An objective of the present invention is to provide a population of cancer stem cells from which cells having no cancer-forming ability have been substantially removed, wherein the population has a feature of reproducing the cancer-tissue hierarchical structure. In addition, another objective of the present invention is to provide a method for producing a population of cancer stem cells from which cells having no cancer-forming ability have been substantially removed, which comprises the step of adherently culturing a cell group containing cancer stem cells. Furthermore, another objective of the present invention is to provide a method of searching for drug target molecules, a method for assessing drugs, and a method for screening for drugs, using a non-human animal model transplanted with the cancer stem cell population or a culture system of the cancer stem cell population under *in vitro* conditions.

Summary

In one aspect, the present invention refers to a population of cancer stem cells from which cells with no cancer-forming ability have been substantially removed by a method comprising the step of adherently culturing a cell group containing cancer stem cells, wherein the cancer stem cells are derived from a human tumor tissue and the population is characterized by reproducing the hierarchical structure of a cancer tissue, and wherein the cell group that reproduces the hierarchical structure of a cancer tissue is a cancer cell line established in a non-human animal, wherein the frequency of cancer stem cells in extreme limiting dilution analysis is 1/20 or higher and wherein the cancer stem cell population comprises 1×10^4 or more cancer stem cells.

In another aspect, the present invention refers to a method of producing a population of cancer stem cells from which cells with no cancer-forming ability have been substantially removed, wherein the method comprises adherently culturing a cell group containing cancer stem cells, wherein the cancer stem cells are derived from a humor tumor tissue and the population is characterized by reproducing the hierarchical structure of a cancer tissue, and wherein the cell group that reproduces the hierarchical structure of a cancer tissue is a cancer cell line established in a non-human animal, wherein the frequency of cancer stem cells in extreme limiting dilution analysis is 1/20 or higher and wherein the cancer stem cell population comprises 1×10^4 or more cancer stem cells.

In yet another aspect, the present invention refers to a method of searching for a target molecule of a drug, wherein assessment is performed in a non-human animal model transplanted with the cancer stem cell population as defined herein, or in a culture system of the cancer stem cell population under *in vitro* conditions, by using as an index a hierarchical structure formed from cancer stem cells, a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells.

In a further aspect, the present invention refers to a method of assessing a drug, wherein assessment is performed in a non-human animal model transplanted with the cancer stem cell population as defined herein, or in a culture system of the cancer stem cell population under in vitro conditions, by using as an index a hierarchical structure formed from cancer stem cells, a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells.

In yet another aspect, the present invention refers to a method of screening for a drug, wherein assessment is performed in a non-human animal model transplanted with the cancer stem cell population as defined herein, or in a culture system of the cancer stem cell population under *in vitro* conditions, by using as an index a hierarchical structure formed from cancer stem cells, a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells.

[Means for Solving the Problems]

To solve the above problems, the present inventors conducted extensive studies.

The inventors established a cancer cell line in severely immunodeficient mice to compare and analyze cancers with or without a hierarchical structure using the established cell line. In addition, due to the lack of methods for isolating, concentrating, homogenizing, and mass-culturing cancer stem cells for cancer with a hierarchical structure that belongs to the cancer stem cell model, analysis and drug screening using cancer stem cells is compromised, and the inventors attempted to solve these problems.

The inventors transplanted a human cancer tissue into NOD/SCID/gamma$_c^{null}$ mice which have no functional T, B, and natural killer cells (Fujii E. et al., Pathol Int. 2008; 58: 559-567) (hereinafter, these mice and NSG mice (NOD-scid, IL-2Rg^{null} mice) are referred to as "NOG mouse" in the present application) to establish several human cancer cell lines. These lines can form cancer tissue with the same morphology as that of the original cancer tissue even after repeated subcutaneous passage in NOG mice, suggesting that human cancer stem cells can be stored in the cell population. Thus, human cancer stem cells that can be passaged in NOG mice are a very useful research tool.

The inventors repeatedly grew human cancer tissues in NOG mice, and then isolated cancer cells for comparing various culture methods. As a result, the present invention is completed by successfully obtaining a homogeneous cancer stem cell composition in which cells having cancer-forming ability and cells without cancer-forming ability does not substantially coexist by an adherent culture method using a serum-free stem cell medium, rather than by a routine floating culture method.

Cancer stem cells obtained by this method were stably maintained for over a month without phenotypic change by repeated passage of the adherent culture using a serum-free stem cell medium. These cells expressed various colon cancer stem cell markers (CD133, CD44,