

Method for obtaining an animal model from conditionally reprogrammed cells and use of the animal model for screening anti-tumor drugs

The present invention relates to a composition for propagating a primary tumor cell obtained from a tumor biopsy sample of a patient under both *in vitro* and *in vivo* conditions, a method for propagating the obtained primary tumor cell in the composition, a method for obtaining an animal model for screening the efficacy of an anti-tumor drug using the animal model.

Background

Conditional reprogramming (CR) is a cell culture technique that can be used to rapidly and efficiently establish patient-derived cell cultures from both normal and diseased cells, including tumor cells. The greatest advantage of CR is its rapid and efficient expansion of cell cultures from patient tissue samples. This allows researchers to screen tumors for sensitivity to anticancer drugs or immunotherapies quickly enough to provide the information for clinical use.

Testing an anti-tumor drug in an animal model for its efficacy is important as such animal model is important for anti-tumor drug screening. Such an animal model will mimic the *in vivo* environment of patients, reflect the patient's response and thus is more effective. To establish an animal models a person skilled in the art usually need to obtain sufficiently large number of tumor cells. However, tumor samples obtained from patients may contain very small or even trace amount of tumor cells depending on how they are obtained. For example, a tumor sample obtained from needle biopsy may contain very small amount of tumor cells and thus very difficult for a person skilled in the art to use them to establish a desired animal model for screening anti-tumor drugs.

Tumor cell immortalization through conditional reprogramming is an invaluable tool to generate propagating tumor cells for cell-based diagnostics, drug sensitivity assay and bio-banking *in vitro*. However, how to effectively and successfully cultivate primary tumor cells obtained from patients, especially low or even trace amount of cell samples from e.g. a needle biopsy, is still a challenge.

Summary of the Invention

To solve the above problem, the inventor has successfully conditionally reprogrammed primary tumor cells from a number of tumor types towards immortalization and use the conditionally reprogrammed tumor cells to establish an animal model for testing anti-tumor drugs. The primary tumor cells are obtained from surgery tissues, biopsy or needle biopsy samples. The conditionally reprogrammed primary tumor cells obtained in the present invention exhibited typical colonized growth, which is well maintained upon cryo-frozen. In some cases, the cells can be passaged for multiple times and become useful cell lines. Like primary tumor cells, conditional reprogrammed tumor cells are reliable to test drug sensitivity *in vitro*. The inventor has successfully implanted conditional reprogrammed tumor cells (CRC) into an animal model for testing drug efficacy, and screened anti-tumor drugs using the animal model. The present invention also discloses the use of conditional reprogrammed tumor cells in drug efficacy tests.

One aspect of the present invention provides a method for obtaining an animal model for screening anti-tumor drugs, comprising

(1) cultivating a primary tumor cell obtained from a tumor biopsy sample of a patient in a composition comprising:

- a) 0.01-1.0 mg/L Hydrocortisone;
- b) 1.0 -10.0 mg/L insulin;
- c) 1.0-20.0 µg/L Cholera toxin;
- d) 2.0-50.0 mg/L Adenine;
- e) 1.0-30.0 µg/L EGF;
- f) 1.0-30.0 µmol/L Y- 27632;
- g) Pen/Strep;
- h) 2-20% FBS;
- i) F12 medium
- j) DMEM with high glucose, and optionally
- k) Non-Essential Amino Acids Solution;
- l) GlutaMAX Supplement;

(2) implanting the primary tumor cell obtained from step (1) into an animal. In embodiments, the composition in step (1) of the method of the present invention comprises:

- a) 0.3-0.5 mg/L, preferably 0.4 mg/L Hydrocortisone;
- b) 4-6 mg/L, preferably 5 mg/L insulin;
- 5 c) 7-9 μ g/L, preferably 8.3 μ g/L Cholera toxin;
- d) 20-30 mg/L, preferably 24.2 mg/L Adenine;
- e) 8-12 μ g/L, preferably 10 μ g/L EGF;
- f) 8-12 μ mol/L, preferably 10 μ mol/L Y- 27632;
- g) Pen/Strep;
- 10 h) 8-12%, preferably 10% FBS;
- i) F12 medium
- j) DMEM with high glucose, and optionally
- k) Non-Essential Amino Acids Solution;
- l) GlutaMAX Supplement.

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One aspect of the present invention provides a method for obtaining an animal model for screening anti-tumor drugs, comprising

(1) cultivating a primary tumor cell obtained from a tumor sample of a patient in a composition comprising:

- 20 a) 0.4 mg/L Hydrocortisone;
- b) 5 mg/L Insulin;
- c) 8.3 μ g/L Cholera toxin;
- d) 24.2 mg/L Adenine;
- e) 10 μ g/L EGF;
- 25 f) 10 μ mol/L Y- 27632;
- g) Pen/Strep;
- h) 10% FBS;
- i) F12 medium
- j) DMEM with high glucose
- 30 k) Non-Essential Amino Acids Solution;
- l) GlutaMAX Supplement; and
- m) a mouse embryonic fibroblast cell that has been treated with mitomycin C; and

(2) implanting the primary tumor cell obtained from step (1) into an animal, wherein the animal is a mouse.

In embodiments, the tumor biopsy sample is a needle biopsy sample. In embodiments, the concentration of the primary tumor cells in the sample obtained from a needle biopsy is less than about $2 \times 10^6/\text{mL}$, less than about $1.9 \times 10^6/\text{mL}$, less than about $1.8 \times 10^6/\text{mL}$, less than about $1.7 \times 10^6/\text{mL}$, less than about $1.6 \times 10^6/\text{mL}$, less than about $1.5 \times 10^6/\text{mL}$, less than about $1.4 \times 10^6/\text{mL}$, less than about $1.3 \times 10^6/\text{mL}$, less than about $1.2 \times 10^6/\text{mL}$, less than about $1.1 \times 10^6/\text{mL}$, less than about $1.0 \times 10^6/\text{mL}$, less than about $0.9 \times 10^6/\text{mL}$, less than about $0.8 \times 10^6/\text{mL}$, less than about $0.7 \times 10^6/\text{mL}$, less than about $0.6 \times 10^6/\text{mL}$, less than about $0.5 \times 10^6/\text{mL}$, less than about $0.4 \times 10^6/\text{mL}$, less than about $0.3 \times 10^6/\text{mL}$, less than about $0.2 \times 10^6/\text{mL}$, less than about $0.1 \times 10^6/\text{mL}$ or less, such as less than about $2 \times 10^6/\text{mL}$, less than about $1.7 \times 10^6/\text{mL}$, less than about $0.8 \times 10^6/\text{mL}$, less than about $0.64 \times 10^6/\text{mL}$, less than about $0.59 \times 10^6/\text{mL}$. In embodiments, the primary tumor cell is cultivated with a feeder cell in the composition of step (1), in particular the feeder cell is a mouse embryonic fibroblast (MEF) cell. In embodiments, the MEF cells have been treated with mitomycin C.

