DESCRIPTION

GENE THERAPY COMPOSITION

TECHNICAL FIELD

[0001] The present invention relates to a gene therapy composition, particularly to a gene therapy composition for promoting cancer cell apoptosis activity by delivering cancer-specific apoptosis-inducing genes into cancer cells, a liposome vector for transferring the gene therapy composition to cancer cells, and a method of preparing the liposome vector.

BACKGROUND ART

[0002] Medical practices in the field of cancer therapy have from the past involved various anticancer therapies, including radiation therapy, chemotherapy using anticancer drugs and the like, and surgical methods, and numerous therapies and anticancer drugs have been researched and developed. Actually in Japan today, one in two people develop cancer and one in three die of the disease. Cancer occurs owing to a single cell accumulating multiple genetic mutations related to carcinogenesis, and kills the host by, for example, metastasizing to other organs.

0003 Three major cancer therapies (surgery, chemotherapy and radiotherapy) are currently performed in the field of cancer treatment. These anticancer therapies are known to cause side effects, and how to relieve the physical burden of the patient is a pressing issue. Heavy burden on the patient is particularly a problem in chemotherapy using anticancer drugs, because patients suffer various side effects such as nausea, hair loss, and leukocyte count reduction.

[0004] The burden on the patient by surgical treatment is also a problem. Moreover, although radiotherapy is a symptomatic therapy that targets tumors confirmed from images or the like that visualize the affected area, it nevertheless leads to significant hematogenous metastasis or lymph node metastasis in the case of surgery or radiation therapy.

[0005] As these therapies are nothing more than symptomatic therapies that treat tumors caused by genetic mutation after reaching visible range, they cannot easily cure cancer completely.

[0006] Gene therapy refers to a therapy for treating cancer that corrects defects of cells made dysfunctional owing to presence of abnormal genes by administering genes or cells incorporating genes and thereby decreasing genetic disorders. Numerous

treatments employing genes have been developed.

[0007] PCT Japanese Publication No. 2010-537973 (Japanese Patent 5 529 021), for example, discloses technology related to compositions (or kits) for diagnosing, preventing, treating or monitoring brain tumors, which are gene therapy compositions containing mesenchymal stem cells derived from umbilical cord blood. The disclosure suggests the possibility of treating brain tumors by inducing/promoting cancer cell apoptosis with anti-tumor genes composed of apoptosis-inducing factor genes that use cytokine or interleukin genes.

[0008] Although this technology can indeed precisely eradicate tumor cells (cancer), it is inadequate from the aspect of selecting compositions matched to the patient's physical constitution, type of cancer, stage of development etc., namely, with regard to customization. It is also inadequate from the viewpoint of the vectors required for precisely transferring the anti-tumor genes to the tumor cell

[0009] In the performance of gene therapy, vectors are necessary for accurately delivering apoptosis-inducing genes into cancer cells. Moreover, a composition that effectively promotes cancer cell apoptosis activity is required. Development of a gene therapy composition for recovering from genetic disorder that overcomes the aforesaid issues has been desired.

Patent document 1: PCT Japanese Publication No. 2010-537973

DISCLOSURE OF THE INVENTION

Problems to be Overcome by the Invention

[0010] The present invention has as an object to overcome the issues set out in the foregoing by providing a gene therapy composition, which gene therapy composition promotes cancer cell apoptosis by delivering apoptosis-inducing genes comprising a combination of multiple genes into cancer cells, and to a liposome vector for transferring the gene therapy composition into cancer cells and a method of preparing the liposome vector.

Means for Overcoming the Problems

[0011] In one configuration of the present invention for achieving the aforesaid object, the gene therapy composition comprises: a gene therapy composition for preventing and/or treating cancer whose anti-tumor genes are apoptosis-inducing genes, wherein the apoptosis-inducing genes comprise p53 genes, FUS-1 genes, TRAIL genes and

IL-24 genes.

Moreover, in one configuration, contained amounts among the apoptosis-inducing genes of p53 genes, FUS-1 genes, TRAIL genes and IL-24 genes are equal.

[0012] In another configuration, in order to activate induction of apoptosis, amounts among the apoptosis-inducing genes of p53 genes, TRAIL genes and IL-24 genes are double amount of FUS-1 genes.

In another configuration, in order to inhibit activation of MDM2 and activate induction of p53-dependent apoptosis, amounts among the apoptosis-inducing genes of p53 genes and FUS-1 genes are double amounts of TRAIL genes and IL-24 genes.

