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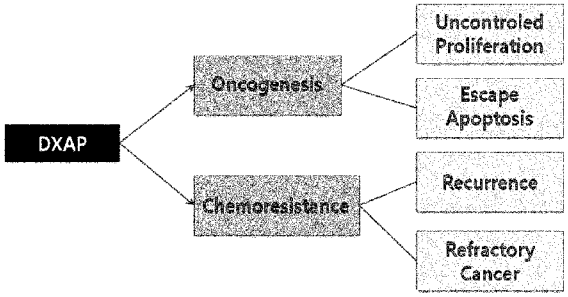
(54) **Title**: ANTICANCER AND CANCER-SENSITIZING COMPOSITION COMPRISING RRP12 INHIBITOR

[Fig. 12]



(57) **Abstract**: Disclosed are an anticancer and cancer-sensitizing composition, comprising as an active ingredient an inhibitor against the expression of an RRP12 (ribosomal RNA processing 12 homolog) gene or an inhibitor against the activity of RRP12, a method for screening an anticancer agent or a cancer sensitizer, and a method for treating cancer and inhibiting anticancer drug resistance, comprising administering the inhibitor to a subject. Showing anticancer and cancer sensitization effects, the composition can be used to treat RRP12-mediated cancer. In addition, the composition inhibits the chemoresistance of cancerous cells to enhance the activity of anticancer agents. Thus, the composition may be applied to the development of effective anticancer agents and anticancer drug aids.

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Description

Title of Invention: ANTICANCER AND CANCER-SENSITIZING COMPOSITION COMPRISING RRP12 INHIBITOR

Technical Field

[1] The present invention relates to an anticancer and cancer-sensitizing composition, comprising as an active ingredient an inhibitor against the expression of an RRP12 (ribosomal RNA processing 12 homolog) gene or an inhibitor against the activity of RRP12, a method for screening an anticancer agent or a cancer sensitizer, and a method for treating cancer and inhibiting anticancer drug resistance, comprising administering the inhibitor to a subject.

[2]

Background Art

[3] Cancer is defined as a malignant tumor and is a disease with one of the highest mortality rates in modern society. Yet, despite the great deal of research that has been made, no effective treatments have been developed. Chemotherapy, a treatment used to treat cancer with an anticancer agent, has achieved results to some degree, but far more advanced research is required because of the various mechanisms of oncogenesis and the resistance against anticancer drugs.

[4] The advances in the diagnosis and treatment of cancer over the last few decades, resulted in improvements in the curative rate and the functional conservation of organs, but the 5-year survival rate still remains as low as 5 ~ 50% in many progressive cancers. These cancers have been characterized by offensive invasion, metastasis to lymph nodes, distal metastasis and the generation of secondary cancer. The survival rate has not significantly changed over the last two decades despite various research and treatments. Recently, many molecular biological approaches are being developed that concern targeted treatment related to the proliferation and metastasis of cancer and apoptosis, and have improved the efficiency at which cancers are cured.

[5] Although a great number of anticancer agents have been developed, there are only a few cancers that can be cured by the means of anticancer agents alone. The reason is that cancerous cells do not respond to anticancer agents, or the cancerous cells show resistance to anticancer agents during or after treatment even if the tumor is effectively reduced in size in the early phase. Hence, effective chemotherapy requires that the resistance of cancerous cells to anticancer agents be overcome.

[6] The RRP12 (ribosomal RNA processing 12 homolog) gene, also known as FLJ20231, KIAA0690 or DKFZp762P1116, is located at position 10q24.1 on human chromosome 10. The protein encoded by the RRP12 gene is known to be involved in

nuclear transport and therefore in the biogenesis of ribosomes. However, the accurate function and mechanism of RRP12 remains unknown. Particularly, the function of RRP12 has not ever been previously reported in regards to cancer therapy.

[7]

Disclosure of Invention

Technical Problem

[8] Leading to the present invention, intensive and thorough research into effective chemotherapy without chemoresistance, resulted in the finding that an inhibitor against the expression of an RRP12 gene or an inhibitor against the activity of RRP12 acts as a cancer sensitizer in addition to exhibiting anticancer activity.

[9]

Solution to Problem

[10] It is an object of the present invention to provide an anticancer composition comprising as an active ingredient an inhibitor against the expression of the RRP12 gene or against the activity of RRP12.

[11] It is another object of the present invention to provide a cancer-sensitizing composition comprising as an active ingredient an inhibitor against the expression of RRP12 gene or the activity of RRP12.

[12] It is a further object of the present invention to provide an anticancer composition comprising the cancer-sensitizing composition and an anticancer agent.

[13] It is still a further object of the present invention to provide an antibody antigen or a method for screening for a cancer sensitizer.

[14] It is still another object of the present invention to provide a method for treating cancer, comprising administering an inhibitor against the expression of RRP12 or the activity of RRP12.

[15] It is yet a further object of the present invention to provide a method for inhibiting anticancer drug resistance, comprising administering an inhibitor against the expression of RRP12 gene or the activity of RRP12.

[16]

Advantageous Effects of Invention

[17] Showing anticancer and cancer sensitization effects, the composition comprising an inhibitor against the expression of RRP12 gene or the activity of RRP12 as an active ingredient in accordance with the present invention can be used to treat RRP12-mediated cancer. In addition, the anticancer and cancer-sensitizing composition inhibits the chemoresistance of cancerous cells to enhance the activity of anticancer agents. Thus, the composition may be applied to the development of effective anticancer agents and anticancer drug aids.

[18]

Brief Description of Drawings

[19] FIG. 1 shows the results of Western blotting with an anti-phosphor (Thr218/Tyr220) antibody in the synovial sarcoma (HSSYII) administered with doxorubicin.

[20] FIGS. 2 to 4 show the expression patterns of an RRP12 gene in normal cells and various tumor cells as measured by RT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serves as a control.

[21] FIG. 5 shows the activity of phosphorylated RRP12 in normal cells (hDF) and synovial sarcoma cells (HSSYII) upon treatment with doxorubicin as measured by Western blotting. β-actin serves as a control.

[22] FIG. 6 shows the activity of phosphorylated RRP12 in doxorubicin-administered osteosarcoma cell lines (MG63, SaOS2, U2OS) as measured by Western blotting. β-actin serves as a control.

[23] FIG. 7 shows the activity of RRP12 in doxorubicin-administered osteosarcoma cells (MG63) as measured by immunohistochemical staining. DAPI staining indicates the location of the nucleus.

[24] FIG. 8 shows the expression patterns of an RRP12 gene in osteosarcoma cells (MG63) transfected with siRNAs of SEQ ID NOS: 5 to 10 as measured by RT-PCR.

[25] FIG. 9 shows RRP12 gene-specific siRNA-mediated inhibition of RRP12 phosphorylation in osteosarcoma cells (MG63) (ne: administered with control siRNA, siDXAP: administered with RRP12 gene-specific siRNA).

[26] FIG. 10 shows the proliferation patterns of normal cells (hDF) and osteosarcoma cells (MG63) upon the RRP12 gene-specific siRNA-mediated inhibition of RRP12 as measured by MTT assay (ne: administered with control siRNA, siDXAP: administered with RRP12 gene-specific siRNA).

[27] FIG. 11 shows the proliferation patterns of osteosarcoma cells (MG63) co-administered with RRP12 gene-specific siRNA and doxorubicin as measured by MTT assay (ne: administered with control siRNA, siDXAP: administered with RRP12 gene-specific siRNA).

[28] FIG. 12 shows the functions of RRP12.

[29] FIG. 13 shows the cell death state of the osteosarcoma cells (MG63) in which the expression of RRP12 was inhibited by RRP12 gene-specific siRNA, as measured by flow cytometric analysis.

[30] FIG. 14 shows the activity of caspase-3 and poly(ADP-ribose)polymerase (PARP) in the osteosarcoma cells (MG63) where the expression of RRP12 was inhibited by RRP12 gene-specific siRNA, as measured by Western blotting (ne: administered with control siRNA, siDXAP: administered with RRP12 gene-specific siRNA).

[31]

Best Mode for Carrying out the Invention

[32] In accordance with an aspect thereof, the present invention provides an anticancer composition comprising as an active ingredient an inhibitor against the expression of the RRP12 gene or the activity of RRP12.

[33] The RRP12 (ribosomal RNA processing 12 homolog) gene is located on human chromosome 10 and may have the mRNA sequence of SEQ ID NO: 1. The protein is known to aid the nuclear transport of ribosomes and thus to influence the biogenesis of ribosomes, but the exact functions and mechanisms still remain unknown. RRP12 may have the amino acid sequence of SEQ ID NO: 2. In the present invention, RRP12 is found to have a relationship with tumor cells and to increase in activity particularly upon treatment with doxorubicin, and is named as the doxorubicin activating protein (DXAP). Hereinafter, DXAP is used interchangeably with RRP12. In addition, the present inventors identified that RRP12 is essential for the survival of tumor cells and the activation activity of RRP12 accounts for the survival pathway of tumor cells against anticancer agents. Accordingly, in accordance with an aspect thereof, the present invention newly reveals the cancer-related properties of RRP12 and provides an anticancer composition or a cancer-sensitizing composition comprising an RRP12 inhibitor as an active ingredient.

[34] Examples of the inhibitor against the expression of the RRP12 gene or the activity of RRP12, used as an active ingredient in the anticancer composition of the present invention, includes RRP12-specific siRNAs, antisense oligonucleotides, aptamers, antibodies and single chain variable fragments, with a preference for siRNAs specific for RRP12.

[35] The term "siRNA," as used herein, refers to a nucleic acid molecule which can mediate RNA interference or gene silencing. Because it is capable of inhibiting the expression of target genes, siRNA is effectively applied to gene knockdown or gene therapy. siRNA was first discovered in plants, insects, drosophila, and parasites and is now used to study mammalian cells.

[36] When used in the present invention, the siRNA molecule may be a double stranded molecule consisting of a sense strand (a sequence corresponding to the RRP12 mRNA (SEQ ID NO: 1) and an antisense strand (a sequence complementary to the RRP12 mRNA) or a single stranded molecule consisting of a self-complementary sense strand or antisense strand.

[37] The siRNA molecule according to the present invention is not limited to the complete matches between an antisense and sense strand or within a self-complementary strand, but may comprise unpaired regions due to mismatches

(non-complementary between corresponding nucleotides) or loop/bulge (no corresponding nucleotides on one strand).