In embodiments, the animal in the present invention is a mouse or a rat, in particular an immunodeficient mouse, such as a nude mouse. In embodiments, the primary tumor cell obtained in step (1) was implanted in to the animal in a hollow fiber. In embodiments, the

hollow fiber is made of modified polyvinylidene fluoride, more particularly the hollow fiber is made of modified polyvinylidene fluoride and has a cut-off value of 500,000 Dalton.

Another aspect of the present invention provides an animal model for screening anti-tumor drugs
5 obtained by the method of the present invention. In embodiments, the animal in the present invention is a mouse or a rat, in particular an immunodeficient mouse, such as a nude mouse.

Another aspect of the present invention provides a method for screening anti-tumor drugs using the animal model of the present invention, comprising administering a candidate drug to the animal
10 model of the present invention. In embodiments, the above method further comprises measuring tumor cell growth in the animal model of the present invention.

Drawing

15 Figure 1 shows the morphology of conditionally reprogrammed cells in growing colonies from various tumor samples. **A**, P0, P4 and P6 passages of conditionally reprogrammed primary cells originated from surgical sample of a lung cancer patient; **B**, P3 conditionally reprogrammed cells originated from biopsy sample of a lung cancer patient; **C**, P4 conditionally reprogrammed cells originated from a needle biopsy sample of an adenoid cystic carcinoma patient; **D**, P12 cell originated
20 from a needle biopsy sample of a peritoneal mesothelioma patient; **E**, P7 cell originated from a surgical sample of a glioblastoma patient; **F**, P6 cell originated from a biopsy sample of a colonrectal cancer patient; **G**, P3 cell originated from a surgical sample of a gallbladder carcinoma patient.

Figure 2 **A**, The results of anti-tumor efficacy of test drug in MDX079 Mini-PDX models; **B**, The
25 results of anti-tumor efficacy of test drug in MDX083 Mini-PDX models; **C**, The results of anti-tumor efficacy of test drug in MDX095 Mini-PDX models; **D**, The results of anti-tumor efficacy of test drug in MDX107 Mini-PDX models; **E**, The results of anti-tumor efficacy of test drug in MDX123 Mini-PDX models.

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Figure 3 A, The results of anti-tumor efficacy of test drug in MDX133 Mini-PDX models; B, The results of anti-tumor efficacy of test drug in MDX154 Mini-PDX models; C, The results of anti-tumor efficacy of test drug in MDX164 Mini-PDX models; D, The results of anti-tumor efficacy of test drug in MDX165 Mini-PDX models; E, The results of anti-tumor efficacy of test drug in MDX168 Mini-PDX models. F, The results of anti-tumor efficacy of test drug in MDX169 Mini-PDX models; G, The results of anti-tumor efficacy of test drug in MDX174 Mini-PDX models; H, The results of anti-tumor efficacy of test drug in MDX186 Mini-PDX models; I, The results of anti-tumor efficacy of test drug in MDX189 Mini-PDX models; J, The results of anti-tumor efficacy of test drug in MDX203 Mini-PDX models.

Detailed Description of the Invention

One aspect of the present invention provides a method for obtaining an animal model for screening anti-tumor drugs, comprising (1) cultivating a primary tumor cell obtained from a tumor biopsy sample of a patient in a composition comprising:

- a) 0.01-1.0 mg/L Hydrocortisone;
- b) 1.0 -10.0 mg/L insulin;
- c) 1.0-20.0 µg/L Cholera toxin;
- d) 2.0-50.0 mg/L Adenine;
- e) 1.0-30.0 µg/L EGF;
- f) 1.0-30.0 µmol/L Y- 27632;
- g) Pen/Strep;
- h) 2-20% FBS;
- i) F12 medium
- j) DMEM with high glucose, and optionally
- k) Non-Essential Amino Acids Solution;
- l) GlutaMAX Supplement; and

(2) implanting the primary tumor cell obtained from step (1) into an animal. In embodiments, the tumor biopsy sample is a needle biopsy sample. In embodiments, the concentration of tumor cells in the sample obtained from the needle biopsy is less than about $2 \times 10^6/\text{mL}$.

The concentration of hydrocortisone in the composition of step (1) can be 0.01-1.0 mg/L, in particular about 0.05-1.0 mg/L, about 0.1-1.0 mg/L, about 0.1-0.9 mg/L, about 0.2-0.8 mg/L, about 0.2-0.7 mg/L, about 0.25-0.65mg/L, about 0.25-0.6mg/L, about 0.3-0.5mg/L, about 0.35-0.55mg/L, about 0.35-0.4mg/L, about 0.4-0.45mg/L, about 0.35-0.45mg/L, about 0.45-0.55mg/L, about 0.05 mg/L, about 0.1 mg/L, about 0.2 mg/L, about 0.3 mg/L, about 0.31 mg/L, about 0.32 mg/L, about 0.33 mg/L, about 0.34 mg/L, about 0.35 mg/L, about 0.36 mg/L, about 0.37 mg/L, about 0.38 mg/L, about 0.39 mg/L, about 0.4 mg/L, about 0.41 mg/L, about 0.42 mg/L, about 0.43 mg/L, about 0.44 mg/L, about 0.45 mg/L, about 0.46 mg/L, about 0.47 mg/L, about 0.48 mg/L, about 0.49 mg/L, about 0.5 mg/L, about 0.6 mg/L, about 0.7 mg/L, about 0.8 mg/L, about 0.9 mg/L, about 1.0 mg/L.

The concentration of insulin in the composition of step (1) can be 1.0-10 mg/L, in particular about 1-10 mg/L, about 2-9 mg/L, about 3-8 mg/L, about 3-7 mg/L, about 4.5-6.5 mg/L, about 4-6 mg/L, about 1 mg/L, about 2 mg/L, about 3 mg/L, about 4 mg/L, about 5 mg/L, about 6 mg/L, about 7 mg/L, about 8 mg/L, about 9 mg/L, about 10 mg/L.

The concentration of cholera toxin in the composition of step (1) can be 1-20 μ g/L, in particular about 2-20 μ g/L, about 3-18 μ g/L, about 4-15 μ g/L, about 5-10 μ g/L, about 6-10 μ g/L, about 7-9 μ g/L, about 7.5-8.5 μ g/L, about 1 μ g/L, about 2 μ g/L, about 3 μ g/L, about 4 μ g/L, about 5 μ g/L, about 6 μ g/L, about 7 μ g/L, about 8 μ g/L, about 8.1 μ g/L, about 8.2 μ g/L, about 8.3 μ g/L, about 8.4 μ g/L, about 8.5 μ g/L, about 8.6 μ g/L, about 8.7 μ g/L, about 8.8 μ g/L, about 8.9 μ g/L, about 9 μ g/L, about 10 μ g/L, about 11 μ g/L, about 12 μ g/L, about 13 μ g/L, about 14 μ g/L, about 15 μ g/L, about 16 μ g/L, about 17 μ g/L, about 18 μ g/L, about 19 μ g/L, about 20 μ g/L.