[0013] In another configuration, in order to activate induction of non-p53-dependent apoptosis, amounts among the apoptosis-inducing genes of p53 genes, FUS-1 genes and TRAIL genes are double amount of IL-24 genes.

In another configuration, in order to activate p53-dependent apoptosis induction, amount among the apoptosis-inducing genes of p53 genes is double amounts of FUS-1 genes, TRAIL genes and IL-24 genes.

[0014] In another configuration, in order to inhibit proliferation of cancer cells through MAPK pathway, amount among the apoptosis-inducing genes of FUS-1 genes is double to five fold amounts of p53 genes, TRAIL genes and IL-24 genes.

In another configuration, in order to achieve anti-tumor effect against melanoma, amount among the apoptosis-inducing genes of IL-24 genes is double amounts of p53 genes, FUS-1 genes and TRAIL genes.

[0015] In another configuration, in order to achieve anti-tumor effect against lung cancer and/or breast cancer, amounts among the apoptosis-inducing genes of FUS-1 genes and IL-24 genes are double amounts of p53 genes and TRAIL genes.

In another configuration, in order to enhance angiogenesis inhibition action and sensitivity to radiotherapy, amounts among the apoptosis-inducing genes of TRAIL genes and IL-24 genes are double amounts of p53 genes and FUS-1 genes.

[0016] In another configuration, for achieving anti-tumor effect and side effect mitigation in combined use with chemotherapy, amounts among the apoptosis-inducing genes of p53 genes and TRAIL genes are double amounts of FUS-1 genes and IL-24 genes.

In another configuration, the gene therapy composition additionally contains p16 genes and/or p21 genes, and contained amounts of the genes are equal.

[0017] In another configuration, the gene therapy composition is encapsulated by a cationic lipid and helper lipid liposome-based vector for gene therapy that targets cancer cells.

In another configuration, the liposome is composed of monolayer membrane having an internal cavity, and the membrane is composed of a monolayer thin membrane compound comprising a capsule-like vector with attached ligand and containing dioleoylphosphatidylethanolamine and dimethylaminoethancarbamoylcholesterol.

[0018] In another configuration, the gene therapy composition further comprises genes as plasmid vectors containing proto-oncogenes and cytokine genes.

In another configuration, the cytokine genes comprise IL-12 genes or IL-18 genes.

[0019] In another configuration, a method of preparing a liposome for a gene therapy composition according to the present invention comprises: a mixing step for preparing a lipid solution by mixing dioleoylphosphatidylethanolamine and dimethylaminoethancarbamoylcholesterol in chloroform; a coating forming step for forming a coating by drying the prepared solution; a vacuum-holding step for maintaining the formed coating under vacuum; a resuspending step for resuspending the thin membrane by addition of water, dextrose or VORTEX®; a culturing step for culturing the suspended suspension at 4°C; an ultrasound step for forming liposomes from the suspension or the cultured suspension by performing ultrasonic irradiation processing; and a shaping step for extrusion-shaping liposomes using a polycarbonate filter of same size as liposomes to be prepared.

EFFECTS OF THE INVENTION

[0020] Being configured in the manner described in detail in the foregoing, the present invention offers the following effects:

1. As p53 genes, FUS-1 genes, TRAIL genes and IL-24 genes are selected as apoptosis-inducing genes, effective cancer cell apoptosis activity can be promoted.

2. As P53 genes, FUS-1 genes, TRAIL genes and IL-24 genes among the apoptosis-inducing genes are mixed in equal amounts, the composition can be customized to the patient’s body without imparting a sudden impact, whereafter the amounts of the respective genes to be combinedly administered can be assessed during ensuing therapy.

3. In one of the configurations, amounts of p53 genes, TRAIL genes and IL-24 genes contained among the apoptosis-inducing genes are about double amount of FUS-1 genes contained, whereby induction of apoptosis can be activated.

[0021] 4. In one of the configurations, amounts of p53 genes and FUS-1 genes contained among the apoptosis-inducing genes are about double amounts of TRAIL genes and IL-24 genes contained, whereby activation of MDM2 can be inhibited and

induction of p53-dependent apoptosis can be activated.

5. In one of the configurations, amounts of p53 genes, FUS-1 genes and TRAIL genes contained among the apoptosis-inducing genes are about double amount of IL-24 genes contained, whereby induction of apoptosis not dependent on p53 genes can be activated.

