

[Claims]

1. A ~~non-human animal~~mouse model, which is obtained by subcutaneously transplanting, into a ~~non-human animal of the same or different species~~mouse, an NOG-established cancer cell line established by transplanting a human tumor tissue into a severely-immunodeficient NOD/SCID/gamma(c)(null) (NOG) mouse, which mimics hierarchical organization and cancer progression process of a human cancer tissue,

wherein the NOG-established cancer cell lines can be transplanted or passaged over three or more generations in NOG mouse, and

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

2. The ~~non-human animal~~mouse model of claim 1, 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.

3. The ~~non-human animal~~mouse model of claim 1, 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.

4. A ~~non-human animal~~mouse model, which enables assessment of the cause of anti-cancer agent resistance by administering an anti-cancer agent into the ~~non-human animal~~mouse model of any one of claims 1 to 3, and assessing, as an indicator, hierarchical organization, cancer progression process, or biological property of a cancer cell, or transition through complete epithelial-mesenchymal transition (complete-EMT).

~~5. The non-human animal model of any one of claims 1 to 3, wherein the human tumor tissue is derived from colon cancer, stomach cancer, breast cancer, lung cancer, or pancreatic cancer.~~

~~6~~5. A method of 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 mimicked human cancer cell in a ~~non-human animal~~mouse model subcutaneously transplanted with an NOG-established cancer cell line prepared as defined in claim 1 established by transplanting human tumor tissue to an NOG mouse or

in an *in vitro* culture system of an NOG-established cancer cell line,

wherein the NOG-established cancer cell lines can be transplanted or passaged over three or more generations in NOG mouse, and

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

76. The method of searching for an anti-cancer agent target of claim **65**, which comprises the steps of (1) to **(42)** below:

- ~~(1) preparing a non-human animal mouse model by subcutaneously transplanting an NOG-established cancer cell line into a non-human animal mouse;~~
- ~~(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;~~
- (31)** assessing the expression of DNA, RNA, or protein in ~~the a~~ collected tissue piece that has been collected from a NOG mouse, wherein said NOG mouse has been in a first step prepared as defined in claim 1 and wherein the collected tissue piece 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 ~~of (2)~~; and
- (42)** identifying a gene/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;

87. The method of searching for an anti-cancer agent target of claim **65**, which comprises the steps of (1) to (3) below:

- (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/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.

98. A method of screening for an anti-cancer agent, wherein assessment is carried out, using as an indicator, hierarchical organization, cancer progression process, or biological property of a mimicked human cancer cell in a ~~non-human animal~~ mouse model subcutaneously transplanted with an NOG-established cancer cell line prepared as

~~defined in claim 1 established by transplanting human tumor tissue into an NOG mouse~~
 or in an *in vitro* culture system of an NOG-established cancer cell line,
wherein the NOG-established cancer cell lines can be transplanted or passaged over
three or more generations in NOG mouse, and
wherein the human tumor tissue is derived from colon cancer, stomach cancer,
breast cancer, lung cancer, or pancreatic cancer.

~~109.~~ The method of screening for an anti-cancer agent of claim 98, which comprises the steps of (1) to (~~5~~2) below:

- ~~(1) preparing a non-human animal mouse model by subcutaneously transplanting an NOG-established cancer cell line into a non-human animal mouse;~~
- ~~(2) administering a test substance to the non-human animal mouse of (1);~~
- ~~(3) 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;~~
- (41) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in ~~the a~~ tissue piece that has been collected from a NOG mouse, wherein said NOG mouse has been in a first step prepared as defined in claim 1 and wherein in a second step a test substance has been administered to said prepared NOG mouse, wherein the collected tissue piece 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; and
- (~~5~~2) identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

~~110.~~ The method of screening for an anti-cancer agent of claim 98, which comprises the steps of (1) to (4) below:

- (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 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.

~~1211.~~ The method of screening for an anti-cancer agent of claim 98, which comprises the

steps of (1) to (~~5~~7) below:

- ~~(1) preparing a non-human animal mouse model by subcutaneously transplanting an NOG-established cancer cell line into a non-human animal mouse;~~
- ~~(2) administering a test substance to the non-human animal mouse model of (1);~~
- ~~(3) 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;~~
- (4) monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in ~~the~~a tissue piece that has been collected from a NOG mouse, wherein said NOG mouse has been in a first step prepared as defined in claim 1 and wherein in a second step a test substance has been administered to said prepared NOG mouse, wherein the collected tissue piece 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; and
- (5) identifying a test substance that inhibits the formation of the hierarchical organization, cancer progression process, or biological property of a specific cancer cell₂.

~~13~~12. The method of screening for an anti-cancer agent of claim ~~9~~8, which comprises the steps of (1) to (4) below:

- (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 in 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.

~~14~~13. A method for assessing a pharmaceutical agent for treating or preventing a human cancer, wherein assessment is carried out, using as an indicator, hierarchical organization, cancer progression process, or biological property of a mimicked human cancer cell in a ~~non-human animal mouse~~ model subcutaneously transplanted with an NOG-established cancer cell line established by transplanting human tumor tissue into an NOG mouse prepared as defined in claim 1 or in an *in vitro* culture system of an NOG-established cancer cell line, wherein the NOG-established cancer cell lines can be transplanted or passaged over three or more generations in NOG mouse, and

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

~~15~~**14.** The method of claim ~~14~~**13** for assessing a pharmaceutical agent, which comprises the steps of (1) to (~~4~~**5**) below:

- ~~(1) preparing a non-human animal mouse model by subcutaneously transplanting an NOG-established cancer cell line into a non-human animal mouse;~~
- ~~(2) administering a test substance to the non-human animal mouse model of (1);~~
- ~~(3) 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;~~
- ~~(4)~~ **(5)** monitoring a time-dependent change of hierarchical organization, cancer progression process, or biological property of a cancer cell in ~~the a~~ tissue piece that has been collected from a NOG mouse, wherein said NOG mouse has been in a first step prepared as defined in claim 1 and wherein in a second step a test substance has been administered to said prepared NOG mouse, wherein the collected tissue piece 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; and
- ~~(5)~~ **(2)** identifying the formation of the hierarchical organization, cancer progression process, or biological property of a cancer cell, which is inhibited by the test substance.

~~16~~**15.** The method of claim ~~14~~**13** for assessing a pharmaceutical agent for treating or preventing a human cancer, which comprises the steps of (1) to (4) below:

- (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 cells 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 in 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.