

CLAIMS

1. A method of (i) screening for an anti-cancer agent, (ii) assessing a pharmaceutical agent for treating or preventing a human cancer, or (iii) searching for an anti-cancer agent target, wherein assessment is carried out, using as an indicator, hierarchical organization, cancer progression process, or biological property of a cancer cell in a non-human animal model transplanted with an NOG-established cancer cell line or in an *in vitro* culture system of an NOG-established cancer cell line, wherein the cancer is derived from colon cancer, stomach cancer, breast cancer, lung cancer or pancreatic cancer.

2. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:

(1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line ;

(2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line, wherein the tissue piece or tissue structure is collected from the non-human animal model of (1) which has been administered a test substance;

(3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece; and

(4) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

3. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:

(1) culturing *in vitro* an NOG-established cancer cell line to mimic a biological property or a structure characteristic of each cancer progression process;

(2) treating the cultured cell of (1) with a test substance;

(3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the cultured cells; and

(4) identifying the hierarchical organization formation, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

4. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:

(1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line;

(2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line, wherein the tissue piece or tissue structure is collected from the non-human animal model of (1) which has been administered a test substance;

(3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece; and

(4) identifying a test substance that inhibits the formation of the hierarchical organization, cancer progression process, or biological property of a specific cancer cell.

5. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:

(1) culturing *in vitro* an NOG-established cancer cell line to mimic a biological property or a structure characteristic of each cancer progression process;

(2) treating the cultured cells of (1) with a test substance;

(3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell among the cultured cells; and

(4) identifying a test substance that inhibits hierarchical organization formation, cancer progression process, or biological property of a specific cancer cell.

6. The method of claim 1 for assessing a pharmaceutical agent for treating or preventing a human cancer, which comprises the steps of:

(1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line;

(2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line, wherein the tissue piece or tissue structure is collected from the non-human animal model of (1) which has been administered a test substance;

(3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece; and

- (4) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

7. The method of claim 1 for assessing a pharmaceutical agent for treating or preventing a human cancer, which comprises the steps of:

- (1) culturing *in vitro* an NOG-established cancer cell line to mimic a structure characteristic of each cancer progression process or a biological property thereof;
- (2) treating the cultured cell of (1) with a test substance;
- (3) monitoring a time-dependent change of the hierarchical organization, cancer progression process, or biological property of a cancer cell among the cultured cells; and
- (4) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

8. The method of searching for an anti-cancer agent target of claim 1, which comprises the steps of:

- (1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line;
- (2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line;
- (3) assessing the expression of DNA, RNA, or protein in the collected tissue piece of (2); and
- (4) identifying a gene or protein that changes in a manner dependent of the hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece.

9. The method of searching for an anti-cancer agent target of claim 1, which comprises the steps of:

- (1) culturing *in vitro* an NOG-established cancer cell line to mimic a biological property or a structure characteristic of each cancer progression process;
- (2) assessing the expression of DNA, RNA, or protein in a cultured cell mimicking a specific structure; and
- (3) identifying a gene or protein in the cultured cell that changes in a manner dependent of the hierarchical organization, cancer progression process, or biological property of

a cancer cell.

10. A non-human animal model, which is obtained by transplanting, into a non-human animal of the same or different species, an NOG-established cancer cell line established by
5 transplanting a human tumor tissue into a severely-immunodeficient NOD/SCID/gamma(c)(null) (NOG) mouse,
wherein the non-human animal model mimics hierarchical organization or cancer progression process of a human cancer tissue,
wherein the human tumor tissue is derived from colon cancer, stomach cancer, breast cancer,
10 lung cancer, or pancreatic cancer.
11. The non-human animal model of claim 10, which enables
detection of a unique cancer stem cell having a characteristic for mimicking
hierarchical organization and cancer progression process of a human cancer tissue,
15 and,
testing of a biological property of the human cancer tissue.
12. The non-human animal model of claim 10, which enables observation of the process
leading to transition of a cancer cell into a tumor stroma-forming fibroblast through complete
20 epithelial-mesenchymal transition (complete-EMT) during the process that mimics
hierarchical organization of a human cancer tissue.