[0022] 6. In one of the configurations, amount of p53 genes contained among the apoptosis-inducing genes is about double amount of FUS-1 genes, TRAIL genes and IL-24 genes contained, whereby induction of apoptosis dependent on p53 genes can be activated

7. In one of the configurations, amount of FUS-1 genes contained among the apoptosis-inducing genes is about double to five fold amounts of p53 genes, TRAIL genes and IL-24 genes contained, whereby proliferation of cancer cells through MAPK pathway can be inhibited.

[0023] 8. In one of the configurations, amount of IL-24 genes contained among the apoptosis-inducing genes is about double amounts of p53 genes, FUS-1 genes and TRAIL genes contained, whereby anti-tumor effect against melanoma can be achieved.

9. In one of the configurations, amounts of FUS-1 genes and IL-24 genes contained among the apoptosis-inducing genes are about double amounts of P53 genes and TRAIL genes contained, whereby anti-tumor effect against lung cancer and/or breast cancer can be achieved.

[0024] 10. In one of the configurations, amounts of P53 genes and TRAIL genes contained among the apoptosis-inducing genes are about double amounts of FUS-1 genes and I-24 genes contained, whereby anti-tumor effect can be achieved in combined use with chemotherapy, and side-effects can be mitigated.

11. In one of the configurations, amounts of TRAIL genes and I-24 genes contained among the apoptosis-inducing genes are about double amounts of p53 genes and FUS-1 genes contained, whereby an effect of enhancing angiogenesis inhibition action and sensitivity to radiotherapy can be achieved.

[0025] 12. In one of the configurations, the gene therapy composition further comprises p16 genes and p21 genes, with contained amounts of the respective genes being made equal, whereby induction of apoptosis of every type of cancer can be activated.

13. Owing to the fact that the gene therapy composition comprising the various genes is encapsulated by a liposome-based liposome vector for gene therapy, stability of the gene therapy composition can be ensured and the gene therapy composition can be reliably delivered to the targeted tumor cells.

[0026] 14. In one of the configurations, the liposome is composed of monolayer membrane having an internal cavity, whereby a vector that stores the gene therapy composition inside can be constituted.

15. In one of the configurations, the gene therapy composition further comprises genes as plasmid vectors containing proto-oncogenes and cytokine genes, whereby proliferation of cancer cells can be inhibited and apoptosis induced.

[0027] 16. As IL-12 genes or IL-18 genes are selected as cytokine genes, effective apoptosis activity can be induced.

17. As the liposome is formed from a suspension by ultrasonic irradiation processing, liposomes of a size on the order of 200 nm can be formed.

BEST MODE FOR WORKING THE INVENTION

[0028] In the following, the gene therapy composition according to the present invention is explained in detail based on an embodiment shown in the drawing.

FIG. 1 is a table showing a list of apoptosis-inducing gene combinations.

[0029] The gene therapy composition according to the present invention is a composition for preventing and/or treating cancer mainly by the action of anti-tumor genes, and is a composition for performing treatment by direct delivery into tumors (cancer cells) through the mediation of vectors, thereby killing the cancer cells.

[0030] The gene therapy composition comprises apoptosis-inducing genes having apoptotic properties. Namely, the gene therapy composition is configured to induce cancer cell apoptosis (spontaneous death) and eradicate cancer cells by delivering genes having apoptotic properties into cancer cells. In other words, a gene therapy composition capable of preventing and/or treating cancer is provided.

[0031] In the present embodiment, the gene therapy composition includes all of four types of apoptosis-inducing genes: p53 genes, FUS-1 genes, TRAIL genes and IL-24 genes. These apoptosis-inducing genes are all cancer-inhibiting genes, and that high anticancer effect is exhibited in cancer cell-killing treatment using these genes was experimentally confirmed. In the present invention, the composition always includes these four types of genes, and the fact that the properties of the composition change with changing content ratios (percentages by weight) of these genes is under elucidation.

[0032] The p53 gene is a typical cancer-inhibiting gene and is the gene responsible for Li-Fraumeni syndrome, an inherited familial disposition that poses a risk of frequent occurrence of malignant tumors in various organs during childhood and adulthood. Also in experiments using p53-deficient mice (deprived of p53 genes), tumor occurrence was observed at high frequency at an early stage after birth, thus experimentally demonstrating that P53 is an important factor in inhibiting cancer. p53 is induced by DNA damage and various kinds of stress, and functions as a transcriptional activator inside the cell nucleus.