The concentration of adenine in the composition of step (1) can be 2-50 mg/L, in particular about 5-40 mg/L, about 10-30 mg/L, about 15-30 mg/L, about 20-30 mg/L, about 22-27 mg/L, about 23-26 mg/L, about 24-25 mg/L, about 2 mg/L, about 6 mg/L, about 8 mg/L, about 10 mg/L, about 12 mg/L, about 14 mg/L, about 16 mg/L, about 18 mg/L, about 20 mg/L, about 21 mg/L, about 22 mg/L, about 23 mg/L, about 24 mg/L, about 24.1 mg/L, about 24.2 mg/L, about 24.3 mg/L, about 24.4 mg/L, about 24.5 mg/L, about 24.6 mg/L,

about 24.7 mg/L, about 24.8 mg/L, about 24.9 mg/L, about 25 mg/L, about 26 mg/L, about 27 mg/L, about 28 mg/L, about 29 mg/L, about 30 mg/L, about 35 mg/L, about 40 mg/L.

5 The concentration of EGF in the composition of step (1) can be 1-30 $\mu\text{g/L}$, in particular about 2-20 $\mu\text{g/L}$, about 4-18 $\mu\text{g/L}$, about 6-16 $\mu\text{g/L}$, about 8-12 $\mu\text{g/L}$, about 9-11 $\mu\text{g/L}$, about 9.5-10.5 $\mu\text{g/L}$, about 2 $\mu\text{g/L}$, about 4 $\mu\text{g/L}$, about 6 $\mu\text{g/L}$, about 7 $\mu\text{g/L}$, about 8 $\mu\text{g/L}$, about 9 $\mu\text{g/L}$ about 10 $\mu\text{g/L}$, about 11 $\mu\text{g/L}$, about 12 $\mu\text{g/L}$, about 14 $\mu\text{g/L}$, about 16 $\mu\text{g/L}$, about 18 $\mu\text{g/L}$, about 20 $\mu\text{g/L}$, about 25 $\mu\text{g/L}$, about 30 $\mu\text{g/L}$.

10 The concentration of Y-27632 in the composition of step (1) can be 1-30 $\mu\text{mol/L}$, in particular about 2-20 $\mu\text{mol/L}$, about 4-18 $\mu\text{mol/L}$, about 6-16 $\mu\text{mol/L}$, about 8-12 $\mu\text{mol/L}$, about 9-11 $\mu\text{mol/L}$, about 9.5-10.5 $\mu\text{mol/L}$, about 2 $\mu\text{mol/L}$, about 4 $\mu\text{mol/L}$, about 6 $\mu\text{mol/L}$, about 7 $\mu\text{mol/L}$, about 8 $\mu\text{mol/L}$, about 9 $\mu\text{mol/L}$, about 10 $\mu\text{mol/L}$, about 11 $\mu\text{mol/L}$, about 12 $\mu\text{mol/L}$, about 14 $\mu\text{mol/L}$, about 16 $\mu\text{mol/L}$, about 18 $\mu\text{mol/L}$, about 20 $\mu\text{mol/L}$, about 25 $\mu\text{mol/L}$, about 30 $\mu\text{mol/L}$.

The concentration of FBS the composition of step (1) can be 2-20%, in particular about 4-15%, about 5-18%, about 6-16%, about 7-14%, about 8-12%, about 9-11%, about 9.5-10.5%, about 2%, about 4 %, about 6%, about 7%, about 8%, about 9%, about 10%,
20 about 11%, about 12%, about 14%, about 16 %, about 18%, about 20%.

In embodiments, the present invention provides a method for obtaining an animal model for screening anti-tumor drugs, comprising (1) cultivating a primary tumor cell obtained from a tumor biopsy sample of a patient in a composition comprising:

- 25 a) 0.3-0.5 mg/L, preferably 0.4 mg/L Hydrocortisone;
b) 4-6 mg/L, preferably 5 mg/L insulin;
c) 7-9 $\mu\text{g/L}$, preferably 8.3 $\mu\text{g/L}$ Cholera toxin;
d) 20-30 mg/L, preferably 24.2 mg/L Adenine;
e) 8-12 $\mu\text{g/L}$, preferably 10 $\mu\text{g/L}$ EGF;
30 f) 8-12 $\mu\text{mol/L}$, preferably 10 $\mu\text{mol/L}$ Y- 27632;
g) Pen/Strep;

- h) 8-12%, preferably 10% FBS;
- i) F12 medium
- j) DMEM with high glucose, and optionally
- k) Non-Essential Amino Acids Solution;
- l) GlutaMAX Supplement; and

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(2) implanting the primary tumor cell obtained from step (1) into an animal. In embodiments, the tumor biopsy sample is a needle biopsy sample. In embodiments, the concentration of the primary tumor cells in the sample obtained from a needle biopsy is less than about $2 \times 10^6/\text{mL}$, less than about $1.9 \times 10^6/\text{mL}$, less than about $1.8 \times 10^6/\text{mL}$, less than about $1.7 \times 10^6/\text{mL}$, less than about $1.6 \times 10^6/\text{mL}$, less than about $1.5 \times 10^6/\text{mL}$, less than about $1.4 \times 10^6/\text{mL}$, less than about $1.3 \times 10^6/\text{mL}$, less than about $1.2 \times 10^6/\text{mL}$, less than about $1.1 \times 10^6/\text{mL}$, less than about $1.0 \times 10^6/\text{mL}$, less than about $0.9 \times 10^6/\text{mL}$, less than about $0.8 \times 10^6/\text{mL}$, less than about $0.7 \times 10^6/\text{mL}$, less than about $0.6 \times 10^6/\text{mL}$, less than about $0.5 \times 10^6/\text{mL}$, less than about $0.4 \times 10^6/\text{mL}$, less than about $0.3 \times 10^6/\text{mL}$, less than about $0.2 \times 10^6/\text{mL}$, less than about $0.1 \times 10^6/\text{mL}$ or less, such as less than about $2 \times 10^6/\text{mL}$, less than about $1.7 \times 10^6/\text{mL}$, less than about $0.8 \times 10^6/\text{mL}$, less than about $0.64 \times 10^6/\text{mL}$, less than about $0.59 \times 10^6/\text{mL}$. In embodiments, the primary tumor cell is cultivated with a feeder cell in the composition of step (1), in particular the feeder cell is a mouse embryonic fibroblast (MEF) cell. In embodiments, the MEF cells have been treated with mitomycin C.

