CLAIMS

1. 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, spheroid, or cells positive for at least one or more cancer stem cell markers selected from CD24, CD29, CD34, CD44, CD49f, CD56, CD90, CD117, CD133, CD135, CD166, CD184, CD271, CD326, Aldefluor, ABCG2, ABCG5, LGR5, and Msi1.

2. The cancer stem cell population of claim 1, wherein the human tumor tissue is derived from epithelial cancer.

3. The cancer stem cell population of claim 2, wherein the epithelial cancer is pancreatic cancer, prostate cancer, breast cancer, skin cancer, gastrointestinal cancer, lung cancer, hepatocellular carcinoma, cervical cancer, endometrial cancer, ovarian cancer, fallopian tube cancer, vaginal cancer, liver cancer, bile duct cancer, bladder cancer, urinary tract cancer, thyroid cancer, adrenal cancer, kidney cancer, or other glandular tissue cancer.

4. The cancer stem cell population of any one of claims 1 to 3, which is substantially homogeneous.

5. The cancer stem cell population of any one of claims 1 to 4, wherein the frequency of cancer stem cells in extreme limiting dilution analysis is 1/20 or higher.

6. The cancer stem cell population of any one of claims 1 to 5, which comprises 1×10^4 or more cancer stem cells.

7. The cancer stem cell population of any one of claims 1 to 6, which is produced by a method comprising the steps of:
(1) transplanting a cell group containing cancer stem cells into a non-human animal that belongs to the same or different species to produce a cancer cell mass;
(2) fragmenting the produced cancer cell mass; and
(3) adherently culturing the cell population obtained in step (2) in a stem cell medium.

8. The cancer stem cell population of any one of claims 1 to 7, wherein the non-human animal is a nude mouse, a SCID mouse, a NOD-SCID mouse, a NOG mouse, or a nude rat.

9. 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, spheroid, or cells positive for at least one or more cancer stem cell markers selected from CD24, CD29, CD34, CD44, CD49f, CD56, CD90, CD117, CD133, CD135, CD166, CD184, CD271, CD326, Aldefluor, ABCG2, ABCG5, LGR5, and Msi1.

10. The method of claim 9, wherein the cell group containing cancer stem cells is allowed to proliferate before performing adherent culture.

11. The method of claim 10, wherein the cell group containing cancer stem cells is allowed to proliferate by spheroid culture.

12. The method of claim 10, wherein the cell group is proliferated by being transplanted to and passaged in a non-human animal.

13. The method of any one of claims 9 to 12, wherein the human tumor tissue is derived from epithelial cancer.

14. The method of claim 13, wherein the epithelial cancer is pancreatic cancer, prostate cancer, breast cancer, skin cancer, gastrointestinal cancer, lung cancer, hepatocellular carcinoma, cervical cancer, endometrial cancer, ovarian cancer, fallopian tube cancer, vaginal cancer, liver cancer, bile duct cancer, bladder cancer, urinary tract cancer, thyroid cancer, adrenal cancer, kidney cancer, or other glandular tissue cancer.

15. The method of any one of claims 9 to 14, wherein the non-human animal is a nude mouse, a SCID mouse, a NOD-SCID mouse, a NOG mouse, or a nude rat.

16. 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 of any one of claims 1 to 8, 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.

17. The method of claim 16, wherein the method comprises the steps of:
(1) producing a non-human animal model by transplanting the cancer stem cell population of any one of claims 1 to 8 to a non-human animal;
(2) collecting a tissue section showing a tissue structure characteristic of a cancer development process of said cancer stem cell population or showing a biological property thereof,
(3) examining the tissue section collected in (2) for the expression of a DNA, RNA, protein, peptide, or metabolite; and
(4) identifying a DNA, RNA, protein, peptide or metabolite that varies depending on a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells, in the tissue section.

18. The method of claim 16, wherein the method comprises the steps of:
(1) culturing the cancer stem cell population of any one of claims 1 to 8 under *in vitro* conditions to reproduce a characteristic structure of a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells;
(2) examining the expression of a DNA, RNA, protein, peptide or metabolite in cultured cells with the reproduced characteristic structure; and
(3) identifying a DNA, RNA, protein, peptide or metabolite that varies depending on a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, and a biological property of the cancer stem cells, in the cultured cells.

19. The method of any one of claims 16 to 18, wherein the drug is an anticancer agent.

20. The method of any one of claims 16 to 19, wherein the target molecule is a cancer cell marker.

21. A method of assessing a drug, wherein assessment is performed in a non-human animal

model transplanted with the cancer stem cell population of any one of claims 1 to 8, 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.