[0033] p53 is activated in a cell that receives DNA damage stimulus, arrests cell division by controlling cell cycle checkpoint function, and in the interim inhibits DNA disorders by promoting DNA repair. When a cell is unrepaired in this process, gene mutation is prevented by inducing apoptosis (cell death). Experiments show that chromosomes lacking p53 function become unstable. Moreover, it is also known from experiments that when a cancer gene is expressed in a normal cell, induction of p53-dependent apoptosis or cell senescence occurs, so that canceration is inhibited by eliminating cells with activated cancer genes.

[0034] The FUS-1 gene is a cancer-inhibiting gene identified in the human chromosome 3p21.3 region, and loss or mutation of alleles (genes defining allelomorphs) for FUS-1 is observed early in many cancers, and FUS-1 protein loss or depletion is observed particularly in lung cancer, lung tumors and breast cancer. There is clear evidence that FUS-1 leads cancer cells to apoptosis by activating mitochondria-dependent Apaf-1 pathway or inhibiting function of tyrosine kinase protein including EGFR, PDGFR, AKT, c-Ab1 and c-Kit.

[0035] In normal cells, FUS-1 is activated by apoptosis stimulation, stress or the like, and apoptosis of endogenous mitochondria is activated. Another characteristic of FUS-1 activation is that it blocks inhibition of p53 by MDM2 gene, thereby increasing p53-dependent apoptosis.

[0036] The FUS-1 gene is a gene that functions to inhibit cell proliferation caused by MDM2 expression and EGFR mutation. It inhibits proliferation of cancer cells through MAPK pathway. The FUS-1 gene is known to have an anti-tumor effect against lung cancer and breast cancer.

[0037] The TRAIL gene is a gene characterized by leading cells to apoptosis through the mediation of a death receptor. TRAIL genes selectively lead various tumor cells and cells that incurred mutation to apoptosis but do not act on normal cells, so that they can be exploited as dedicated cancer therapy genes. The TRAIL gene is a homo-trimeric structure that interacts with members of the TNF receptor family.

[0038] The IL-24 (interleukin-24) gene is located on the first chromosome long arm (1p32) and is a gene manifesting proliferation-inhibiting action and apoptosis-inducing action against many kinds of cancer cells, including melanoma, breast cancer and lung cancer. IL-24 does not act on normal cells, and further works to reinforce sensitivity of cancer cells to radiation. IL-24 manifests anti-tumor effect against melanoma, lung cancer and breast cancer. It is also known to have an angiogenesis-inhibiting effect.

0039 The formulation of the gene therapy composition according to this invention is defined by specifying individual content ratios of the four genes, i.e., p53 gene, FUS-1 gene, TRAIL gene and IL-24 gene, based on genetic abnormality, determined diagnosis,

presence / absence of metastasis, and other information ascertained by gene diagnosis. In one embodiment of the present invention, the p53 gene, FUS-gene, TRAIL gene and IL-24 gene among the apoptosis-inducing genes are incorporated in equal amounts. This combining of the genes in equal amounts enables formulation of a composition that can be customized while minimizing impact on the patient's body. In addition, dosage can be adjusted to enhance the effect of the gene therapy composition while maintaining a good balance. Moreover, data obtained by monitoring expression of MDM2 and various other genes can be used to decide gene content ratios in future formulations.

[0040] In another embodiment of the gene therapy composition according to the present invention, a configuration can be adopted wherein p53 genes, TRAIL genes and IL-24 genes are contained among the apoptosis-inducing genes in double amount of FUS-1 genes. When the aforesaid gene therapy composition containing equal amounts of the respective genes is used, it is necessary upon observation of p53 mutation to administer increased dose of p53 and promote p53-independent apoptosis induction. In this case, it is possible by incorporating p53 genes, TRAIL genes and IL-24 genes in double the amount of FUS-1 genes to realize activation of p53 and p53-independent apoptosis induction and provide a more effective gene therapy composition.

