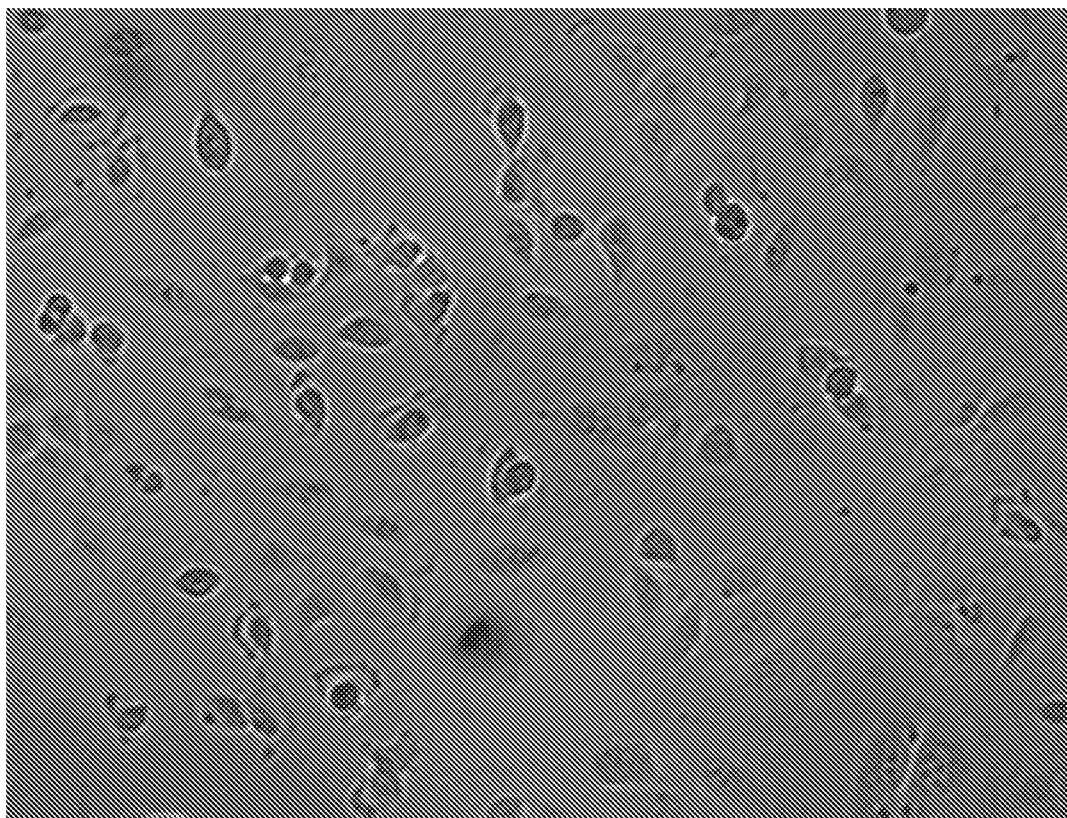


FIG. 7

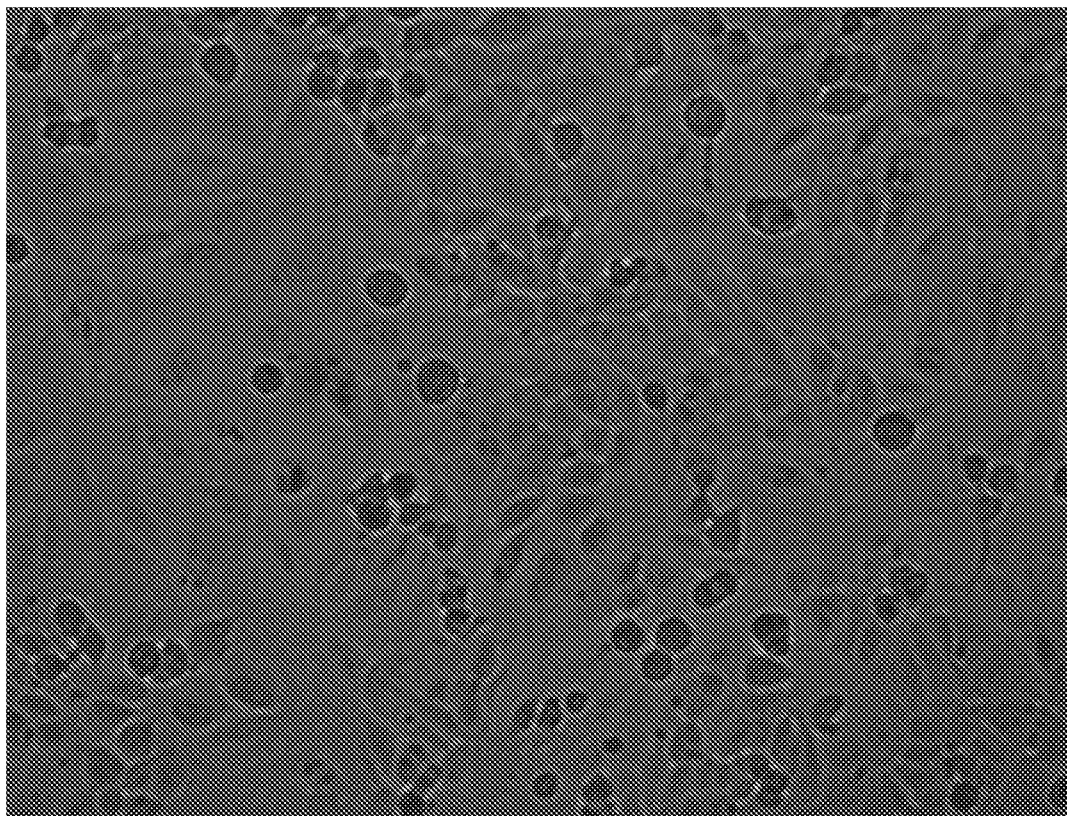
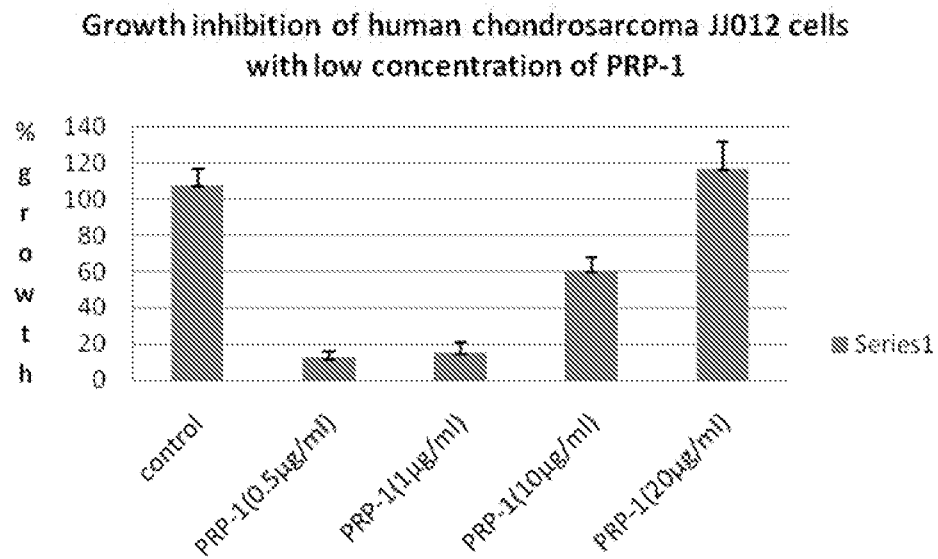


FIG. 8

**FIG. 9**