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In embodiments, the animal in the method of the present invention is a mouse or a rat, in particular an immunodeficient mouse, such as a nude mouse. In embodiments, the primary tumor cell obtained in step (1) was implanted in to the animal in a hollow fiber. In embodiments, the hollow fiber is made of modified polyvinylidene fluoride, more particularly the hollow fiber is made of modified polyvinylidene fluoride and has a cut-off value of 500,000 Dalton.

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Another aspect of the present invention provides an animal model for screening anti-tumor drugs obtained by the method of the present invention. In embodiments, the animal in the present invention is a mouse or a rat, in particular an immunodeficient mouse, such as a nude mouse.

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Another aspect of the present invention provides a method for screening anti-tumor drugs using the animal model of the present invention, comprising administering a candidate drug to the animal model of the present invention. In embodiments, the above method further comprises measuring tumor cell growth in the animal model of the present invention.

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The tumor cells can be obtained from a variety of tumor types, including but not limited to tumors in the digestive tract (such as the stomach, intestines, duodenum, colon, pancreas, etc.), the breast, lung, liver, and endocrine glands (such as the adrenal gland, parathyroid gland, pituitary, testis, ovaries, and thymus, thyroid gland), urinary and reproductive systems (such as kidney, bladder, ovary, testis, prostate, etc.), skeletal muscle system (such as bone, smooth muscle, striated muscle, etc.), skin, and so on. For example, the tumor cells can be derived from gastric cancer, biopsy specimen of colorectal cancer and lung cancer.

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The conditionally reprogrammed primary tumor cells can be implanted into the animal via a syringe or other methods or devices known in the art. In embodiments, the conditionally reprogrammed primary tumor cells are implanted into an animal by a method for producing a PDX model for tumor growth in vivo. See e.g. “Melanoma patient-derived xenografts accurately model the disease and develop fast enough to guide treatment decisions” , Oncotarget, Vol. 5, No. 20, Berglind O. Einarsdottir et.al., published on Sep 8 2014, “Personalizing Cancer Treatment in the Age of Global Genomic Analyses: PALB2 Gene Mutations and the Response to DNA Damaging Agents in Pancreatic Cancer”, Molecular Cancer Therapies, published on Dec 6 2010; DOI: 10.1158/1535-7163.MCT-10-0893, Maria C. Villarroel. In embodiments, the conditionally reprogrammed primary tumor cells were transferred into a hollow-fiber tube, which is then implanted into an animal. The hollow fiber can be made of modified polyvinylidene fluoride and has a cut-off value of 500,000 Dalton. In the present invention, a mini-PDX device in the refers to a hollow fiber can be made of modified polyvinylidene fluoride and has a cut-off value of 500,000 Dalton, which may contain the primary tumor cells obtained from a patient and be implanted into a candidate animal, e.g. implanted into a candidate animal subcutaneously. In the present invention, a

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mini-PDX model refers to an animal that has been implanted with the mini-PDX device of the present invention. The mini-PDX animal model can be used in the methods/tests described in the present invention.

5 Another aspect of the present invention provides a method for screening anti-tumor drugs using the animal model of the present invention, comprising administering a candidate drug to the animal model of the present invention. In some embodiments, the method further comprises measuring the growth of the tumor cells which is carried by the animal model of the present invention before and after said administration. In some embodiments, the size of
10 the tumor is measured before and 5-14 days, in particular 5-7 days after administering the drug to the animal model of the present invention. In some embodiments, tumor cell apoptosis is measured before and 5-14 days, in particular 5-7 days after administering the drug to the animal model of the present invention. In some embodiments, tumor cell differentiation is measured before and 5-14 days, in particular 5-7 days after administering
15 the drug to the animal model of the present invention. A person skilled in the art will choose a suitable method to measure the growth of the tumor cells which is carried by the animal model of the present invention before and after said administration.

The drug can be administered via any suitable route, either oral or parental. For example, the
20 candidate drug is administered to the animal model of the present invention by oral administration or injection (such as through intramuscular, subcutaneous or intravenous infusion), local administration, inhalation, and transdermal delivery such as skin patches, implants, suppositories, etc. A skilled person in the art will choose a suitable route of administration according to their needs.

25 The candidate drugs to be screened in the present invention may be a known antitumor drug or its combination, a new antitumor or a combination, or a new combination of known antitumor drugs. In the method of the invention, the drug to be screened can be used in solid, semi-solid or liquid form.

30 The candidate animals can be administered with the drugs by oral administration or

injection (such as through intramuscular, subcutaneous or intravenous infusion), local administration, inhalation, and transdermal delivery such as skin patches, implants, suppositories, etc. Technicians in this field will choose a suitable route of administration according to their needs

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Examples

The present invention will be better described below with reference to the accompanying drawings.

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Conditional reprogramming medium

Medium contains the following ingredients:

- 375 mL F12 medium (commercially available), 125 mL DMEM with high glucose (commercially available), 25 mL 10% FBS (commercially available), 5 mL hydrocortisone, 5 mL insulin, 5 mL cholera toxin, 5 mL adenine, 5 mL Pen/Strep (commercially available), 5 mL EGF, 1mL Y- 27632.
- Optional ingredients: Non-Essential Amino Acids Solution (commercially available); and GlutaMAX Supplement (commercially available).

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Preparation:

The following ingredients are prepared:

- Hydrocortisone: dissolve 25 mg commercially available Hydrocortisone in 5 mL cold 100% ethanol to make a 5 mg/mL solution. Add 0.8 mL of said 5 mg/mL solution to 100 mL Hanks' Balanced Salt Solution (HBSS) containing 5% fetal bovine serum (FBS). Filter sterilize and store at -20°C in 10 mL aliquots. The final concentration of hydrocortisone is 0.4mg/L.
- Cholera toxin: add 1.2 mL of sterile water to a vial containing 1-mg cholera toxin (commercially available), obtaining a 10 μM solution. Dilute 50 μL of said 10 μM solution into 50 mL HBSS containing 0.1% bovine serum albumin. Filter sterilize and store at 4°C in 10 mL aliquots. The final concentration of cholera toxin is 8.3 $\mu\text{g/L}$.

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- Insulin: dissolve 12.5 mg commercially available insulin in 25 mL of 0.005 M HCL. Filter sterilize with a syringe filter pre-wet with FBS. The final concentration of insulin is 5 mg/L.
- Adenine: dissolve 121 mg commercially available adenine in 50 mL of 0.05 M HCl by stirring for 1 h. Filter sterilize and store at -20°C in 10 mL aliquots. The final concentration of adenine is 24.2 mg/L.
- Epidermal growth factor (EGF): dissolve 100 μg commercially available EGF in 10 mL sterile water. Add 90 mL HBSS containing 0.1% bovine serum albumin. Filter sterilize and store at -20°C in 10 mL aliquots. The final concentration of EGF is 10 $\mu\text{g/L}$.
- Y- 27632; 10 μmol in DMSO. Commercially available.