[0041] In still another embodiment of the present invention, a configuration is adopted wherein p53 genes and FUS-1 genes are contained among the apoptosis-inducing genes of the gene therapy composition in double the amounts of TRAIL genes and IL-24 genes. When the aforesaid gene therapy composition containing equal amounts of the respective genes is used, it is necessary upon observation of overexpression of any of MDM2, AKT1 (activated kinase) and EGFR to inhibit cell proliferation and/or promote apoptosis induction. By incorporating FUS-1 genes in double the amounts of TRAIL genes and IL-24 genes, MDM2 expression can be inhibited and p53-dependent caspase pathway can be activated, whereby cancer cells can be led to apoptosis. In addition, since increasing amount of FUS-1 inhibits cell proliferation caused by EGFR mutation, a tumor growth inhibiting effect can be anticipated. Moreover, since overexpression of MDM2, AKT1 and EGFR affects cancer cell proliferation owing to inhibition of p53 apoptosis induction, increasing amount p53 helps to activate p53-dependent apoptosis induction and enable provision of a more effective gene therapy composition.

[0042] Still further, a configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention can be adopted wherein p53 genes, FUS-1 genes and TRAIL genes are contained in double the amount of IL-24 genes. When the aforesaid gene therapy composition is used, it is necessary upon observation of overexpression of BCL2 to inhibit anti-apoptosis. Therefore, since doubling p53 gene and FUS-1 gene content can lead cancer cells to apoptosis as

mentioned above, a tumor growth inhibiting effect can be anticipated. In addition, since presence of a double content of TRAIL genes activates non-P53-dependent apoptosis induction, a more effective gene therapy composition can be provided.

[0043] Further, a configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention can be adopted wherein p53 genes are contained in double the amounts of FUS-1 genes, TRAIL genes and IL-24 genes. When the aforesaid gene therapy composition is used, it is necessary upon observation of APC gene methylation and/or mutation of BRCA-1 and/or BRCA-2 cancer-inhibiting genes to increase p53-dependent apoptosis activity. Therefore, p53-dependent apoptosis induction can be activated by doubling p53 gene content relative to the other genes. In this case, p53 gene content is preferably double or more than double the contents of the other genes.

[0044] Further, a configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention can be adopted wherein FUS-1 genes are contained in double to five fold amounts of p53 genes, TRAIL genes and IL-24 genes. When the aforesaid gene therapy composition is used, it is necessary upon observation of overexpression of any of RAS (H-RAS, K-RAS, N-RAS), MAP2K5, MAP3K10 and RAF (B-RAF, A-RAF) to inhibit cancer cell proliferation through the MAPK pathway. The configuration therefore defines FUS-1 content as double to five fold depending on the number of the aforesaid overexpressions. In the present embodiment, content is increased two fold (doubled) for one overexpression, three fold for up to three overexpressions, four fold for up to five overexpressions, and five fold for greater than five overexpressions. A tumor growth inhibiting effect can be anticipated by this.

[0045] Further, a configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention can be adopted wherein IL-24 genes are contained in double amount of p53 genes, FUS-1 genes and TRAIL genes. In the present embodiment, a configuration is adopted wherein IL-24 genes are contained in double amount because angiogenesis-inhibiting effect can be anticipated when overexpression of VEGF-A is observed during use of the aforesaid gene therapy composition. As a result, growth and/or metastasis of cancer tissue due to angiogenesis can be prevented. Moreover, since IL-24 genes exhibit anti-tumor effect against melanoma, induction of cancer cell apoptosis and/or tumor growth inhibiting effect owing to doubling of IL-24 gene content can be anticipated.

[0046] Further, in one configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention, FUS-1 genes and IL-24 genes are contained in double the amounts of p53 genes and TRAIL genes. FUS-1 genes

and IL-24 genes can be expected to exhibit anti-tumor effect against lung cancer and breast cancer. The present embodiment is therefore configured to contain double amounts of FUS-1 genes and IL-24 genes when the determined clinical diagnosis is lung cancer and/or breast cancer. As a result, induction of cancer cell apoptosis and/or tumor growth inhibiting effect owing to doubling of IL-24 gene content can be anticipated particularly with respect to lung cancer and breast cancer.

[0047] Further, a configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention can be adopted wherein TRAIL genes and IL-24 genes are contained in double amounts of p53 genes and FUS-1 genes. In a case where metastases are present or a case where radiation therapy is used concomitantly, the present embodiment is configured to contain TRAIL genes and IL-24 genes in double amounts. By adopting this configuration, an angiogenesis-inhibiting effect can be anticipated, and since an effect of possibly achieving enhanced sensitivity to radiation therapy also arises, more effective induction of cancer cell apoptosis and tumor growth inhibiting effect can be anticipated in cases of advanced cancer.