[38] The terminal structure of siRNA is not limited and can be blunt or cohesive so long as it can inhibit the expression of RRP12 through the RNA interference (RNAi) pathway. The cohesive terminal structure may be 3'-overhang or 5'-overhang.

[39] The siRNA molecule according to the present invention may have a total length of from 10 to 50 nt, preferably from 15 to 30 nt, and more preferably from 18 to 25 nt. In one embodiment of the present invention 19-nt long siRNA was synthesized using siDESIGN SOFTWARE and used to examine the expression level of the RRP12 gene. The siRNA of the present invention may be selected from the group consisting of RNAs represented by SEQ ID NOS: 5 to 10.

[40] As used herein, the term "antisense oligonucleotide" refers to a DNA or RNA molecule or a derivative thereof which has a nucleotide sequence complementary to a sequence of specific mRNA and binds to its complementary mRNA sequence to inhibit the translation of the mRNA into a protein. That is, the term "antisense oligonucleotide sequence" means a DNA or RNA sequence that is complementary to and that can bind to RRP12 mRNA, thus functioning to inhibit the biologically essential activity of RRP12 mRNA, such as translation, translocation into cytoplasm, maturation or other activities. The antisense oligonucleotide may be 6 to 100 nucleotides long, preferably 8 to 60 nucleotides long and more preferably 10 to 40 nucleotides long.

[41] For functional enhancement purposes, the antisense oligonucleotide may be modified at one or more bases, the sugar moiety, or the backbone. Specific examples of some preferred modified oligonucleotides envisioned for this invention include those containing phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. In addition, the antisense oligonucleotide may comprise at least one modified sugar moiety. Further, the antisense oligonucleotide may comprise a modified base. Exemplary among the modified bases are hypoxanthine, 6-methyladenine, 5-methylpyrimidine (e.g., 5-methylcytosine), 5-hydroxymethylcytosine (HMC), glycosyl HMC, gentiobiosyl HMC, 2-aminoadenine, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine and 2,6-diaminopurine. The antisense oligonucleotide of the present invention may also involve chemically linking the oligonucleotide to one or more lipophilic moieties which enhance the activity and cellular uptake of the antisense oligonucleotide. Examples of such lipophilic moieties include a cholesterol moiety, a cholesteryl moiety, cholic acid, a thioether, a thiocholesterol, an aliphatic chain, a phospholipid, a polyamine chain, a polyethylene glycol chain, adamantine acetic acid, a palmityl moiety, an octadecylamine moiety, and a hexylamino-

carbonyl-oxycholesterol moiety, but are not limited thereto. Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides are well known in the art (U.S. Patent Nos. 5,138,045, 5,218,105 and 5,459,255). The stability of the modified oligonucleotide to nucleases may be improved as well as its binding affinity for target mRNA.

[42] The antisense oligonucleotide may be synthesized in vitro using a conventional method and also in vivo. One approach to the in vitro synthesis of the antisense oligonucleotide is to use RNA polymerase I. For in vivo biosynthesis, a vector comprising a multiple cloning site the origin of which is situated in the reverse direction is used to transcribe the antisense RNA. It is preferred that the antisense RNA encompass a stop codon therein lest it could be translated into a protein.

[43] With reference to the nucleotide sequence of RRP12 gene, the antisense oligonucleotide useful in the present invention may be readily designed using a well-known technique.

[44] As used herein, the term "aptamer" refers to a nucleic acid molecule having affinity for a specific molecule. The aptamer may bind to an RRP12 polynucleotide or protein to inhibit the activity of the polynucleotide or protein. The aptamer of the present invention may be RNA, DNA, a modified nucleic acid or a combination thereof, and may have a straight or circular structure. No particular limitations are imparted to the length of the aptamer of the present invention, but it may typically be 15~200 nucleotides long, or have a length not more than 100 nucleotides, or preferably 80 nucleotides, even more preferably 60 nucleotides, and far more preferably 45 nucleotides. The aptamer of the present invention may be, for example, 18, 20 or 25 nucleotides long. A smaller number of nucleotides is more advantageous in terms of chemical synthesis and mass production as well as cost. In addition, the aptamer with fewer nucleotides can be more easily chemically modified and is more stable and less toxic in vivo.

[45] The aptamer of the present invention may be engineered by utilizing SELEX or an improved version thereof. SELEX is a technique by which oligonucleotides specifically binding to a target ligand are selected from a pool of oligonucleotides having 10~14 different nucleotide sequences, each containing a stretch of 40 random nucleotides that serve as primers. The pool of oligonucleotide is exposed to the target substance, followed by recovering the oligonucleotides bound to the target substance by means of filtering. The recovered oligonucleotides are amplified by RT-PCR and used as templates in subsequent rounds of selection. This procedure is repeated 10 times to evolve aptamers specifically binding to the target substance. In the context of SELEX, an aptamer exhibiting a stronger binding force for the target substance is concentrated and selected by increasing the number of rounds or using a competing

substance. Hence, aptamers with different binding forces, aptamers with different binding modes, and aptamers with the same binding force and binding mode but different base sequences can be obtained in some cases by adjusting the number of rounds of SELEX, and/or changing the competitive condition. The SELEX method comprises a process of amplification by PCR; it is possible to perform SELEX of more diverse methods by causing a mutation by using manganese ions and the like in the process.

[46] In addition to the conventional SELEX method, a Cell-SELEX technique is applied to a complex target, that is, to the living cells and tissues to obtain aptamers. The Cell-SELEX technique enjoys the advantage of developing aptamers against disease-related cells even though surface marker targets are unknown. Further, the Cell-SELEX technology is advantageous compared to conventional SELEX because it has more functional access to target proteins under physiological conditions because they may not exhibit their inherent properties in a dissociated state.

[47] An aptamer binds to the target substance in a wide variety of binding modes, such as ionic bonds based on the negative charge of the phosphate group, hydrophobic bonds and hydrogen bonds based on a ribose, and hydrogen bonds and stacking bonds based on nucleic acid bases. Particularly, ionic bonds based on the negative charge of the phosphate group, the number of which is the same as the number of constituent nucleotides, are strong, and bonds to lysine and arginine are present on the surface of the positive charge of the protein. For this reason, nucleic acid bases not involved in the direct binding to the target substance can be substituted for. In particular, because the stem structure region has already formed base pairs and faces the inside of the double helical structure, nucleic acid bases are unlikely to bind directly to the target substance. Therefore, even when a base pair is replaced with another base pair, the activity of the aptamer does not decrease in many cases. In structures wherein no base pairs are formed, such as loop structures, given that the nucleic acid base is not involved in the direct binding to the target molecule, base substitution is possible. For instance, with regard to modifications of the 2'-position of ribose, the hydroxyl group may be substituted with any atom or group. Examples of such a substituent atom or group include a hydrogen atom, a fluorine atom, or ?O-alkyl group (e.g., -O-CH3), -O-acyl group (e.g., -O-CHO), and an amino group (e.g., -NH2). Hence, an aptamer, unless the functional group involved in the direct binding to the target molecule is substituted or deleted, often retains the activity thereof.

[48] Moreover, aptamers are easily alterable because they permit chemical synthesis. For aptamers, by predicting the secondary structure using the MFOLD program, or by predicting the steric structure by X-ray analysis or NMR analysis, it is possible to predict to some extent which nucleotide can be substituted or deleted, and where to

insert a new nucleotide. An aptamer with the predicted new sequence can easily be chemically synthesized, and it can be determined whether or not the aptamer has retained its activity using existing assay systems.

[49] The term "antibody," as used herein, refers to a substance which is produced by the immune system in response to the stimulus of an antigen and binds to the antigen to form an antigen-antibody complex. In the present invention, an antibody specifically binding to RRP12 may be used to inhibit the activity of RRP12. Particularly, the antibody may be inhibitive of the phosphorylation of RRP12 by specifically binding to the conserved threonine-glutamic acid-tyrosine (Thr-Glu-Tyr, TEY) motif, which is phosphorylated to activate RRP12.

[50] The RRP12-specific antibodies useful in the present invention may be polyclonal or monoclonal, the latter being preferred. The RRP12-specific antibodies may be prepared using a method well known in the art, such as a fusion method (Kohler and Milstein, European Journal of Immunology, 6:511-519(1976)), a recombinant DNA method (U.S. Patent No. 4,816,567) or a phage antibody library method (Clackson et al, Nature, 352:624-628(1991); and Marks et al, J. Mol. Biol., 222:58, 1-597(1991)). For antibody production, general processes known in the art may be employed. For example, an immortalized cell line is fused with antibody-producing lymphocyte to prepare a hybridoma that produces monoclonal antibodies. Techniques necessary for this procedure are well known to those skilled in the art and can be readily conducted. Polyclonal antibodies may be prepared by injecting an RRP12 antigen into an appropriate animal, taking antisera from the animal and isolating the antibody from the antisera by affinity technology.

[51] The antibody of the present invention may comprise a single chain variable fragment (scFv). The single chain variable fragment may consist of a light chain variable region (VL)-linker-heavy chain variable region (VH). The term "linker" refers to an amino acid sequence of predetermined length that functions to link a light chain variable region and a heavy chain variable region.

[52] The term "anticancer," as used herein, means pertaining to inhibiting or preventing the growth of cancer. Inhibiting or preventing the growth of cancer is a concept that encompasses reducing cancer growth and metastasis, compared to an untreated case. The metastasis of cancer means the spread of tumor cells to a distal part of the body.

[53] As used herein, the term "cancer" refers to a diverse group of diseases, characterized by the hyperplasia of cells that under normal conditions would be dead, but due to problems associated with the function of self-regulation, leading to invasion into nearby tissues and organs to form mass and the destruction and transformation of the existing tissues, and has the same meaning as a malignant tumor.

[54] The cancer treated in the present invention may be selected from the group consisting

of osteosarcoma, giant cell tumor, chondroma, synovial sarcoma, bladder cancer, stomach cancer, breast cancer, colorectal cancer, uterine cervical cancer, prostate cancer and epidermoid carcinoma and preferably from among osteosarcoma and synovial sarcoma. More preferable is osteosarcoma. In addition, the cancer may encompass those that have occurred because of metastasis.

[55] The anticancer composition of the present invention may comprise a pharmaceutically acceptable carrier suitable to the mode of administration.

