cancer tissue or a tissue analogous to a cancer tissue which was treated with an anticancer agent is three-dimensionally cultured, and elucidated the properties thereof. The newly discovered small cell clusters resemble the survival pattern of cancer cells in a patient's body when treated with an anticancer agent, which are found in cancer tissues removed from cases in which chemotherapy has been extremely effective. Furthermore, they found that the cells in the small cell clusters found in the culture were similar to surviving cancer cells in a patient's body when treated with an anticancer agent in terms of CSC phenotype and mRNA expression level. Thus, the present inventors succeeded in artificially regenerating, as a model of an anticancer agent-resistant cancer tissue, treatment-resistant cell clusters having the characteristics of surviving cancer cells during treatment with an anticancer agent in a patient's cancer tissue, and completed the present disclosure.

To date, there have been reports of the establishment of organoids from cancer tissues (J N. Buzzelli et al., Stem Cell Res (2018) 27: 109-120), C. S Verissimo et al., Elife (2016) 5. Pii. : e18489). However, there are no known examples of attempting three-dimensional culture after anticancer agent treatment. That is, the present disclosure provides the following culture methods:

[0009] In one aspect, the present invention refers to a culture method, wherein the method comprises a step of three-dimensionally culturing a cancer tissue or a tissue analogous to a cancer tissue which was treated with at least one anticancer agent, and a step of recovering the cancer tissue or the tissue analogous to a cancer tissue comprising three dimensional tissue, wherein the cancer is colorectal cancer. In another aspect, the present invention refers to a method of forming a cell cluster, which comprises a step of three-dimensionally culturing a cancer tissue or a tissue analogous to a cancer tissue which was treated with at least one anticancer agent, and a step of recovering the cancer tissue or the tissue analogous to a cancer tissue comprising three dimensional tissue, wherein the cancer is colorectal cancer. Also, disclosed herein are:

(A1) a culture method, wherein the method comprises a step of three-dimensionally culturing a cancer tissue or a tissue analogous to a cancer tissue which was treated with at least one anticancer agent;

(A2) the method according to (A1), wherein treatment of the cancer tissue or tissue analogous to a cancer tissue with an anticancer agent is carried out by incubating the cancer tissue or tissue analogous to a cancer tissue in the presence of at least one anticancer agent;

(A3) the method according to any one of (A1) and (A2), wherein the anticancer agent is one or more anticancer agents selected from agents that inhibit the growth of cancer cells;

(A4) the method according to any one of (A1) to (A3), wherein the anticancer agent is a chemotherapeutic agent;

(A5) the method according to any one of (A1) to (A4), wherein the anticancer agent is 7-ethyl-10-hydroxycamptothecin, and the anticancer agent treatment is a 72-h incubation in the presence of the anticancer agent at a concentration of 300 nM;

(A6) the method according to any one of the (A1) to (A5), wherein the three-dimensional culture step comprises allowing the cancer tissue, or the tissue analogous to a cancer tissue, treated with at least one anticancer agent to adhere to a first extracellular matrix and culturing by adding a first medium;

(A7) the method according to (A6), wherein the first medium is a medium capable of three-dimensionally culturing cells or tissues;

[CLAIMS]

1. A culture method, wherein the method comprises a step of three-dimensionally culturing a cancer tissue or a tissue analogous to a cancer tissue which was treated with at least one anticancer agent, and a step of recovering the cancer tissue or the tissue analogous to a cancer tissue comprising three dimensional tissue, wherein the cancer is colorectal cancer.

2. The method according to claim 1, wherein the anticancer agent is one or more anticancer agents selected from agents that inhibit the growth of cancer cells.

3. The method according to claim 1 or 2, wherein the three-dimensional culture step comprises allowing the cancer tissue or the tissue analogous to a cancer tissue which was treated with at least one anticancer agent to adhere to a first extracellular matrix and culturing by adding a first medium.

4. The method according to any one of claims 1 to 3, wherein the tissue analogous to a cancer tissue is formed by a pre-culture step of culturing one or more cancer stem cells.

5. A method of forming a cell cluster, which comprises a step of three-dimensionally culturing a cancer tissue or a tissue analogous to a cancer tissue which was treated with at least one anticancer agent, and a step of recovering the cancer tissue or the tissue analogous to a cancer tissue comprising three dimensional tissue, wherein the cancer is colorectal cancer.

6. The method according to claim 5, wherein the anticancer agent is one or more anticancer agents selected from agents that inhibit the growth of cancer cells.

7. The method according to claim 5 or 6, wherein the three-dimensional culture step comprises allowing the cancer tissue or the tissue analogous to a cancer tissue which was treated with at least one anticancer agent to adhere to a first extracellular matrix and culturing by adding a first medium.

8. The method according to any one of claims 5 to 7, wherein the tissue analogous to a cancer tissue is formed by a pre-culture step of culturing one or more cancer stem cells.

9. A cell cluster formed by a method according to any one of claims 5 to 8.

10. The cell cluster according to claim 9, wherein at least one of the cells comprised in the cell cluster expresses at least one protein selected from LGR5, CD44 (including CD44v9), CD34, CD133, Sca-1, and CD24.

11. A method for analyzing the gene expression level, protein expression level, protein modification, protein localization, epigenome, or metabolomics state of a cell comprised in the cell cluster according to claim 9 or 10.

12. A method of identifying an agent having a therapeutic activity against cancer, wherein the method comprises: selecting at least one test agent; contacting the cell cluster according to claim 9 or 10 with the test agent; and measuring the cytotoxic activity of the test agent against a cell comprised in the cell cluster.

13. A method for identifying an agent having a therapeutic activity against cancer, wherein the method comprises: selecting at least one test agent; contacting the cell cluster according to claim 9 or 10 with the test agent; and comparing the protein expression of a cell comprised in the cell cluster in the presence and absence of the agent.

14. A method for identifying the mechanism of action of an agent, wherein the method comprises: selecting at least one test agent; contacting the cell cluster according to claim 9 or 10 with the test agent; and comparing the protein expression of a cell comprised in the cell cluster in the presence and absence of the agent.

15. A method for identifying a combination for combined use of an anticancer agent and an agent, wherein the method comprises: providing a cell cluster according to claim 9 or 10 which is resistant to a specific anticancer agent; contacting the provided cell cluster with a test agent; and measuring the cytotoxic activity of the test agent against a cell comprised in the cell cluster.