[0048] Further, a configuration of the apoptosis-inducing genes of the gene therapy composition in accordance with the present invention can be adopted wherein p53 genes and TRAIL genes are contained in double amounts of FUS-1 genes and IL-24 genes. By adopting this configuration, anti-tumor effect in combined use with chemotherapy can be anticipated and side effects can be mitigated.

[0049] The gene therapy composition according to the present invention can be configured to further comprise p16 genes and/or p21 genes. The p16 gene is a cancer-inhibiting gene that plays an important role in adjusting cell cycle. p16 cell mutation increases occurrence risk of numerous cancers, particularly melanoma. The p21 gene is a gene that impedes cell cycle progression by impeding cyclin-dependent kinase that has an effect on cell cycle rotation. As these genes are incorporated in amounts equal to other genes, induction of cancer cell apoptosis and tumor growth inhibiting effect can be anticipated.

[0050] The gene therapy composition according to the present invention can have a configuration encapsulated by a liposome vector for gene therapy. The gene therapy composition of the present invention is configured for direct delivery into targeted cancer cells by encapsulation in nonviral vector liposomes.

[0051] The liposome vector for gene therapy comprises a liposome of cationic lipid and helper lipid and comprises a capsule-like vector constituted of a monolayer thin membrane compound having an internal cavity. In the present embodiment, this liposome has a diameter of 70 nm to 100 nm and the internal cavity and average diameter of 50 nm. This internal cavity is configured to store plasmid, gene therapy

composition or other genes.

[0052] A ligand is attached to the monolayer membrane of the liposome. As binding to a specific receptor (cancer cell) can therefore be anticipated, the gene therapy composition can be more effectively delivered to the targeted cancer cell.

[0053] In the present embodiment, this liposome contains at least dioleoylphosphatidylethanolamine and dimethylaminoethancarbamoylcholesterol, and, as a lipid for assisting their membrane fusion, is formed cationic. In addition, this liposome can contain phosphatidylethanolamine or other lipid.

[0054] By adopting this configuration, stability of the gene therapy composition can be ensured and the gene therapy composition can be protected from all types of disturbances outside the cell, whereby therapeutic DNA or RNA (gene therapy composition) can be reliably delivered into the targeted cancer cells.

[0055] The gene therapy composition according to the present invention can further adopt a configuration incorporating genes as plasmid vectors containing proto-oncogenes. The plasmid vector containing the proto-oncogene ascertained by gene diagnosis is inserted into the nucleus of the cell, and a protein acting as an endogenous antigen is produced through the process of transcription and translation from DNA. The antigen is thereafter presented through an MHC class 1 molecule, cancer cell rejection occurs, and the cell is damaged by a T killer cell. Further, a cell having protein acting as an endogenous antigen incurs apoptosis, whereupon the antigen is expelled to outside the cell to cause voracious phagocytic activity by a dendritic cell or other phagocyte, and is presented to a helper T cell through an MHC class 2 molecule. As the results in killer T cell and macrophage activation and massive production of antibodies, cancer cell depletion and tumor growth inhibition by a so-called DNA vaccine effect can be anticipated.

[0056] Moreover, the gene therapy composition can additionally contain cytokine genes. In this embodiment, a configuration is adopted whereby IL-12 genes or IL-18 genes are contained as cytokine genes. Effects exhibited by IL-12 include cell proliferating effect with respect to natural killer cells (NK cells) that operate as primary factors of T cells, and natural immunity and cytotoxic activity inducing effects. In light of its role in these cellular immune functions, IL-12 can be expected to exhibit effects in areas such as infection protection, anti-cancer therapy, and immunodeficiency treatment.

[0057] IL-18 is a gene related to massive production of INF-γ in response to poison stimulation, and also stimulates macrophages to kill germs by voracious ingestion. In light of these roles, IL-18 can be anticipated to exhibit effects in areas such as infection protection and anti-cancer therapy.

[0058] When stimulated by a cytokine such as IL-2 or IL-18, the NK cell expresses Fas ligand. The Fas ligand is cytokine that by trimerizing a Fas receptor permeating the targeted cell membrane transmits a signal for inducing apoptosis, and as a THF receptor family molecule, triggers apoptosis in a tumor cell expressing Fas. In other words, the Fas ligand can be expected to exhibit cancer cell reducing and tumor growth inhibiting effects.