The medium was then properly kept in a cool place for use.

Feeder cell preparation:

MEF (mouse embryonic fibroblast) cells were isolated from e13.5 embryos of C57 mouse and were grown in DMEM supplemented with 10% FBS. Isolated MEF cells in 3-5 passages were treated with mitomycin C (10 $\mu\text{g/ml}$) for 2h and were washed with PBS. The treated MEF were harvested and cryopreserved as feeder cell.

Example 1

1. Materials

1.1 Tumors for conditional reprogramming

Conditional reprogramming cells from MDX079, MDX083, MDX095, MDX107, MDX123 were collected separately. MDX079 originated from a female lung cancer patient, MDX083 model originated from a 27-year old male adenoid cystic carcinoma patient, MDX095 model originated from a 54-year old male peritoneal malignant mesothelioma patient, MDX107 model originated from a 54-year old male glioblastoma patient, MDX123 model originated from a 53-year old male colonrectal cancer patient.

1.2 Animals

1.2.1 Balb/c nude, female, were purchased from Shanghai Laboratory Animal Center

(Lingchang, Shanghai, China, SCXK(SH)2013-0018).

Age: 6-8 weeks

Sex: female

Body weight: 18-22 g

5 1.2.2 Housing condition

The mice were kept in SPF room at constant temperature and humidity with 3 animals in each cage.

- Temperature: 20~26°C .

- Humidity: 40~70 %.

10 - Light cycle: 12 hours light and 12 hours dark.

Cages: Made of polycarbonate. The size is 325 mm x 210 mm x 180 mm. The bedding material is corn cob, which is changed twice per week.

Diet: Animals had free access to irradiation sterilized dry granule food during the entire study period.

15 Water: Animals had free access to sterile drinking water.

Cage identification: the identification labels for each cage contain the following information: number of animals, sex, strain, date received, treatment, study number, group number, and the starting date of the treatment. Animal identification: Animals were marked by ear coding.

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1.3 Equipment

Reverse microscope DMIL, LEICA. Balance ALC-310.3, Acclulab. Microbalance BSA224S, Sartorius. Centrifuge 5810R, Eppendorf.

25 Mini-PDX device: a hollow fiber made of modified polyvinylidene fluoride which has a cut-off value of 500,000 Dalton, internal diameter 1-2 mm. Cut into desired length.

2. Procedure and methods

2.1 Cell Culture

2.1.1

30 Wash the tumor tissue sample with buffer solution and remove away non-tumor tissue and necrotic tumor tissue in biosafety cabinet. Cut the tumor into 1~3 mm³ fragments, suspend pellet with 1X collagenase solution and incubate in 37°C for 1-2 hours. Collect single cells through 70uM strainer, count cell numbers to calculate the tumor cell concentration (See 9. Tumor cell concentration obtained from surgery and biopsy/needle biopsy). Cultivate the

cells with the conditional reprogramming medium, and after tumor cells proliferation, harvest the conditional reprogramming cells.

2.1.2

Suspend tumor tissues obtained from biopsy/needle biopsy in 1X collagenase solution and incubated in 37°C for 1-2 hours. Collect single cells through 70uM strainer, count cell numbers to calculate the tumor cell concentration (See 9. Tumor cell concentration obtained from surgery and biopsy/needle biopsy). Cultivate the cells with the conditional reprogramming medium and after tumor cells proliferation harvest the conditional reprogramming cells.

2.2 Mini-PDX device Inoculation

The cells suspension was filled into Mini-PDX device and the devices were inoculated subcutaneously into both flanks of Nu/Nu-nude mice, to establish the Mini-PDX model. The inoculation day was defined as day 0. Mice were randomized in groups according to the bodyweights and the treatments were initiated at day 0. The test article administration and the mini-PDX device numbers in each study group are shown in the following experimental design tables.

Table 1 MDX079 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	5	Vehicle	0	<i>i.p.</i>	qd*5
2	6	Gemcitabine+Carboplatin	60+50	<i>i.p.</i>	q4d*2 +qw
3	6	Docetaxel +Carboplatin	20+50	<i>i.p.</i>	q4d*2 +qw
4	6	Pemetrexed+ Carboplatin	200+50	<i>i.p.</i>	qd*5 +qw
5	6	Pemetrexed+ Carboplatin+ Bevacizumab	200+50 +10	<i>i.p.</i>	qd*5 +qw+ q4d*2

6 6 Etoposide+ Carboplatin 20+50 *i.p.* q4d*2 +qw

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

Table 2 MDX083 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5
2	6	Paclitaxel +Cisplatin	20+5	<i>i.p.</i>	q4d*2 +qw
3	6	Gemcitabine + Cisplatin	60+5	<i>i.p.</i>	q4d*2 +qw
4	6	Docetaxel+ Carboplatin	20+50	<i>i.p.</i>	q4d*2 +qw
5	6	5-Fu +Cisplatin	25+5	<i>i.p.</i>	qd*5 +qw
6	6	Epirubicin+ 5-Fu+ Cisplatin	5+25+5	<i>i.p.</i>	qw + qd*5+qw

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

Table 3 MDX095 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5

2	4	Gemcitabine + Cisplatin	60+5	<i>i.p.</i>	q4d*2 +qw
3	6	5-Fu	25	<i>i.p.</i>	qd*5
4	6	Cisplatin	5	<i>i.p.</i>	q4d*2
5	6	Paclitaxel	20	<i>i.p.</i>	q4d*2
6	6	Epirubicin	10	<i>i.p.</i>	q4d*2
7	6	Etoposide	20	<i>i.p.</i>	q4d*2

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

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Table 4 MDX107 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>p.o.</i>	qd*5
2	6	Temozolo mide	50	<i>p.o.</i>	qd*5

Note: N: mini-PDX device number;

p.o.: per os;

qd: Once every day.

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Table 5 MDX123 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
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1	6	Vehicle	0	<i>i.p.</i>	qd*5
2	6	5-Fu	25	<i>i.p.</i>	qd*5
3	6	Oxaliplatin	5	<i>i.p.</i>	q4d*2
4	6	Irinotecan	50	<i>i.p.</i>	q4d*2
5	6	Raltitrexed	30	<i>i.p.</i>	q4d*2
6	6	Bevacizumab	10	<i>i.p.</i>	q4d*2
7	6	Cetuximab	30	<i>i.p.</i>	q4d*2

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

qd: Once every day;

q4d: Once every 4 days.