22. The method of claim 21, wherein the method comprises the steps of:
(1) producing a non-human animal model by transplanting the cancer stem cell population of any one of claims 1 to 8 to a non-human animal;
(2) administering a test substance to the non-human animal model of (1);
(3) collecting a tissue section showing a tissue structure characteristic of a cancer development process originating from cancer stem cells or showing a biological property thereof;
(4) observing a change in the cancer stem cells over time, cancer development process, or a biological property thereof, in the tissue section; and
(5) identifying formation of a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells, that is inhibited by the test substance.

23. The method of claim 21, wherein the method comprises the steps of:
(1) culturing the cancer stem cell population of any one of claims 1 to 8 under *in vitro* conditions to reproduce a characteristic structure of a cancer development process originating from cancer stem cells or a biological property of cancer stem cells;
(2) treating the cultured cells of (1) with a test substance;
(3) observing changes in a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells; and
(4) identifying a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells, that is inhibited by the test substance.

24. A method of screening for a drug, wherein assessment is performed in a non-human animal model transplanted with the cancer stem cell population of any one of claims 1 to 8, 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.

25. The method of claim 24, wherein the method comprises the steps of:

(1) producing a non-human animal model by transplanting the cancer stem cell population of any one of claims 1 to 8 to a non-human animal;
(2) administering a test substance to the non-human animal model of (1);
(3) collecting a tissue section that shows a tissue structure characteristic of a cancer development process originating from cancer stem cells, or shows a biological property thereof;
(4) observing a change in the cancer stem cells over time, cancer development process, or a biological property thereof, in the tissue section; and
(5) identifying a test substance that inhibits formation of a hierarchical structure formed from specific cancer stem cells, a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells.

26. The method of claim 24, wherein the method comprises the steps of:
(1) culturing the cancer stem cell population of any one of claims 1 to 8 under *in vitro* conditions to reproduce a characteristic structure of a cancer development process originating from cancer stem cells or a biological property of cancer stem cells;
(2) treating the cultured cells of (1) with a test substance;
(3) observing a change in 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; and
(4) identifying a test substance that inhibits formation of a hierarchical structure formed from specific cancer stem cells, a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells.

27. The method of any one of claims 21 to 26, wherein the drug is an anticancer agent.

28. The method of any one of claims 16, 18, 21, 23, 24, and 26, wherein the culture system under *in vitro* conditions is spheroid culture.

CLAIMS

1. 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.

2. The cancer stem cell population of claim 1, wherein the human tumor tissue is derived from epithelial cancer.

3. The cancer stem cell population of claim 2, wherein the epithelial cancer is pancreatic cancer, prostate cancer, breast cancer, skin cancer, gastrointestinal cancer, lung cancer, hepatocellular carcinoma, cervical cancer, endometrial cancer, ovarian cancer, fallopian tube cancer, vaginal cancer, liver cancer, bile duct cancer, bladder cancer, urinary tract cancer, thyroid cancer, adrenal cancer, kidney cancer, or other glandular tissue cancer.

4. The cancer stem cell population of any one of claims 1 to 3, which is substantially homogeneous.

5. The cancer stem cell population of any one of claims 1 to 4, which is produced by a method comprising the steps of:
(1) transplanting a cell group containing cancer stem cells into a non-human animal that belongs to the same or different species to produce a cancer cell mass;
(2) fragmenting the produced cancer cell mass; and
(3) adherently culturing the cell population obtained in step (2) in a stem cell medium.

6. The cancer stem cell population of any one of claims 1 to 5, wherein the non-human animal is a nude mouse, a SCID mouse, a NOD-SCID mouse, a NOG mouse, or a nude rat.

7. 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.

8. The method of claim 7, wherein the cell group containing cancer stem cells is allowed to proliferate before performing adherent culture.

9. The method of claim 8, wherein the cell group containing cancer stem cells is allowed to proliferate by spheroid culture.

10. The method of claim 8, wherein the cell group is proliferated by being transplanted to and passaged in a non-human animal.

11. The method of any one of claims 7 to 10, wherein the human tumor tissue is derived from epithelial cancer.

12. The method of claim 11, wherein the epithelial cancer is pancreatic cancer, prostate cancer, breast cancer, skin cancer, gastrointestinal cancer, lung cancer, hepatocellular carcinoma, cervical cancer, endometrial cancer, ovarian cancer, fallopian tube cancer, vaginal cancer, liver cancer, bile duct cancer, bladder cancer, urinary tract cancer, thyroid cancer, adrenal cancer, kidney cancer, or other glandular tissue cancer.