[56] In detail, the anticancer composition of the present invention may be formulated with a pharmaceutically acceptable carrier, excipient or additive. Examples of the pharmaceutically acceptable carrier include saline, sterile water, Ringer's solution, buffered saline, dextrose solution, maltodextrin solution, glycerol, ethanol, liposomes, and a combination thereof. Optionally, conventional additives, such as antioxidants, buffers and the like, may be added to the composition. For the preparation of dosage forms including injections, such as aqueous solutions, suspensions and emulsions, pills, capsules, granules and tablets, the active ingredients may be admixed with a diluent, a dispersant, a surfactant, a binder and/or a lubricant. Further, the carrier may be conjugated with antibodies or ligands specific for target organs or tissues so that the active ingredient is directed toward the target organs or tissues. As long as it is typically used in the art, any carriers, excipients or additives may be added to the composition, and thus they are not limited to the examples enumerated above.

[57] Because the RRP2 inhibitor of the present invention can reduce the resistance of cancerous cells to anticancer agents as well as kill cancerous cells, the anticancer composition of the present invention exhibits excellent anticancer effects compared to conventional anticancer agents.

[58]

[59] In accordance with another aspect thereof, the present invention provides a cancer-sensitizing composition comprising an inhibitor against the expression of RRP12 gene or the activity of RRP12.

[60] The term "cancer sensitization," as used herein, has the same meaning as chemosensitization and refers to the reinforcement or enhancement of toxicity of an anticancer agent against cancer, as compared to the anticancer agent itself, by reducing the chemoresistance of cancerous cells. In the present invention, the cancer-sensitizing composition comprising an RRP12 inhibitor sensitizes cancerous cells to an anticancer agent to reduce the chemoresistance of the cells and to improve the therapeutic effect of the anticancer agent.

[61] Chemoresistance refers to a condition in which when administered, an anticancer agent cannot kill cancerous cells or kills only a small number of cancer cells. The term "resistance to an anticancer agent" or "anticancer resistance" means the condition in

which when administered to a cancer patient, an anticancer agent has no therapeutic effect on cancer from the beginning or shows a therapeutic effect in the early phase, but gradually loses its pharmaceutical activity as the number of times of administration increases. Generally, an anticancer agent decreases in pharmaceutical effect with an increase in the number of administrations thereof. This chemoresistance is attributed to the appearance of cancerous cells that have turned resistant to an anticancer agent over the course of continuous exposure to the anticancer agent. In the present invention, the cancer-sensitizing composition is characterized by an ability to inhibit resistance against anticancer agents.

[62] In one embodiment of the present invention, the osteosarcoma cell line (MG63), known to have resistance to anticancer agents, was observed to grow very poorly when doxorubicin was added in combination with siRNA specific for an RRP12 gene, demonstrating that the RRP12 gene-specific siRNA inhibits the resistance of the osteosarcoma cell line (MG63) to doxorubicin to increase the anticancer effect of doxorubicin (FIG. 11).

[63] In the present invention, the cancer on which the cancer-sensitizing effect takes place may be selected from the group consisting of osteosarcoma, giant cell tumor, chondroma, synovial sarcoma, bladder cancer, stomach cancer, breast cancer, colorectal cancer, uterine cervical cancer, prostate cancer and epidermoid carcinoma and preferably from among synovial sarcoma and osteosarcoma, the latter being more preferred. In addition, the cancer may encompass those that have arisen because of metastasis.

[64] Examples of the inhibitor against the expression of RRP12 gene or the activity of RRP12, used as an active ingredient in the cancer-sensitizing composition of the present invention, includes RRP12-specific siRNAs, antisense oligonucleotides, aptamers, antibodies and single chain variable fragments, with a preference for siRNAs specific for RRP12. The siRNA of the present invention may be selected from the group consisting of RNAs represented by SEQ ID NOS: 5 to 10. The siRNAs, antisense oligonucleotides, aptamers, antibodies and single chain variable fragments are as described above.

[65]

[66] In accordance with a further aspect thereof, the present invention provides an anticancer composition comprising the cancer-sensitizing composition and an anticancer agent. Functioning to reduce the resistance of cancerous cells to anticancer agents, the RRP12 inhibitor of the present invention may be used as an aid to enhance the pharmaceutical effect of anticancer agents.

[67] As used herein, the term “anticancer agent” refers to any drugs that are used to kill cancerous cells. Most anticancer agents block the replication, transcription and

translation of DNA in cancerous cells. The anticancer agent used in the composition of the present invention is not limited to any particular kind. The standard criteria that are taken into consideration when selecting anticancer agents, such as the type of cancerous cells, the uptake rate of anticancer agents (period of time of treatment, administration route, etc.), the location of tumors, the size of tumors, etc., are applied to the anticancer agent used in the present invention. Exemplary among the anticancer agents useful in the present invention are DNA alkylating agents such as mechlorethamine, chlorambucil, phenylalanine, mustard, cyclophosphamide, ifosfamide, carmustine (BCNU), lomustine (CCNU), streptozotocin, busulfan, thiotepa, cisplatin and carboplatin; anti-cancer antibiotics, such as dactinomycin, doxorubicin, daunorubicin, idarubicin, mitoxantrone, plicamycin, mitomycin and C Bleomycin; and plant alkaloids, such as vincristine, vinblastine, paclitaxel, docetaxel, etoposide, teniposide, topotecan and iridotecan, with a preference for doxorubicin. However, the anticancer agent used in the present invention is not limited thereto.

[68]

[69] In accordance with yet a further aspect thereof, the present invention provides a method for screening an anticancer agent or a cancer sensitizer. The method comprises (a) analyzing the expression level of RRP12 gene or the activity of RRP12 after treatment with a candidate; and (b) determining the candidate as an anticancer agent or a cancer sensitizer if the expression level of RRP12 gene or the activity of RRP12 is inhibited after treatment with the candidate, as compared to that before treatment with the candidate. The "inhibitor against the expression of the RRP12 gene" or "inhibitor against the activity of RRP12" can be obtained by this screening method.

[70] In step (a) of the screening method, a candidate is analyzed for its ability to affect the expression of RRP12 gene or the ability of RRP12. This analysis may be performed within the cells or in vitro by a technique, illustrative, non-limiting examples of which include RT-PCR (Reverse Transcription Polymerase Chain Reaction), Northern blotting, cDNA microarray hybridization, in situ hybridixation, radioimmunoassay, immunoprecipitation, ELISA (enzyme-linked immunosorbent assay), and Western blotting.

[71] As used therein, the term "candidate" refers to an unknown substance that is screened to examine whether it has influence on the expression of the RRP12 gene or the activity of RRP12. The candidate may include, but is not limited to, chemicals, peptides and natural extracts. The candidate to be analyzed by the screening method may also be a single compound or a mixture of compounds and may be obtained from a library of synthetic or natural compounds.

[72] In step (b) of the screening method, the candidate is determined to be an anticancer agent or a cancer sensitizer if the expression level of RRP12 or the activity of RRP12

is inhibited after treatment with the candidate, as compared to that before the treatment. When observed to down-regulate the expression of the RRP12 gene or the activity of RRP12, a candidate can be judged as an anticancer agent or cancer sensitizer.

[73] The screening method of the present invention may be carried out in various manners, particularly, in a high-throughput manner using various binding assays known in the art.

[74] In the screening method of the present invention, a candidate or an RRP12 protein may be coupled with a detectable label. Among the detectable labels are chemical labels (e.g., biotin), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase, peroxidase, luciferase, β-galactosidase and β-glucosidase), radiolabels (e.g, C14, I125, P32 and S35), fluorescent labels (e.g., coumarin, fluorescein, FITC (fluoresein Isothiocyanate), rhodamine 6G, rhodamine B, TAMRA (6-carboxy-tetramethyl-rhodamine), Cy-3, Cy-5, Texas Red, Alexa Fluor, DAPI(4,6-diamidino-2-phenylindole), HEX, TET, Dabsyl and FAM), luminescent labels, chemiluminescent labels, FRET (fluorescence resonance energy transfer) labels and metal labels (e.g., gold, silver, etc.).

[75] When a detectable label-coupled RRP12 or candidate is used, the signal from the label may be detected to determine the ability of the candidate to interact with RRP12. For example, when alkaline phosphatase is used as a label, a signal may be detected with a colorimetric substrate such as bromochloroindolyl phosphate (BCIP), nitroblue tetrazolium (NBT), naphthol-AS-B1-phosphate and ECF (enhanced chemifluorescence). The use of horseradish peroxidase as a label requires a substrate, such as chloronaphthol, aminoethylcarbazole, diaminobenzidine, D-luciferin, lucigenin (bis-N-methylacridium nitrate) resorufin benzyl ether, luminal, amplex red reagent (10-acetyl-3,7-dihydroxyphenoxazine), HYR (p-phenylenediamine-HCl and pyrocatechol), TMB (tetramethylbenzidine), ABTS (2,2'-Azine-di[3-ethylbenzthiazoline sulfonate]), o-phenylenediamin (OPD) and naphthol/pyronin, for signal detection.

[76] Alternatively, the ability of a candidate to interact with RRP12 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a candidate with RRP12 without the labeling of either the candidate or RRP12. As used herein, a "microphysiometer" is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in the acidification rate can be used as an indicator of the interaction between a candidate and RRP12 (McConnell et al., Science 257:19061912(1992)).

[77] The ability of a candidate to bind to RRP12 can be determined using real-time Biomolecular Interaction Analysis (BIA) (Sjolander et al., Anal. Chem., 63:2338-2345

(1991), and Szabo et al., Curr. Opin. Struct. Biol., 5:699-705 (1995)). BIA detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore™). Alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)) result in a detectable signal which can be used as an indication of the real-time reactions between biological molecules.

[78]

[79] In accordance with still another aspect thereof, the present invention provides a method for treating cancer, comprising administering an inhibitor against the expression of the RRP12 gene or the activity of RRP12 to a subject in need thereof.

[80] As used herein, the term "subject" refers to any animal including a human that is suffering from cancer or is apt to suffer from cancer. By administering the inhibitor against the expression of RRP12 gene or the activity of RRP12 to a subject, cancer can be effectively treated. The inhibitor against the expression of RRP12 gene or the activity of RRP21 is as described above.

[81] The term "administration," as used herein, refers to the introduction of the inhibitor of the present invention into a subject in an appropriate manner. As long as it leads the inhibitor to a target tissue, any administration route, whether oral or parenteral, may be used.