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2.3 Observation

The protocol and any amendment(s) or procedures involving the care and use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai LIDE prior to conduct. During the study, the care and use of animals were conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). After inoculation, the animals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption, body weight gain/loss (body weight were measured twice weekly or every other day), eye/hair matting and any other abnormal effect. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

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2.4 Endpoints

2.4.1 Body weight were measured every day, the treatment periods of these studies were 7 days, at the termination, all mice were euthanized and the Mini-PDX devices were removed for CTG (cell viability) assay. Tumor relative proliferation rate (%) is the indication of the anti-tumor activity.

2.4.2 When $V_{Cd7} - V_{d0} > 0$, Tumor relative proliferation rate(%) = $(V_{Td7} - V_{d0}) / (V_{Cd7} - V_{d0}) \times 100\%$; When $V_{Cd7} - V_{d0} < 0$, Tumor relative proliferation rate(%) = $V_{Td7} / V_{Cd7} \times 100\%$ (V_{Td7} : the cell viability on day 7 of treatment group; V_{Cd7} : the cell viability on day 7 of control group; V_{d0} : the cell viability on day 0).

3. Results (see Figure 2)

4. Summary and Discussion

In MDX079 Mini-PDX models, Docetaxel and Carboplatin combination treatment (T/C%=-21%) could result in a significant decrease in tumor cell viability; Gemcitabine and Carboplatin combination treatment (T/C%=55%) also shown an anti-tumor activity; Pemetrexed and Carboplatin combination treatment (T/C%=103%) treatment, Pemetrexed, Carboplatin and Bevacizumab combination treatment (T/C%=119%) treatment, Etoposide and Carboplatin combination treatment (T/C%=114%) shown no anti-tumor activity.

In MDX083 Mini-PDX models, all the treatment group include Paclitaxel and Cisplatin combination treatment (T/C%=44%), Gemcitabine and Cisplatin combination treatment (T/C%=41%), Docetaxel and Carboplatin combination treatment (T/C%=50%), 5-Fu and Cisplatin combination treatment (T/C%=45%), Epirubicin, 5-Fu and Cisplatin combination treatment (T/C%=45%) could result in a decrease in tumor cell viability.

In MDX095 Mini-PDX models, 5-Fu treatment (T/C%=80%), Paclitaxel treatment (T/C%=79%) result in a little decrease in tumor cell viability; Gemcitabine and Cisplatin combination treatment (T/C%=119%) shown no anti-tumor activity, Cisplatin treatment (T/C%=128%), Epirubicin treatment (T/C%=120%), Etoposide treatment (T/C%=109%) shown no anti-tumor activity.

In MDX107 Mini-PDX models, Temozolomide treatment shown no anti-tumor activity.

In MDX123 Mini-PDX models, 5-Fu treatment (T/C%=75%), Oxaliplatin treatment (T/C%=61%), Irinotecan treatment (T/C%=68%), Bevacizumab treatment (T/C%=76%)

could result in a decrease in tumor cell viability; Raltitrexed treatment (T/C%=92%), Bevacuzimab treatment (T/C%=101%) shown no anti-tumor activity.

Example 2

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5. Material

5.1 Tumors for conditional reprogramming

Conditional reprogramming cells from MDX133, MDX154, MDX164, MDX165, MDX168, MDX169, MDX174, MDX186, MDX189, MDX203 were collected separately. MDX133 originated from a 45-year old male gastric cancer patient, MDX154 model originated from a female gallbladder carcinoma patient, MDX164 model originated from a 43-year old male glioblastoma patient, MDX165 model originated from a 64-year old male glioblastoma patient, MDX168 model originated from a 66-year old female gallbladder carcinoma patient, MDX169 model originated from a 52-year old male lung cancer patient, MDX174 model originated from a 59-year old male lung cancer patient, MDX186 model originated from a 54-year old female pancreatic cancer patient, MDX189 model originated from a 44-year old male esophagus cancer patient, MDX203 model originated from a 61-year old male gallbladder carcinoma patient.

20 5.2 Animals

5.2.1 Balb/c nude, female, were purchased from Shanghai Laboratory Animal Center (Lingchang, Shanghai, China, SCXK(SH)2013-0018).

Age: 6-8 weeks

Sex: female

25 Body weight: 18-22 g

5.2.2 Housing condition

The mice were kept in SPF room at constant temperature and humidity with 3 animals in each cage.

- Temperature: 20~26 °C.

30 - Humidity: 40~70 %.

- Light cycle: 12 hours light and 12 hours dark.

Cages: Made of polycarbonate. The size is 325 mm x 210 mm x 180 mm. The bedding material is corn cob, which is changed twice per week.

Diet: Animals had free access to irradiation sterilized dry granule food during the entire study period.

Water: Animals had free access to sterile drinking water.

- 5 Cage identification: the identification labels for each cage contain the following information: number of animals, sex, strain, date received, treatment, study number, group number, and the starting date of the treatment. Animal identification: Animals were marked by ear coding.

5.3 Equipment

- 10 Reverse microscope DMIL, LEICA. Balance ALC-310.3, Acclulab. Microbalance BSA224S, Sartorius. Centrifuge 5810R, Eppendorf.

Mini-PDX device: a hollow fiber made of modified polyvinylidene fluoride which has a cut-off value of 500,000 Dalton, internal diameter 1-2 mm. Cut into desired length.

15 6. Procedure and methods

6.1 Cell Culture

6.1.1

- Wash the tumor tissue sample with buffer solution and remove away non-tumor tissue and necrotic tumor tissue in biosafety cabinet. Cut the tumor into 1~3 mm³ fragments, suspend
20 pellet with 1X collagenase solution and incubate in 37°C for 1-2 hours. Collect single cells through 70uM strainer, count cell numbers to calculate tumor cell concentration (See 9. Tumor cell concentration obtained from surgery and biopsy/needle biopsy). Cultivate the cells with conditional reprogramming medium, after tumor cells proliferation, and harvest the conditional reprogramming cells.

25 6.1.2

- Suspend tumor tissues obtained from biopsy/needle biopsy in 1X collagenase solution and incubate in 37°C for 1-2 hours. Collect single cells through 70uM strainer, count cell
30 numbers to calculate tumor cell concentration (See 9. Tumor cell concentration obtained from surgery and biopsy/needle biopsy). Cultivate the cells with conditional reprogramming medium, after tumor cells proliferation, and harvest the conditional reprogramming cells.

6.2 Mini-PDX device Inoculation

The cells suspension was filled into Mini-PDX device and the devices were inoculated subcutaneously into both flanks of Nu/Nu-nude mice, to establish the Mini-PDX model. The inoculation day was defined as day 0. Mice were randomized in groups according to the bodyweights and the treatments were initiated at day 0. The test article administration and the mini-PDX device numbers in each study group are shown in the following experimental design tables.