CLAIMS

1. A method of (i) screening for an anti-cancer agent, (ii) assessing a pharmaceutical agent for treating or preventing a human cancer, or (iii) searching for an anti-cancer agent target, wherein assessment is carried out, using as an indicator, hierarchical organization, cancer progression process, or a biological property of a donor cancer tissue that is mimicked in a non-human animal model transplanted with an NOG-established cancer cell line or in an *in vitro* culture system of an NOG-established cancer cell line, wherein the cancer is colon cancer, stomach cancer, breast cancer, lung cancer or pancreatic cancer.
2. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:
 - (1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line ;
 - (2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line, wherein the tissue piece or tissue structure is collected from the non-human animal model of (1) which has been administered a test substance;
 - (3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece; and
 - (4) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.
3. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:
 - (1) culturing *in vitro* an NOG-established cancer cell line to mimic a biological property or a structure characteristic of each cancer progression process;
 - (2) treating the cultured cell of (1) with a test substance;
 - (3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the cultured cells; and
 - (4) identifying the hierarchical organization formation, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

4. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:

(1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line;

5 (2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line, wherein the tissue piece or tissue structure is collected from the non-human animal model of (1) which has been administered a test substance;

10 (3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece; and

(4) identifying a test substance that inhibits the formation of the hierarchical organization, cancer progression process, or biological property of a specific cancer cell.

15 5. The method of screening for an anti-cancer agent of claim 1, which comprises the steps of:

(1) culturing *in vitro* an NOG-established cancer cell line to mimic a biological property or a structure characteristic of each cancer progression process;

(2) treating the cultured cells of (1) with a test substance;

20 (3) monitoring a time-dependent change of hierarchical organization, cancer progression process, or a biological property of a donor cancer tissue among the cultured cells; and

(4) identifying a test substance that inhibits hierarchical organization formation, cancer progression process, or a biological property of a specific donor cancer tissue.

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6. The method of claim 1 for assessing a pharmaceutical agent for treating or preventing a human cancer, which comprises the steps of:

(1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line;

30 (2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line, wherein the tissue piece or tissue structure is collected from the non-human animal model of (1) which has been administered a test substance;

35 (3) monitoring a time-dependent change of hierarchical organization, cancer progression

process, or biological property of a cancer cell in the tissue piece; and

- (4) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

5 7. The method of claim 1 for assessing a pharmaceutical agent for treating or preventing a human cancer, which comprises the steps of:

- (1) culturing *in vitro* an NOG-established cancer cell line to mimic a structure characteristic of each cancer progression process or a biological property thereof;
- (2) treating the cultured cell of (1) with a test substance;
- 10 (3) monitoring a time-dependent change of the hierarchical organization, cancer progression process, or biological property of a cancer cell among the cultured cells; and
- (4) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

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8. The method of searching for an anti-cancer agent target of claim 1, which comprises the steps of:

- (1) preparing a non-human animal model which is a non-human animal transplanted with an NOG-established cancer cell line;
- 20 (2) collecting a tissue piece that exhibits a biological property or a tissue structure characteristically seen in the cancer progression process in each area, when starting from a cell line same as the NOG-established cancer cell line;
- (3) assessing the expression of DNA, RNA, or protein in the collected tissue piece of (2); and
- 25 (4) identifying a gene or protein that changes in a manner dependent of the hierarchical organization, cancer progression process, or biological property of a cancer cell in the tissue piece.

9. The method of searching for an anti-cancer agent target of claim 1, which comprises the steps of:

- (1) culturing *in vitro* an NOG-established cancer cell line to mimic a biological property or a structure characteristic of each cancer progression process;
- (2) assessing the expression of DNA, RNA, or protein in a cultured cell mimicking a specific structure; and
- 35 (3) identifying a gene or protein in the cultured cell that changes in a manner dependent

of the hierarchical organization, cancer progression process, or biological property of a cancer cell.

10. A non-human animal model, which is obtained by transplanting, into a non-human
5 animal of the same or different species, an NOG-established cancer cell line established by transplanting a human tumor tissue into a severely-immunodeficient NOD/SCID/gamma(c)(null) (NOG) mouse,

wherein the non-human animal model mimics hierarchical organization or cancer progression process of a human cancer tissue,

10 wherein the human tumor tissue is derived from colon cancer, stomach cancer, breast cancer, lung cancer, or pancreatic cancer, and

wherein the NOG-established cancer cell line is one that was established so as to meet the criterion that it can be transplanted or passaged over three or more generations.

15 11. The non-human animal model of claim 10, which enables

detection of a unique cancer stem cell having a characteristic for mimicking hierarchical organization and cancer progression process of a human cancer tissue, and,

testing of a biological property of the human cancer tissue.

20 12. The non-human animal model of claim 10, which enables observation of the process leading to transition of a cancer cell into a tumor stroma-forming fibroblast through complete epithelial-mesenchymal transition (complete-EMT) during the process that mimics hierarchical organization of a human cancer tissue.

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