[82] The inhibitor may be administered in a suitable amount to subjects through a suitable route according to purpose or necessity. The inhibitor may be administered, for example, orally, parenterally, subcutaneously, intraperitoneally, intrapulmonarily, or intranasally. For local immunosuppressive therapy, the composition may, if desired, be administered using a suitable method, including intralesional administration. Parenteral injections include intramuscular, intravenous, intraarterial, intraperitoneal and subcutaneous routes. The therapeutically effective amount and the number of administration of the inhibitor may vary depending on various factors well known in the medical art, including the kind and degree of the response to be achieved, the patient's age, body weight, and state of health, etc.

[83]

[84] In accordance with yet another aspect thereof, the present invention provides a method for inhibiting anticancer drug resistance, comprising administering an inhibitor against the expression of the RRP12 gene or the activity of RRP12 to a subject in need thereof.

[85] As used herein, the term "subject" refers to any animal including a human that is suffering from cancer or is apt to suffer from cancer. By administering the inhibitor against the expression of the RRP12 gene or the activity of RRP12 to a subject, anticancer drug resistance can be effectively inhibited. The inhibitor against the expression of RRP12 gene or the activity of RRP21 is as described above.

[86]

[87] In accordance with yet a further aspect thereof, the present invention provides use of an inhibitor against the expression of RRP12 gene or the activity of RRP12 in preparing an anticancer agent. The inhibitor against the expression of RRP12 gene or the activity of RRP21 is as described above and may be used to prepare an anticancer agent.

[88]

[89] In accordance with yet still another aspect thereof, the present invention provides for the use of an inhibitor against the expression of the RRP12 gene or the activity of RRP12 in preparing a cancer sensitizer. The inhibitor against the expression of the RRP12 gene or the activity of RRP21 is as described above and may be used for preparing a cancer sensitizer.

[90]

Mode for the Invention

[91] A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as limiting the present invention.

[92]

[93] **Preparation of Cells**

[94] The synovial sarcoma cell line HSSYII (obtained from the Seoul National University Hospital, Korea) and human-derived fibroblast cell line (hDF, provided from the Samsung Medical Hospital Transplant Center, Korea), and osteosarcoma cell lines (MG63, SaOS2, U2OS) were maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum, 50 U/ml penicillin, and 50 μg/ml streptomycin.

[95]

[96] **EXAMPLE 1: Isolation of RRP1 Protein**

[97] Synovial sarcoma (HSSYII) treated with doxorubicin was subjected to Western blotting using a phosphor-antibody (Thr218/Tyr220). In this regard, the synovial sarcoma cells were lysed and 30 μg of the cell lysate was separated by 10% SDS-PAGE (polyacrylamide gel electrophoresis) and transferred to a polyvinylidene di-fluoride (PVDF) membrane (Bio-rad) which was then blocked at room temperature for 1 hour with a buffer (TBST containing 5% bovine serum albumin). Thereafter, the membrane was incubated for an additional two hours in a solution containing the phosphor ((Thr218/Tyr220)-antibody (Cell signaling, USA) and a primary antibody against β-actin (Santa Cruz Biotechnology) (rabbit polyclonal antibody). After being washed, the membrane was incubated for 1 hour with an HRP (horseradish peroxidase,

Santa Cruz Biotechnology)-conjugated secondary antibody (1:5,000) in TBST (Tris-buffered Saline with tween-20). The membrane was washed again, followed by color development with ECL (enhanced chemiluminescence) reagent (Intron Biotechnology). The results are shown in FIG. 1. As seen in FIG. 1, constant activity was detected at 140 kD. All the proteins at 140 kD were obtained by excision from the gel and subjected to mass analysis (Gel MS) to discover 13 candidate proteins (Table 1). Among them, one protein was found to have the phosphor (Thr218/Tyr220)-antibody-detected amino acid sequence, that is threonine-glutamic acid-tyrosine (Thr-Glu-Tyr, TEY). The protein was identified as RRP12, also called KIAA0690, having the amino acid sequence of SEQ ID NO: 2, which was phosphorylated to show activity, but with unknown functions.

[98]

[99] Table 1

[Table 1]

gi|194376150	unnamed protein product [Homo sapiens]
gi|189054178	unnamed protein product [Homo sapiens]
gi|221040426	unnamed protein product [Homo sapiens]
gi|119573109	coatomer protein complex, subunit alpha, isoform CRA_b [Homo sapiens]
gi|194380132	unnamed protein product [Homo sapiens]
gi|435476	cytokeratin 9 [Homo sapiens]
gi|31657129	phosphoribosylformylglycinamidine synthase [Homo sapiens]
gi|4758012	clathrin heavy chain 1 [Homo sapiens]
gi|3327194	KIAA0690 protein [Homo sapiens]
gi|307383	RNA helicase A [Homo sapiens]
gi|30582355	dynactin 1 (p150, glued homolog, Drosophila) [Homo sapiens]
gi|203800096	Non-SMC condensin I complex, subunit D2 [Homo sapiens]
gi|3420277	glypican 4 [Homo sapiens]

[100]

[101] **EXAMPLE 2: Detection of Expression of RRP12 in Various Tumor Cells**

[102] To examine whether the gene encoding the RRP12 protein isolated in Example 1 is expressed in other tumor cells, RT-PCR was performed. In this context, RNA was isolated from normal cells (hDF, HNSC) and various cancer cells including epidermoid carcinoma (A431), osteosarcoma (SaOs2, U2OS, MG63), giant cell tumor (giant tumor), synovial sarcoma (HSSYII), bladder cancer (T24), stomach cancer (MKN1), breast cancer (MCF7, sKBR3), colorectal cancer (HCT116), uterine cervical cancer (HeLa), prostate cancer (PC3) and chondroma cells, using an easy-blue™ total RNA extraction Kit (Intron biotechbology) according to the manufacturer's instruction.

cDNA was synthesized using a cDNA synthesis kit (superscript III reverse transcriptase, invitrogen) according to the manufacturer's manual. PCR was performed using the RRP12 primers F-GACGCCCATGGAAGAAGAGGC (SEQ ID NO: 3), R-GCAGCGAAGTACTCAGTCTCC (SEQ ID NO: 4), with the synthesized cDNA serving as a template.

[103] As a result, RRP12 was found to be expressed at a very low level in the normal cells, but at a high level in the tumor cells (FIGS. 2 to 4). These data indicate that the RRP12 gene is overexpressed in various tumor cells and is associated with the survival or proliferation of tumor cells.

[104]

[105] **EXAMPLE 3: Activity of RRP12 in Tumor Cells upon Administration of Anticancer Agent**

[106] 3-1. Measurement of RRP12 Activity in Synovial Sarcoma (HSSYII)

[107] RRP12 activity in normal cells (hDF) and synovial sarcoma cells (HSSYII) administered with or without doxorubicin was measured by Western blotting. For this, the normal cells and the synovial sarcoma cells were lysed and 30 μg of each of the cell lysates was separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-rad) which was then blocked at room temperature for 1 hour with a buffer (TBST containing 5% bovine serum albumin). Thereafter, the membrane was incubated for an additional two hours in a blocking solution containing the phosphor (Thr218/Tyr220)-antibody (Cell signaling, USA), an anti-RRP12 antibody (Novus, USA) and a primary antibody against β-actin (Santa Cruz Biotechnology) (rabbit polyclonal antibody). After being washed, the membrane was incubated for 1 hour with an HRP (horseradish peroxidase, Santa Cruz Biotechnology)-conjugated secondary antibody (1:5,000) in TBST (Tris-buffered Saline with tween-20). The membrane was washed again, followed by color development with ECL (enhanced chemiluminescence) reagent (Intron Biotechnology). The results are shown in FIG. 5. as can be seen in FIG. 5, when phosphorylated RRP12 activity was measured in normal cells and synovial sarcoma cells treated with or without 50 nM doxorubicin, the phosphorylated activity of RRP12 was detected only in doxorubicin-administered synovial sarcoma cells.

[108]

[109] 3-2. Measurement of RRP12 Activity in Osteosarcoma

[110] The activity of phosphorylated RRP12 was measured in three different osteosarcoma cell lines (MG63, SaOS2, U2OS) treated with or without 50 nM doxorubicin. For this, the three osteosarcoma cell lines were incubated for 24 hours with 50 nM doxorubicin and harvested. Proteins from the cells were subjected to Western blotting in a similar manner to that of Example 3-1. The activity of phosphorylated RRP12 was observed in

the doxorubicin-administered cells whereas the cells treated without doxorubicin showed no RRP12 activity (FIG. 6).

[111] Immunohistochemical staining was performed on the osteosarcoma cell line (MG63) after incubation with 50 nM doxorubicin for 24 hours. The osteosarcoma cell line was fixed with 5% paraformaldehyde for 10 minutes, permeabilized with a buffer containing 0.1% Triton X-100, and treated with the primary antibody p-RRP12 (Cell signaling, USA), anti-RRP12 antibody (Novus, USA) and then with a secondary antibody (Alexa Fluor 488- or Alexa Fluor 546-conjugated anti-rabbit, mouse, goat or chicken antibody). Cell images were taken with a fluorescence microscope or a confocal microscope. DAPI staining (blue fluorescence) indicated the location of the nucleus while phosphorylated RRP12 was stained green. As can be seen in FIG. 7, RRP12 was detected around the nucleus in the absence of doxorubicin, but when treated with doxorubicin, the RRP12 was activated and localized into the nucleus.

[112] Taken together, these data indicate that there is a mechanism by which the anticancer agent doxorubicin induces the phosphorylation of RRP12 and this allows for the expectation that the doxorubicin-induced RRP12 phosphorylation would be associated with the specific responses of tumor cells to anticancer agents, that is, their apoptosis as caused by or their survival to anticancer agents.

[113]

[114] **EXAMPLE 4: Inhibition of RRP12 Expression Using siRNA**

[115] 4-1. Construction of siRNA and Inhibition of RRP12 Gene by siRNA

[116] To make an inhibitor the expression of RRP12 gene, siRNAs specific for RRP12 were constructed using the siDESIGN SOFTWARE. As a result, six siRNA candidates having the nucleotide sequence of SEQ ID NOS: 5 to 10 were synthesized (Table 2). The six siRNA candidates were transfected into the osteosarcoma cell lines (MG63) three days after which RT-PCR was performed in a similar manner to that of Example 2 to analyze mRNA levels. All of the cells transfected with the six siRNA candidates were observed to decrease in RRP12 expression level (FIG. 8).