Table 6 MDX133 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5
2	6	Oxaliplatin	5	<i>i.p.</i>	q4d*2
3	6	5-Fu	25	<i>i.p.</i>	qd*5
4	6	S-1	10	<i>p.o.</i>	qd*5
5	6	Paclitaxel	20	<i>i.p.</i>	q4d*2
6	6	Irinotecan	50	<i>i.p.</i>	q4d*2

Note: N: mini-PDX device number;
i.p.: intraperitoneal injection;
p.o.: per os;
 qd: Once every day;
 q4d: Once every 4 days.

Table 7 MDX154 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5

2	6	Gemcitabine	60	<i>i.p.</i>	q4d*2
3	6	Oxaliplatin	5	<i>i.p.</i>	q4d*2
4	6	5-Fu	25	<i>i.p.</i>	qd*5
5	6	Nab-Paclitaxel	20	<i>i.v.</i>	qd*5
6	6	Irinotecan	50	<i>i.p.</i>	q4d*2

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

i.v.: intravenous injection;

qd: Once every day;

q4d: Once every 4 days.

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Table 8 MDX164 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>p.o.</i>	qd*5
2	6	Temozolomide	50	<i>p.o.</i>	qd*5

Note: N: mini-PDX device number;

p.o.: per os;

qd: Once every day.

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Table 9 MDX165 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>p.o.</i>	qd*5

2	6	Temozolomide	50	<i>p.o.</i>	qd*5
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Note: N: mini-PDX device number;

p.o.: per os;

qd: Once every day.

Table 10 MDX168 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5
2	6	Gemcitabine	60	<i>i.p.</i>	q4d*2
3	5	Oxaliplatin	5	<i>i.p.</i>	q4d*2
4	5	5-Fu	25	<i>i.p.</i>	qd*5
5	5	Nab-Paclitaxel	20	<i>i.v.</i>	qd*5
6	6	Irinotecan	50	<i>i.p.</i>	q4d*2

5 Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

i.v.: intravenous injection;

qd: Once every day;

q4d: Once every 4 days.

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Table 11 MDX169 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5
2	6	Pemetrexed+ Carboplatin	200+50	<i>i.p.</i>	qd*5 +qw

3	6	Paclitaxel + Carboplatin	20+50	<i>i.p.</i>	q4d*2 +qw
4	6	Docetaxel +Carboplatin	20+50	<i>i.p.</i>	q4d*2 +qw
5	6	Gemcitabine+Carboplatin	60+50	<i>i.p.</i>	q4d*2 +qw
6	6	Vinorelbine+Carboplatin	5+50	<i>i.p.</i>	q4d*2+qw

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

p.o.: per os;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

Table 12 MDX174 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	6	Vehicle	0	<i>i.p.</i>	qd*5
2	6	Pemetrexed+ Carboplatin	200+50	<i>i.p.</i>	qd*5 +qw
3	6	Paclitaxel + Carboplatin	20+50	<i>i.p.</i>	q4d*2 +qw
4	6	Docetaxel +Carboplatin	20+50	<i>i.p.</i>	q4d*2 +qw
5	6	Gemcitabine+Carboplatin	60+50	<i>i.p.</i>	q4d*2 +qw
6	6	Vinorelbine+Carboplatin	5+50	<i>i.p.</i>	q4d*2+qw

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

p.o.: per os;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

Table 13 MDX186 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	5	Vehicle	0	<i>i.p.</i>	qd*5
2	5	Gemcitabine	60+5	<i>i.p.</i>	q4d*2 +qw
3	5	Oxaliplatin	5	<i>i.p.</i>	q4d*2
4	5	5-Fu	25	<i>i.p.</i>	qd*5
5	5	Nab-Paclitaxel	20	<i>i.v.</i>	qd*5
6	5	Irinotecan	50	<i>i.p.</i>	q4d*2

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

i.v.: intravenous injection;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

Table 14 MDX189 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	5	Vehicle	0	<i>i.p.</i>	qd*5
2	5	S-1+Oxaliplatin	10+5	<i>p.o. + i.p.</i>	qd*5+qw
3	5	Docetaxel +Cisplatin+5-Fu	20+5+5	<i>i.p.</i>	qw+qw+ qd*5

4 5 5-Fu+Cisplatin 25+5 *i.p.* qd*5+qw

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

p.o.: per os;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

Table 15 MDX203 Groups and Treatment

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Schedule
1	5	Vehicle	0	<i>i.p.</i>	qd*5
2	5	Gemcitabine	60+5	<i>i.p.</i>	q4d*2 +qw
3	5	Oxaliplatin	5	<i>i.p.</i>	q4d*2
4	5	5-Fu	25	<i>i.p.</i>	qd*5
5	5	Nab-Paclitaxel	20	<i>i.v.</i>	qd*5
6	5	Irinotecan	50	<i>i.p.</i>	q4d*2

Note: N: mini-PDX device number;

i.p.: intraperitoneal injection;

i.v.: intravenous injection;

qd: Once every day;

q4d: Once every 4 days;

qw: Once every week.

6.3 Observation

The protocol and any amendment(s) or procedures involving the care and use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee

(IACUC) of Shanghai LIDE prior to conduct. During the study, the care and use of animals were conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). After inoculation, the animals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption, body weight gain/loss (body weight were measured twice weekly or every other day), eye/hair matting and any other abnormal effect. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

6.4 Endpoints

6.4.1 Body weight were measured every day, the treatment periods of these studies were 7 days, at the termination, all mice were euthanized and the Mini-PDX devices were removed for CTG (cell viability) assay. Tumor relative proliferation rate (%) is the indication of the anti-tumor activity.

6.4.2 When $V_{Cd7} - V_{d0} > 0$, Tumor relative proliferation rate(%) = $(V_{Td7} - V_{d0}) / (V_{Cd7} - V_{d0}) \times 100\%$; When $V_{Cd7} - V_{d0} < 0$, Tumor relative proliferation rate(%) = $V_{Td7} / V_{Cd7} \times 100\%$ (V_{Td7} : the cell viability on day 7 of treatment group; V_{Cd7} : the cell viability on day 7 of control group; V_{d0} : the cell viability on day 0).

7. Results (See Figure 3)

8. Summary and Discussion

In MDX133 Mini-PDX models, Paclitaxel treatment (T/C%=52%) could result in a decrease in tumor cell viability, Irinotecan treatment (T/C%=72%), S-1 treatment (T/C%=86%), Oxaliplatin treatment (T/C%=89%) also shown a little anti-tumor activity; 5-Fu treatment (T/C%=103%) shown no anti-tumor activity.