13. The method of any one of claims 7 to 12, wherein the non-human animal is a nude mouse, a SCID mouse, a NOD-SCID mouse, a NOG mouse, or a nude rat.

14. 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 of any one of claims 1 to 6, 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.

15. The method of claim 14, wherein the method comprises the steps of:
(1) producing a non-human animal model by transplanting the cancer stem cell population of any

one of claims 1 to 6 to a non-human animal;
(2) collecting a tissue section showing a tissue structure characteristic of a cancer development process of said cancer stem cell population or showing a biological property thereof,
(3) examining the tissue section collected in (2) for the expression of a DNA, RNA, protein, peptide, or metabolite; and
(4) identifying a DNA, RNA, protein, peptide or metabolite that varies depending on a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells, in the tissue section.

16. The method of claim 14, wherein the method comprises the steps of:
(1) culturing the cancer stem cell population of any one of claims 1 to 8 under *in vitro* conditions to reproduce a characteristic structure of a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells;
(2) examining the expression of a DNA, RNA, protein, peptide or metabolite in cultured cells with the reproduced characteristic structure; and
(3) identifying a DNA, RNA, protein, peptide or metabolite that varies depending on a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, and a biological property of the cancer stem cells, in the cultured cells.

17. The method of any one of claims 14 to 16, wherein the drug is an anticancer agent.

18. The method of any one of claims 14 to 17, wherein the target molecule is a cancer cell marker.

19. A method of assessing a drug, wherein assessment is performed in a non-human animal model transplanted with the cancer stem cell population of any one of claims 1 to 6, 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.

20. The method of claim 19, wherein the method comprises the steps of:
(1) producing a non-human animal model by transplanting the cancer stem cell population of any one of claims 1 to 6 to a non-human animal;
(2) administering a test substance to the non-human animal model of (1);

(3) collecting a tissue section showing a tissue structure characteristic of a cancer development process originating from cancer stem cells or showing a biological property thereof;
(4) observing a change in the cancer stem cells over time, cancer development process, or a biological property thereof, in the tissue section; and
(5) identifying formation of a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells, that is inhibited by the test substance.

21. The method of claim 19, wherein the method comprises the steps of:
(1) culturing the cancer stem cell population of any one of claims 1 to 6 under *in vitro* conditions to reproduce a characteristic structure of a cancer development process originating from cancer stem cells or a biological property of cancer stem cells;
(2) treating the cultured cells of (1) with a test substance;
(3) observing changes in a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells; and
(4) identifying a hierarchical structure formed from the cancer stem cells, a cancer development process originating from the cancer stem cells, or a biological property of the cancer stem cells, that is inhibited by the test substance.

22. A method of screening for a drug, wherein assessment is performed in a non-human animal model transplanted with the cancer stem cell population of any one of claims 1 to 6, 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.

23. The method of claim 22, wherein the method comprises the steps of:
(1) producing a non-human animal model by transplanting the cancer stem cell population of any one of claims 1 to 6 to a non-human animal;
(2) administering a test substance to the non-human animal model of (1);
(3) collecting a tissue section that shows a tissue structure characteristic of a cancer development process originating from cancer stem cells, or shows a biological property thereof;
(4) observing a change in the cancer stem cells over time, cancer development process, or a biological property thereof, in the tissue section; and
(5) identifying a test substance that inhibits formation of a hierarchical structure formed from specific cancer stem cells, a cancer development process originating from cancer stem cells, or a

biological property of cancer stem cells.

24. The method of claim 22, wherein the method comprises the steps of:
(1) culturing the cancer stem cell population of any one of claims 1 to 6 under *in vitro* conditions to reproduce a characteristic structure of a cancer development process originating from cancer stem cells or a biological property of cancer stem cells;
(2) treating the cultured cells of (1) with a test substance;
(3) observing a change in 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; and
(4) identifying a test substance that inhibits formation of a hierarchical structure formed from specific cancer stem cells, a cancer development process originating from cancer stem cells, or a biological property of cancer stem cells.

25. The method of any one of claims 19 to 24, wherein the drug is an anticancer agent.

26. The method of any one of claims 14, 16, 19, 21, 22, and 24, wherein the culture system under *in vitro* conditions is spheroid culture.