[117]

[118] Table 2

[Table 2]

siDXAP #1	GCAACAAGATGGAGGAAGA	SEQ ID NO : 5
siDXAP #2	GTGCTTATTAAGAAGTTCT	SEQ ID NO : 6
siDXAP #3	CAGTGAATTCATGTTTGAA	SEQ ID NO : 7
siDXAP #4	GGAGAAAGCCCACATCAAC	SEQ ID NO : 8
siDXAP #5	CCTTTTCGAGTTTAAAGGT	SEQ ID NO : 9
siDXAP #6	TTTGGAAACTCCAAATGGA	SEQ ID NO : 10

[119]

[120] These results imply that RRP12 gene-siRNAs effectively inhibit the expression of RRP12 gene. Particularly, siRNAs having the nucleotide sequences of SEQ ID NOS: 5, 7 and 10 were used in subsequent experiments because of their significant effects.

[121]

[122] 4-2. Inhibition of Phosphorylated RRP12 by siRNA

[123] RRP12 gene-specific siRNAs were analyzed for the ability to inhibit the expression of phosphorylated RRP12. Western blotting was performed to analyze the doxorubicin-induced phosphorylation of RRP12 in an osteosarcoma cell line (MG63). After being treated for two hours with 120 pmol RRP12-specific siRNA, the cells were incubated for 24 hours with 50 nM doxorubicin, and harvested. Proteins extracted from the cells were analyzed for activity using Western blotting in a similar manner to that of Example 3-1.

[124] In the cells treated without doxorubicin, no phosphorylated RRP12 proteins were detected. Phosphorylated RRP12 was detected in the cells administered with doxorubicin, but at a significantly low level when the cells were treated with RRP12 gene-specific siRNA (FIG. 9).

[125] These data indicate that RRP12 gene-specific siRNA can inhibit the anticancer agent-induced phosphorylation of RRP12.

[126]

[127] 4-3. Proliferation of Tumor Cells upon siRNA-Mediated Inhibition of RRP12

[128] When RRP12 was inhibited by RRP12 gene-specific siRNA in the osteosarcoma cell line (MG63), the growth of the cells was examined. Normal cells (hDF) and osteosarcoma cells (MG63) were treated for two days with control siRNA and RRP12-gene specific siRNA, followed by performing an MTT assay to measure the growth of the normal cells and the osteosarcoma cells (MG63). The normal fibrous cells and the osteosarcoma cells were separately incubated at 37℃ for one hour with 50 μg/ml MTT solution. After the MTT solution was removed, 200 μl of DMSO

(dimethyl sulfoxide) was added to dissolve formazan crystals. Optical density was measured at 595 nm using a spectrophotometer. The ratios of O.D. values of the cells transfected with the control siRNA to those of the cells trasnfected with RRP12 siRNA were calculated and the results are listed. Student's T test was carried out to determine the statistical significance of the data ($p < 0.05$).

[129] As a result, the cells transfected with the RRP12 gene-specific siRNA were observed to show poor growth. Particularly, the growth of the osteosarcoma cells was significantly decreased (FIG. 10). These results indicate that RRP12 gene-specific siRNA regulates the expression of RRP12 gene in osteosarcoma cells to inhibit the growth of the cells and that the down-regulation of RRP12 gene leads to anticancer activity because the RRP12 gene is essential for the growth of osteosarcoma cells.

[130] In addition, after the osteosarcoma cell line (MG63) was co-administered with the RRP12 gene-specific siRNA and doxorubicin, their growth was examined. In this regard, osteosarcoma cells were transfected with control siRNA or RRP12 gene-specific siRNA and additionally treated with or without doxorubicin, followed by an MTT assay for measuring cell growth.

[131] The growth of the osteosarcoma cells were observed to be decreased more significantly when administered with RRP12 gene-specific siRNA than with doxorubicin. The growth was far more significantly decreased by co-administration with doxorubicin and the RRP12 gene-specific siRNA (FIG. 11).

[132] Taken together, these results indicate that the RRP12 inhibitor has greater anticancer activity and that when administered in combination with the anticancer agent doxorubicin to osteosarcoma cells, the RRP12 gene-specific siRNA enhances the anticancer activity of doxorubicin to inhibit cell growth. On the basis of the results obtained in the Examples, the functions of RRP12 are summarized in FIG. 12.

[133]

[134] 4-4. Cell Death of Tumor Cells Upon siRNA-mediated Inhibition of RRP12 Gene

[135] When RRP12 was down regulated by RRP12 gene-specific siRNA in osteosarcoma cells (MG63), the cells were observed to examine whether they underwent cell death. The osteosarcoma cells were transfected with control siRNA or RRP12 gene-specific siRNA and additionally treated with or without doxorubicin. Flow cytometric analysis was carried out to examine the cell death of the cells and the activity of caspase-3 and poly(ADP-ribose)polymerase (PARP), both being activated during programmed cell death, was analyzed using Western blotting (FIGS. 13 and 14).

[136] As a result, the administration of RRP12 gene-specific siRNA was found to induce the osteosarcoma cells (MG63) to undergo cell death. These data indicate that RRP12 is essential for the survival of the osteosarcoma cell line and that RRP12 phosphorylation is a survival pathway of tumor cells over anticancer agents such as dox-

orubicin.

[137]

[138] Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

[139]

Claims

[Claim 1] An anticancer composition, comprising as an active ingredient an inhibitor against expression of a RRP12 (ribosomal RNA processing 12 homolog) gene or an inhibitor against the activity of RRP12.

[Claim 2] The anticancer composition of claim 1, wherein the inhibitor is selected from the group consisting of siRNA, antisense oligonucleotide, aptamers, antibody, and a single chain variable fragment, all being specific for RRP12.

[Claim 3] The anticancer composition of claim 2, wherein the siRNA is selected from the group consisting of nucleic acid molecules having the nucleotide sequences represented by SEQ ID NOS: 5 to 10.

[Claim 4] The anticancer composition of claim 1, showing a therapeutic effect on a cancer selected from the group consisting of osteosarcoma, giant cell tumor, chondroma, synovial sarcoma, bladder cancer, stomach cancer, breast cancer, colorectal cancer, uterine cervical cancer, prostate cancer and epidermoid carcinoma.

[Claim 5] A cancer-sensitizing composition, comprising an inhibitor against expression of an RRP12 (ribosomal RNA processing 12 homolog) gene or an inhibitor against the activity of RRP12.

[Claim 6] The cancer-sensitizing composition of claim 5, being inhibitive of anticancer drug resistance.

[Claim 7] The cancer-sensitizing composition of claim 5, wherein the inhibitor is selected from the group consisting of siRNA, antisense oligonucleotide, aptamers, antibody, and a single chain variable fragment, all being specific for RRP12.

[Claim 8] The cancer-sensitizing composition of claim 7, wherein the siRNA is selected from the group consisting of nucleic acid molecules having the nucleotide sequences represented by SEQ ID NOS: 5 to 10.

[Claim 9] The cancer-sensitizing composition of claim 5, showing a therapeutic effect on a cancer selected from the group consisting of osteosarcoma, giant cell tumor, chondroma, synovial sarcoma, bladder cancer, stomach cancer, breast cancer, colorectal cancer, uterine cervical cancer, prostate cancer and epidermoid carcinoma.

[Claim 10] An anticancer composition, comprising the composition as in one of claims 5-9 and an anticancer agent.

[Claim 11] The anticancer composition of claim 10, wherein the anticancer agent is doxorubicin.

[Claim 12] A method for screening an anticancer agent or a cancer sensitizer, comprising:
(a) analyzing an expression level of RRP12 gene or activity of RRP12 after treatment with a candidate; and
(b) determining the candidate as an anticancer agent or a cancer sensitizer if the expression level of RRP12 gene or the activity of RRP12 is inhibited after treatment with the candidate, as compared to that before treatment with the candidate.

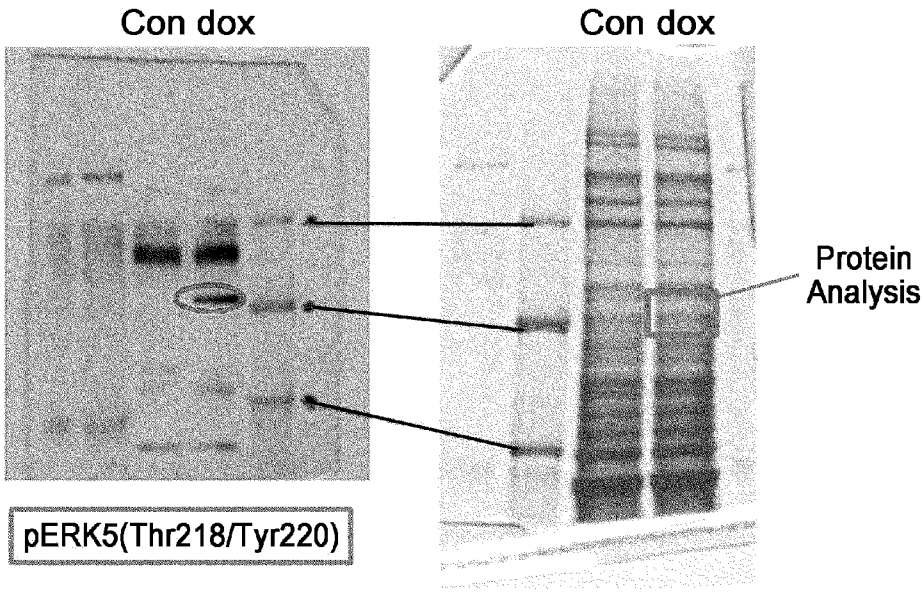
[Claim 13] A method for treating cancer in a subject in need thereof, comprising administering an inhibitor against expression of an RRP12 gene or an inhibitor against the activity of RRP12.

[Claim 14] A method for inhibiting anticancer drug resistance in a subject in need thereof, comprising administering an inhibitor against expression of an RRP12 gene or an inhibitor against the activity of RRP12.