In MDX154 Mini-PDX models, all the treatment group include Gemcitabine treatment (T/C%=100%), Oxaliplatin treatment (T/C%=174%), 5-Fu treatment (T/C%=111%), Nab-paclitaxel treatment (T/C%=162%), Irinotecan treatment (T/C%=227%) shown no anti-tumor activity.

In MDX164 Mini-PDX models, Temozolomide treatment (T/C%=102%) shown no anti-tumor activity.

In MDX165 Mini-PDX models, Temozolomide treatment (T/C%=29%) could result in a decrease in tumor cell viability.

- 5 In MDX168 Mini-PDX models, Nab-paclitaxel treatment (T/C%=15%), Irinotecan treatment (T/C%=12%) could result in a decrease in tumor cell viability; Gemcitabine treatment (T/C%=53%), Oxaliplatin treatment (T/C%=75%) also shown a little anti-tumor activity; 5-Fu treatment (T/C%=146%) shown no anti-tumor activity.

- 10 In MDX169 Mini-PDX models, Docetaxel and Carboplatin combination treatment (T/C%=20%), Paclitaxel and Carboplatin combination treatment (T/C%=40%) could result in a decrease in tumor cell viability; Pemetrexed and Carboplatin combination treatment (T/C%=55%), Gemcitabine and Carboplatin combination treatment (T/C%=82%), Vinorelbine and Carboplatin combination treatment (T/C%=91%) also shown a little anti-tumor activity.

- 15 In MDX174 Mini-PDX models, Gemcitabine and Carboplatin combination treatment (T/C%=86%) shown a little anti-tumor activity; Pemetrexed and Carboplatin combination treatment (T/C%=104%), Paclitaxel and Carboplatin combination treatment (T/C%=120%), Docetaxel and Carboplatin combination treatment (T/C%=110%), Vinorelbine and Carboplatin combination treatment (T/C%=134%) shown no anti-tumor activity.

- 20 In MDX186 Mini-PDX models, Nab-paclitaxel treatment (T/C%=0%), Irinotecan treatment (T/C%=5%) could result in a significant decrease in tumor cell viability; Oxaliplatin treatment (T/C%=47%) shown a anti-tumor activity; Gemcitabine treatment (T/C%=83%), 5-Fu treatment (T/C%=90%) also shown a little anti-tumor activity.

- 25 In MDX189 Mini-PDX models, S-1 and Oxaliplatin combination treatment (T/C%=-2%), Docetaxel, Cisplatin and 5-Fu combination treatment (T/C%=14%), 5-Fu and Cisplatin combination treatment (T/C%=19%) could result in a significant decrease in tumor cell viability.

- 30 In MDX203 Mini-PDX models, Oxaliplatin treatment (T/C%=43%), Irinotecan treatment (T/C%=48%), Nab-paclitaxel treatment (T/C%=52%) could result in a decrease in tumor cell viability; Gemcitabine treatment (T/C%=77%), 5-Fu treatment (T/C%=83%) also showed a little anti-tumor activity.

9. Tumor cell count for samples obtained from surgery and biopsy/needle biopsy tumor samples

Tumor cells numbers are counted using methods known in the art and then converted to

5 tumor cell concentration in the suspension. M: $\times 10^6/\text{mL}$.

	sample ID	Source of sample	cancer type	tumor cell concentration
1	MDX079	surgical	lung cancer	-
2	MDX083	needle biopsy	adenoid cystic carcinoma	2M
3	MDX095	needle biopsy	peritoneal malignant mesothelioma	1.7M
4	MDX107	surgical	Glioblastoma	0.87M
5	MDX123	biopsy	colonrectal cancer	0.8M
6	MDX133	surgical	gastric cancer	6M
7	MDX154	surgical	gallbladder carcinoma	0.42M
8	MDX164	surgical	glioblastoma	7M
9	MDX165	surgical	glioblastoma	5.9M
10	MDX168	surgical	gallbladder carcinoma	4M
11	MDX169	biopsy	lung cancer	0.64M
12	MDX174	biopsy	lung cancer	0.59M
13	MDX186	surgical	pancreatic cancer	0.5M
14	MDX189	surgical	esophagus cancer	2.1M
15	MDX203	surgical	gallbladder carcinoma	3.2M

Claims

1. A method for obtaining an animal model for screening anti-tumor drugs, comprising
 - (1) cultivating a primary tumor cell obtained from a tumor sample of a patient in a composition comprising:
 - a) 0.4 mg/L Hydrocortisone;
 - b) 5 mg/L Insulin;
 - c) 8.3 μ g/L Cholera toxin;
 - d) 24.2 mg/L Adenine;
 - e) 10 μ g/L EGF;
 - f) 10 μ mol/L Y- 27632;
 - g) Pen/Strep;
 - h) 10% FBS;
 - i) F12 medium
 - j) DMEM with high glucose
 - k) Non-Essential Amino Acids Solution;
 - l) GlutaMAX Supplement; and
 - m) a mouse embryonic fibroblast cell that has been treated with mitomycin C;and
 - (2) implanting the primary tumor cell obtained from step (1) into an animal, wherein the animal is a mouse.
2. The method of claim 1, wherein the tumor sample is a surgical sample or a biopsy sample.
3. The method of claim 1, wherein the concentration of the primary tumor cells in the tumor sample obtained from a needle biopsy is less than about 2×10^6 /mL, less than about 1.9×10^6 /mL, less than about 1.8×10^6 /mL, less than about 1.7×10^6 /mL, less than about 1.6×10^6 /mL, less than about 1.5×10^6 /mL, less than about 1.4×10^6 /mL, less than about 1.3×10^6 /mL, less than about 1.2×10^6 /mL, less than about 1.1×10^6 /mL, less than about 1.0×10^6 /mL, less than about 0.9×10^6 /mL, less than about 0.8×10^6 /mL, less than about 0.7×10^6 /mL, less than about 0.6×10^6 /mL, less than about 0.5×10^6 /mL, less than about 0.4×10^6 /mL, less than about 0.3×10^6 /mL, less than about 0.2×10^6 /mL, less than about 0.1×10^6 /mL, such as less than about 2×10^6 /mL, less than about 1.7×10^6 /mL, less than about 0.8×10^6 /mL, less than about 0.64×10^6 /mL, less than about 0.59×10^6 /mL.
4. The method of claim 1, wherein the mouse is an immunodeficient mouse.

5. The method of claim 1, wherein the primary tumor cell obtained in step (1) is implanted in to the animal in a hollow fiber, in particular the hollow fiber is made of modified polyvinylidene fluoride, more particularly the hollow fiber is made of modified polyvinylidene fluoride and has a cut-off value of 500,000 Dalton.
6. The method of claim 5, wherein the hollow fiber is implanted subcutaneously into the mouse.