[0059] It is known that the gene therapy composition according to the present invention can be expected to exhibit strong effect by introduction together with NK cells. NK cells selectively eliminate abnormal cells overlooked by T cell immune surveillance owing to lowered MHC class 1 molecule expression. However, some tumor cell lines and normal cells that do not express MHC class 1 molecules are not necessarily NK-sensitive, so that that even if ordinary NK cells are cultured and delivered into cancer cells, the NK cells are apt not to activate from the viewpoint of anti-tumor effect. In the present embodiment, proto-oncogenes are delivered into NK cells based on gene diagnosis, thereby enabling surface receptors corresponding to the protein (cancer antigen, ligand) expressed on the cancer cells to be expressed on the NK cells, so that even if the cancer cells should have MHC class 1 molecules, receptors binding to activated NK receptor ligands come to be expressed, whereby the NK cells can discriminatingly kill cancer cells and achieve more effective cancer cell reduction and tumor growth inhibition.

[0060] The gene therapy composition according to the present invention can additionally be introduced together with dendritic cells. The dendritic cell is a type of immune cell that functions as an antigen-presenting cell whose role is to present an antigen acquired by the dendritic cell itself to another immune system cell. Upon acquiring an antigen, the dendritic cell activates and moves to a lymph node, the spleen or some other secondary lymphoid organ. In the lymphoid organ, a T cell specific to the acquired antigen is activated. As this activation is efficient, dendritic cells can be considered superior to macrophages with regard to T cell activation. In this embodiment, plasmids containing the proto-oncogene as an antigen obtained by diagnosis are encapsulated in a liposome and delivered into the dendritic cell. This has the advantage of making removal of cancer tissue unnecessary.

[0061] The gene therapy composition according the present invention can be introduced together with the aforesaid NK cells or dendritic cells, or with both NK cells and dendritic cells. Moreover, strong anti-tumor effect can also be expected by introduction together with genes as plasmid vectors containing proto-oncogenes (DNA vaccine) of cytokine genes.

[0062] There next follows an explanation of a method of preparing a liposome for a gene therapy composition according to the present invention. A method of preparing a liposome for a gene therapy comprises: a mixing step, a coating forming step, a vacuum-holding step, a resuspending step, a culturing step, an ultrasound step, and a shaping step. The mixing step is a step for preparing a lipid solution by mixing dioleoylphosphatidylethanolamine and dimethylaminoethancarbamoylcholesterol in chloroform. This prepares a lipid solution.

[0063] In the ensuing coating forming step, the prepared lipid solution is dried by N^2 gas blowing. This forms a membrane. In the ensuing vacuum-holding step, the membrane formed by drying in the coating forming step is held under vacuum. In the present embodiment, a vacuum state is maintained for five hours with the vessel opening in a covered state.

[0064] In the resuspending step the vacuum-held membrane is suspended. In the present embodiment, resuspension is performed by adding water, dextrose or VORTEX® to the membrane. The suspended suspension is cultured in the culturing step. In the present embodiment, culturing is done at 4°C for 24 hours but is not limited to this and culture time can be suitably varied. Optionally in this embodiment, the method can go directly to the following ultrasound step without performing the culturing step.

[0065] In the ultrasound step, ultrasonic irradiation processing is performed for forming liposomes from the suspension or the cultured suspension having passed through the culturing step. Size of formed liposomes can be controlled by performing ultrasonic irradiation. In the present embodiment, formation of 200 nm (nanometer) liposomes can be formed. In the shaping step, in order to perform additional shape control on the liposomes, shaping size is decided and liposomes are extrusion-shaped using a polycarbonate filter of the same size as that of the liposomes to be prepared. In the present embodiment, temperature management is performed during extrusion-shaping. This process enables preparation of monolayer liposomes. In the present embodiment, the size of the prepared liposomes is smaller than 100 nm (nanometer).

[0066] As this method of preparing a liposome for gene therapy can reliably prepare liposomes to serve as gene therapy composition vectors, it facilitates delivery of the gene therapy composition into targeted cancer cells.

BRIEF EXPLANATION OF THE DRAWING

[0067] FIG. 1 is a table showing a list of apoptosis-inducing gene combinations.