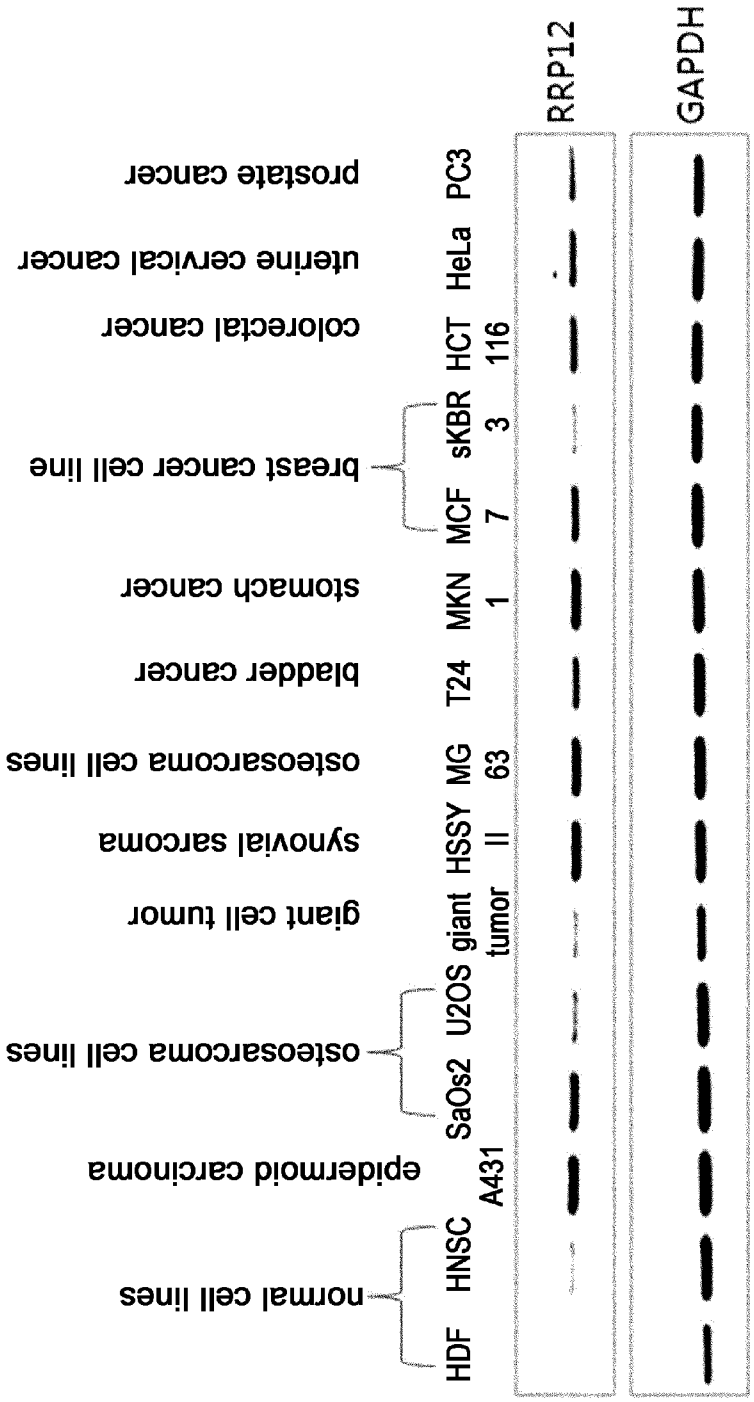
[Claim 15] Use of an inhibitor against expression of an RRP12 gene or an inhibitor against the activity of RRP12 in preparing an anticancer agent.

[Claim 16] Use of an inhibitor against expression of an RRP12 gene or an inhibitor against the activity of RRP12 in preparing a cancer sensitizer.

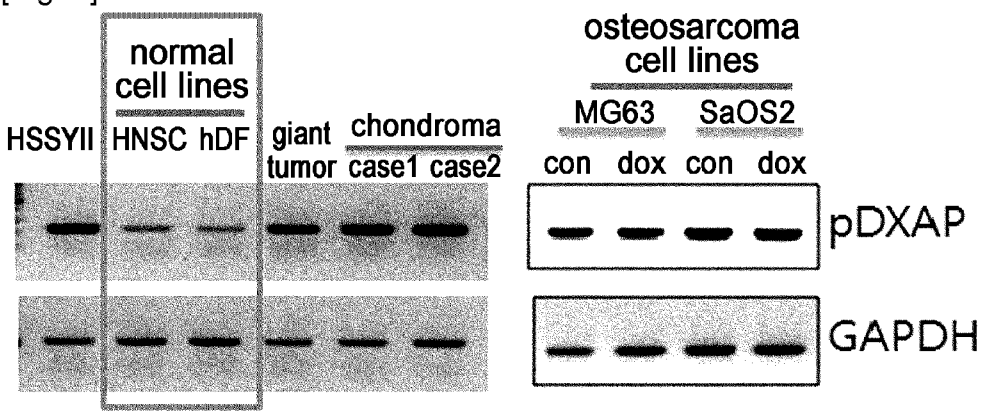
[Fig. 1]



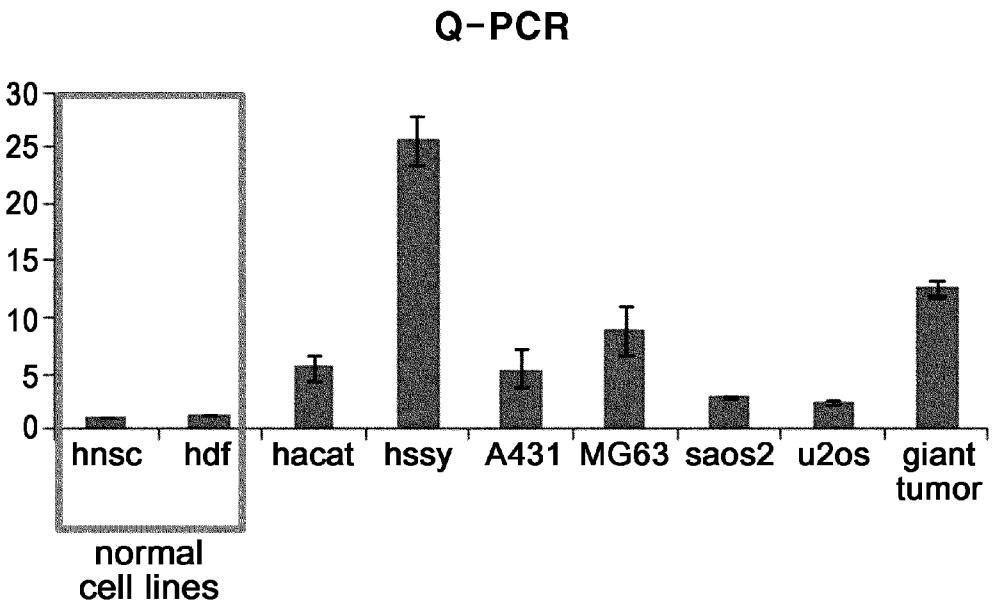
[Fig. 2]


RRP12
GAPDH
prostate cancer
PC3
uterine cervical cancer
HeLa
colorectal cancer
HCT 116
breast cancer cell line
sKBR 3
MCF 7
stomach cancer
MKN 1
bladder cancer
T24
osteosarcoma cell lines
MG 63
synovial sarcoma
HSSY II
giant cell tumor
giant tumor
osteosarcoma cell lines
U2OS
SaOs2
epidermoid carcinoma
A431
normal cell lines
HNSC
HDF

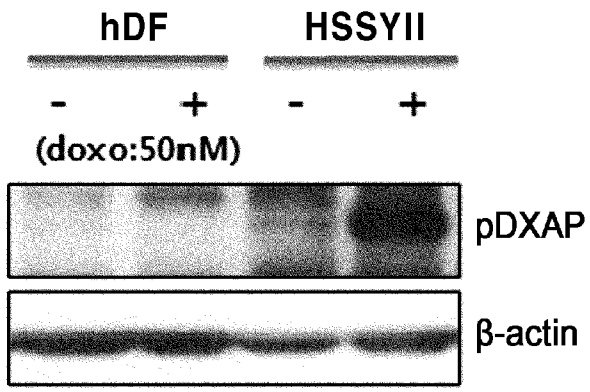
[Fig. 3]


normal cell lines
HSSYII
HNSC
hDF
giant tumor
chondroma
case1
case2
osteosarcoma cell lines
MG63
SaOS2
con
dox
con
dox
pDXAP
GAPDH

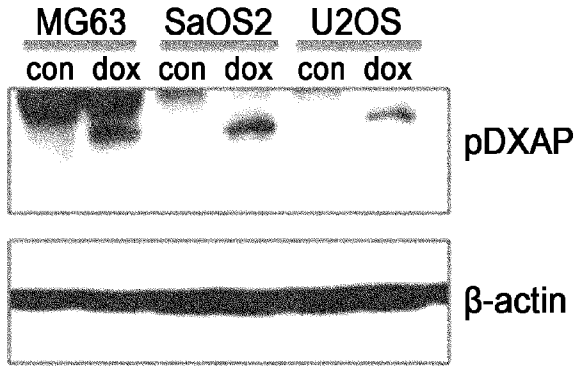
[Fig. 4]



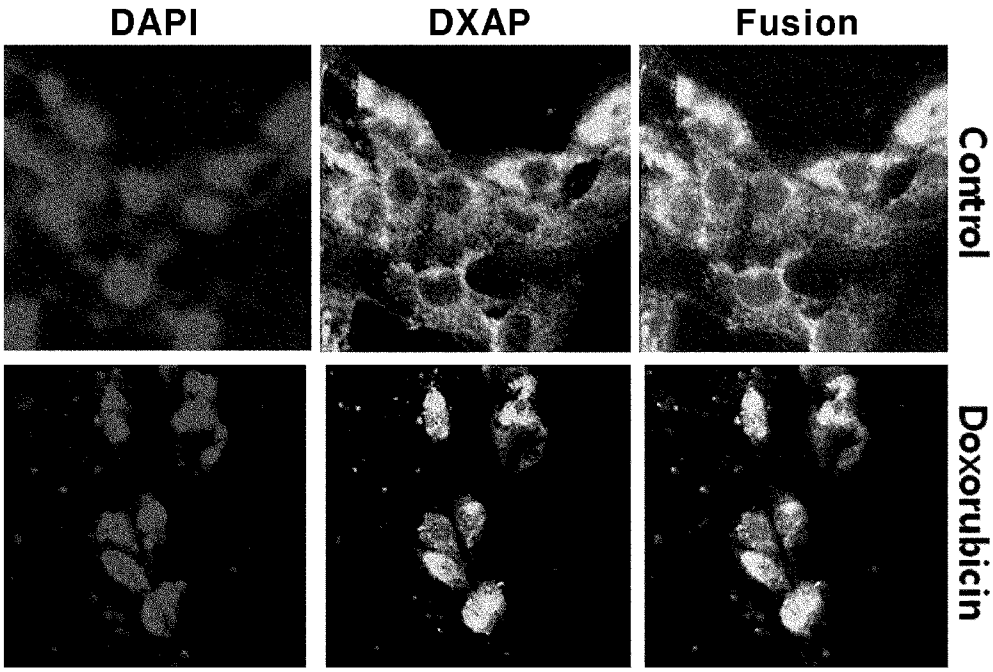
[Fig. 5]