CLAIMS

1. A gene therapy composition for cancer prevention and/or therapy comprising apoptosis-inducing genes as anti-tumor genes, wherein the gene therapy composition is characterized in that the apoptosis-inducing genes comprise p53 genes, FUS-1 genes, TRAIL genes and IL-24 genes.

2. A gene therapy composition of claim 1, characterized in that contained amounts among the apoptosis-inducing genes of p53 genes, FUS-1 genes, TRAIL genes and IL-24 genes are equal.

3. A gene therapy composition of claim 1, characterized in that, in order to activate induction of apoptosis, amounts among the apoptosis-inducing genes of p53 genes, TRAIL genes and IL-24 genes are double amount of FUS-1 genes.

4. A gene therapy composition of claim 1, characterized in that, in order to inhibit activation of MDM2 and activate induction of p53-dependent apoptosis, amounts among the apoptosis-inducing genes of p53 genes and FUS-1 genes are double amounts of TRAIL genes and IL-24 genes.

5. A gene therapy composition of claim 1, characterized in that, in order to activate induction of non-p53-dependent apoptosis, amounts among the apoptosis-inducing genes of p53 genes, FUS-1 genes and TRAIL genes are double amount of IL-24 genes.

6. A gene therapy composition of claim 1, characterized in that, in order to activate p53-dependent apoptosis induction, amount among the apoptosis-inducing genes of p53 genes is double amounts of FUS-1 genes, TRAIL genes and IL-24 genes.

7. A gene therapy composition of claim 1, characterized in that, in order to inhibit proliferation of cancer cells through MAPK pathway, amount among the apoptosis-inducing genes of FUS-1 genes is double to five fold amounts of p53 genes, TRAIL genes and IL-24 genes.

8. A gene therapy composition of claim 1, characterized in that, in order to achieve anti-tumor effect against melanoma, amount among the apoptosis-inducing genes of IL-24 genes is double amounts of p53 genes, FUS-1 genes and TRAIL genes.

9. A gene therapy composition of claim 1, characterized in that, in order to achieve anti-tumor effect against lung cancer and/or breast cancer, amounts among the apoptosis-inducing genes of FUS-1 genes and IL-24 genes are double amounts of p53 genes and TRAIL genes.

10. A gene therapy composition of claim 1, characterized in that, in order to enhance angiogenesis inhibition action and sensitivity to radiotherapy, amounts among the apoptosis-inducing genes of TRAIL genes and IL-24 genes are double amounts of

p53 genes and FUS-1 genes.

11. A gene therapy composition of claim 1, characterized in that, for achieving anti-tumor effect and side effect mitigation in combined use with chemotherapy, amounts among the apoptosis-inducing genes of p53 genes and TRAIL genes are double amounts of FUS-1 genes and IL-24 genes.

12. A gene therapy composition of claim 1, characterized in that the gene therapy composition additionally contains p16 genes and/or p21 genes, and contained amounts of the genes are equal.

13. A gene therapy composition of any of claim 1 to claim 12, characterized in that the gene therapy composition is encapsulated by a cationic lipid and helper lipid liposome-based vector for gene therapy that targets cancer cells.

14. A gene therapy composition of claim 13, characterized in that the liposome is composed of monolayer membrane having an internal cavity, and the membrane is composed of a monolayer thin membrane compound comprising a capsule-like vector with attached ligand and containing dioleoylphosphatidylethanolamine and dimethylaminoethancarbamoylcholesterol.

15. A gene therapy composition of any of claim 1 to claim 14, characterized in that the gene therapy composition further comprises genes as plasmid vectors containing proto-oncogenes and cytokine genes.

16. A gene therapy composition of claim 15, characterized in that the cytokine genes comprise IL-12 genes or IL-18 genes.

17. A method of preparing a liposome for a gene therapy composition according to the present invention comprising:

a mixing step for preparing a lipid solution by mixing dioleoylphosphatidylethanolamine and dimethylaminoethancarbamoylcholesterol in chloroform;

a coating forming step for forming a coating by drying the prepared solution;

a vacuum-holding step for maintaining the formed coating under vacuum;

a resuspending step for resuspending the thin membrane by addition of water, dextrose or VORTEX®;

a culturing step for culturing the suspended suspension at 4°C;

an ultrasound step for forming liposomes from the suspension or the cultured suspension by performing ultrasonic irradiation processing; and

a shaping step for extrusion-shaping liposomes using a polycarbonate filter of same size as liposomes to be prepared.