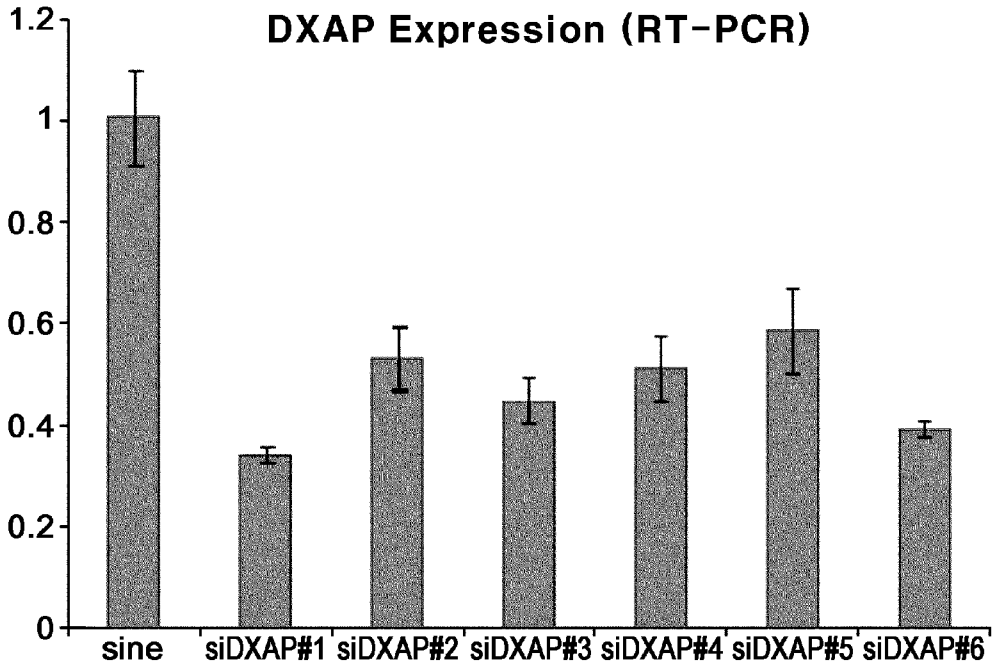
[Fig. 6]



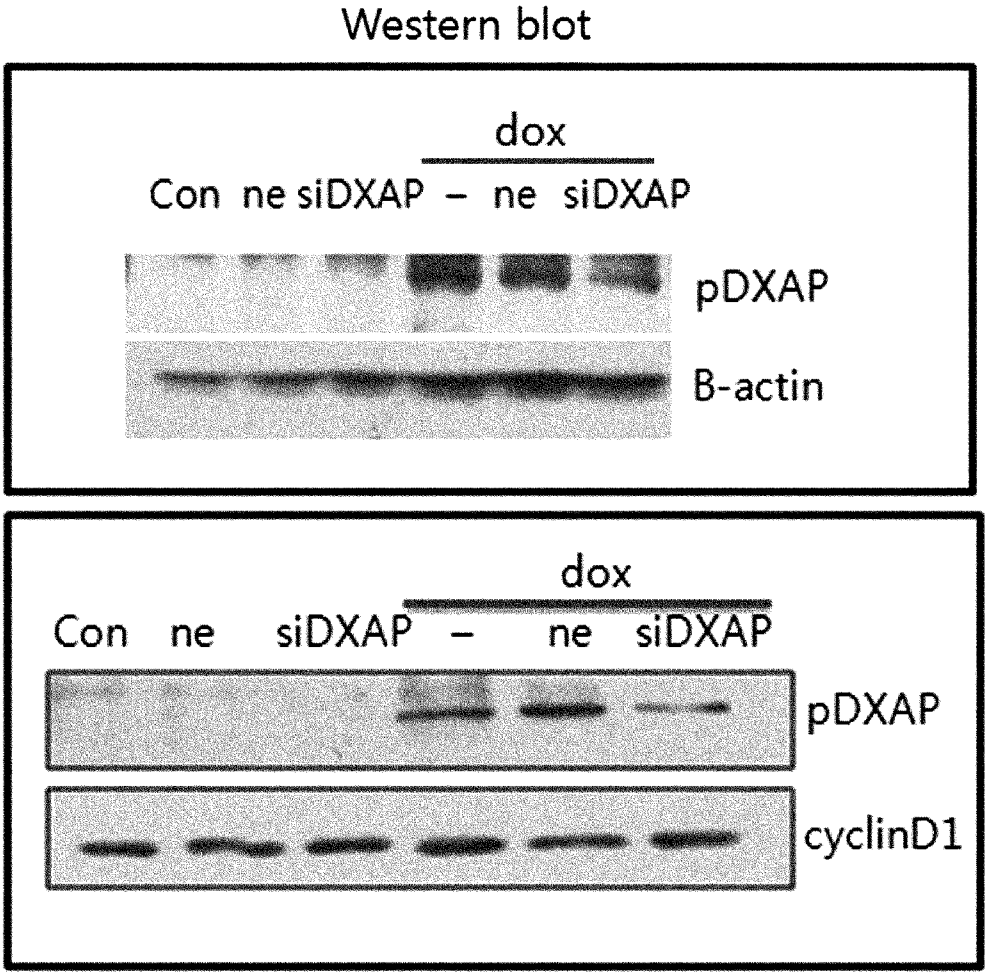
[Fig. 7]


DAPI
DXAP
Fusion
Control
Doxorubicin

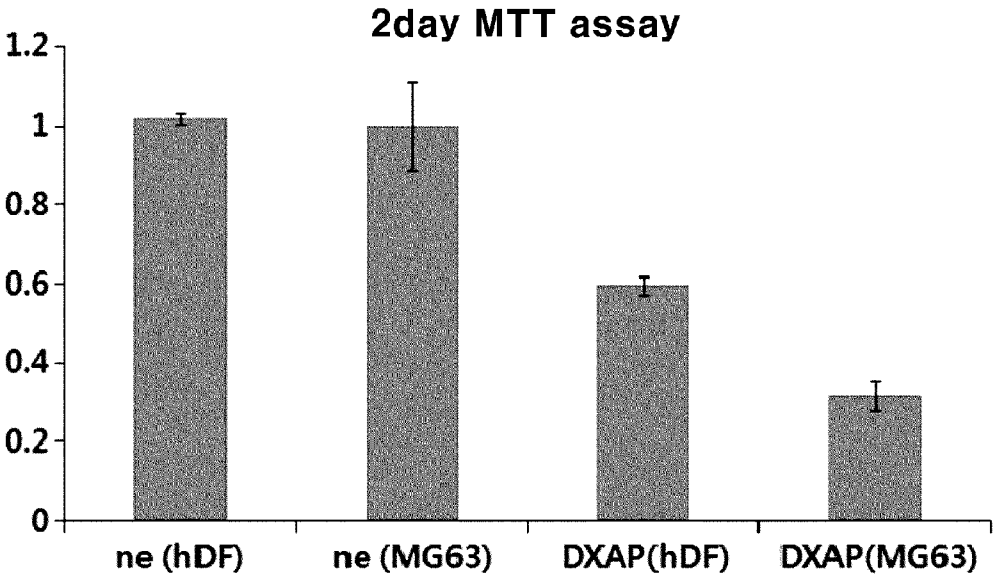
[Fig. 8]


DXAP Expression (RT-PCR)
1.2
1
0.8
0.6
0.4
0.2
0
sine
siDXAP#1
siDXAP#2
siDXAP#3
siDXAP#4
siDXAP#5
siDXAP#6

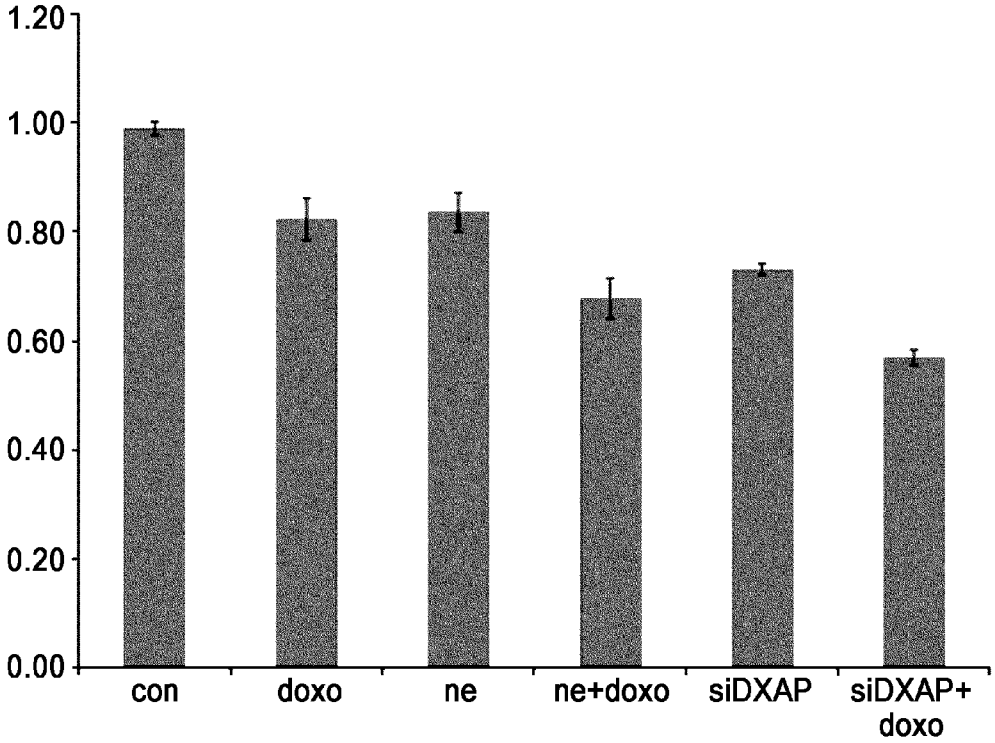
[Fig. 9]


Western blot
dox
Con ne siDXAP – ne siDXAP
pDXAP
B-actin
dox
Con ne siDXAP – ne siDXAP
pDXAP
cyclinD1

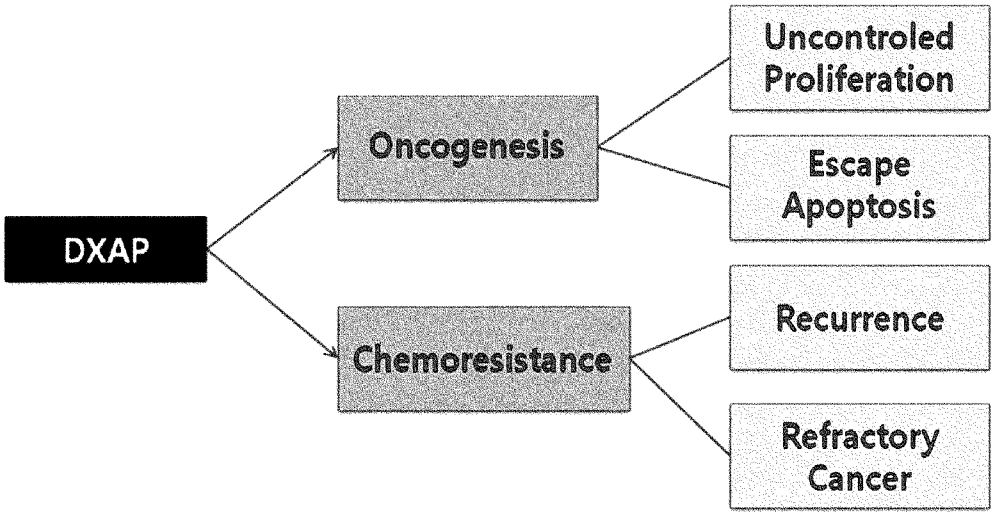
[Fig. 10]


2day MTT assay
1.2
1
0.8
0.6
0.4
0.2
0
ne (hDF)
ne (MG63)
DXAP(hDF)
DXAP(MG63)

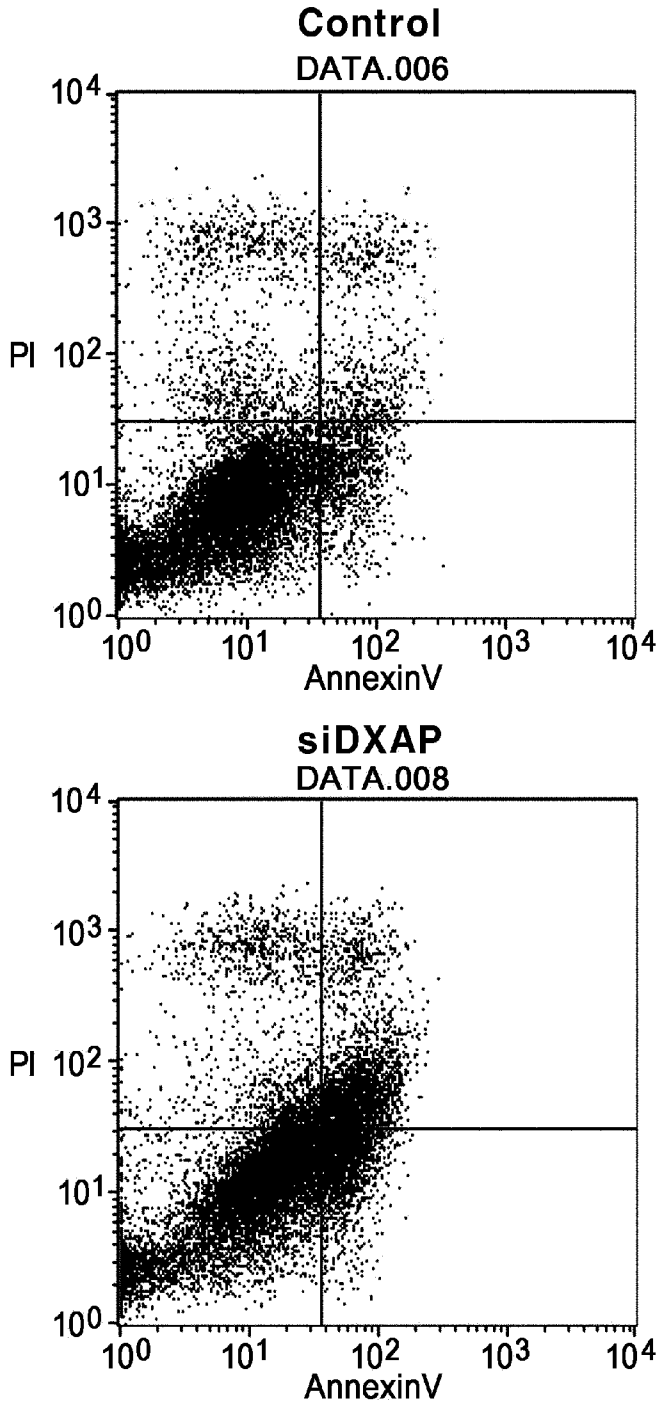
[Fig. 11]


1.20
1.00
0.80
0.60
0.40
0.20
0.00
con
doxo
ne
ne+doxo
siDXAP
siDXAP+ doxo

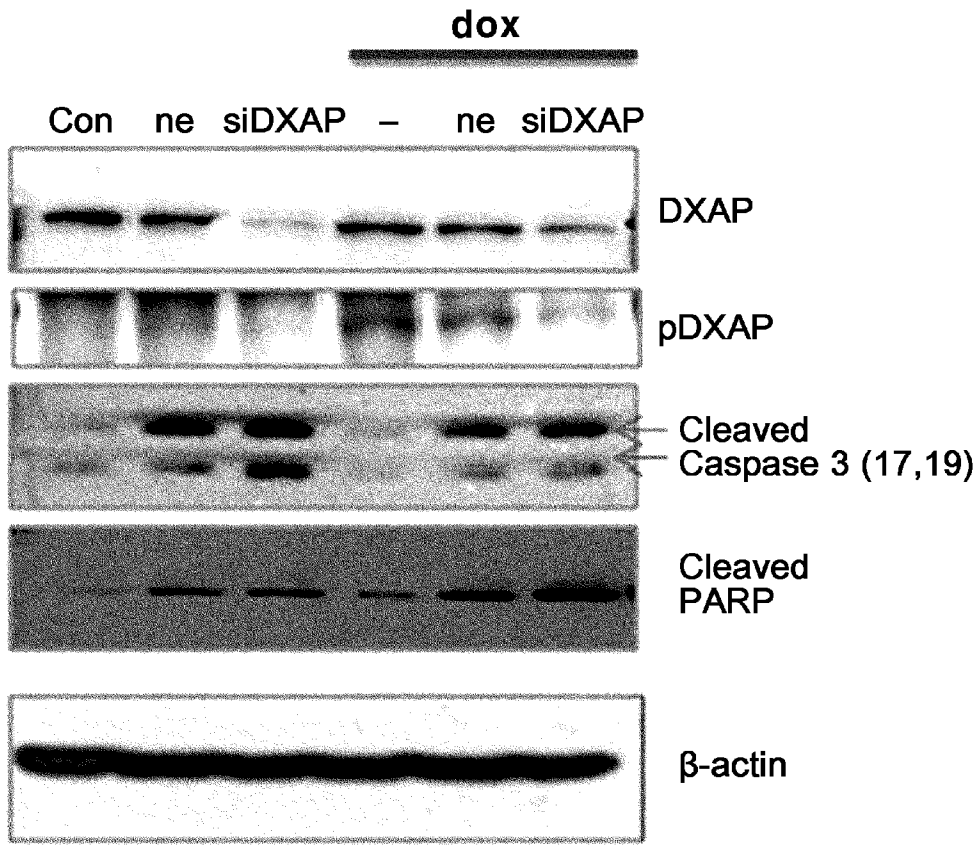
[Fig. 12]


DXAP
Oncogenesis
Chemoresistance
Uncontroled Proliferation
Escape Apoptosis
Recurrence
Refractory Cancer

[Fig. 13]



[Fig. 14]


dox
Con
ne
siDXAP
–
ne
siDXAP
DXAP
pDXAP
Cleaved
Caspase 3 (17,19)
Cleaved
PARP
β-actin

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/006715

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i, A61K 38/17(2006.01)i, A61K 48/00(2006.01)i, A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 39/395

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

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

eKOMPASS(KIPO internal) & Keywords: anticancer, cancer-sensitizing, RRP12, KIAA0690, doxorubicin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Y.-W. CHOI et al. `Gene expression profiles in squamous cell cervical carcinoma using array-based comparative genomic hybridization analysis' Int. J. Gynecol. Cancer, 2007, Vol. 17, No. 3, pp. 687-696, ISSN 1048-891x. See table 3; discussion.	1-12,15,16
A	MERCEDES DOSIL. 'Ribosome synthesis-unrelated functions of the preribosomal factor Rrp12 in cell cycle progression and the DNA damage response' Molecular and Cellular Biology, 11 April 2011 (E-pub.), Vol. 31, No. 12, pp. 2422-2438, ISSN 0270-7306. See abstract; results.	1-12,15,16
A	DIETER KRESSLER et al. 'Driving ribosome assembly' Biochimica et Biophysica Acta, 2010, Vol. 1803, No. 6, pp. 673-683, ISSN 0167-4889. See p. 676, right-column - p. 677, left-column.	1-12,15,16
A	STEPHEN J. FREEDLAND et al. 'Heterogeneity of molecular targets on clonal cancer lines derived from a novel hormone-refractory prostate cancer tumor system' The Prostate, 2003, Vol. 55, No. 4, pp. 299-307, ISSN 0270-4137. See table I.	1-12,15,16

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 APRIL 2012 (26.04.2012)	**27 APRIL 2012 (27.04.2012)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
Government Complex-Daejeon, 189 Cheongsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
Choi Sung Hee
Telephone No. 82-42-481-8740

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/006715

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. a sequence listing filed or furnished

- ☐ on paper
- ☒ in electronic form

b. time of filing or furnishing

- ☒ contained in the international application as filed
- ☐ filed together with the international application in electronic form
- ☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2011/006715

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☒ Claims Nos.: 13, 14
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 13 and 14 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

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

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2011/006715

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
None			