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~~Inulin~~;

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~~Inulin~~;

c) 7-9μg/L, preferably 8.3 μg/L Cholera toxin;

d) 20-30 mg/L, preferably 24.2 mg/L Adenine;

e) 8-12μg/L, preferably 10 μg/L EGF;

f) 8-12μmol/L, preferably 10 μmol/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.

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:

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

m) a feeder cell; 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 the primary tumor cells in the sample obtained from a needle biopsy is less than about 2*10^6/mL~~mol/L~~, less than about 1.9*10^6/mL~~mol/L~~, less than about 1.8*10^6/mL~~mol/L~~, less than about 1.7*10^6/mL~~mol/L~~, less than about 1.6*10^6/mL~~mol/L~~, less than about 1.5*10^6/mL~~mol/L~~, less than about 1.4 *10^6/mL~~mol/L~~, less than about 1.3 *10^6/mL~~mol/L~~, less than about 1.2 *10^6/mL~~mol/L~~, less than about 1.1 *10^6/mL~~mol/L~~, less than about 1.0 *10^6/mL~~mol/L~~, less than about 0.9 *10^6/mL~~mol/L~~, less than about 0.8 *10^6/mL~~mol/L~~, less than about 0,7 *10^6/mL~~mol/L~~, less than about 0.6 *10^6/mL~~mol/L~~, less than about 0.5 *10^6/mL~~mol/L~~, less than about 0.4 *10^6/mL~~mol/L~~, less than about 0.3 *10^6/mL~~mol/L~~, less than about 0.2 *10^6/mL~~mol/L~~, less than about 0.1 *10^6/mL ~~mol/L~~ or less, such as less than about 2 *10^6/mL~~mol/L~~, less than about 1.7 *10^6/mL~~mol/L~~, less than about 0.8 *10^6/mL~~mol/L~~, less than about 0.64 *10^6/mL~~mol/L~~, less than about 0.59 *10^6/mL~~mol/L~~. 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 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 model of the present invention. In embodiments, the above method further comprises measuring tumor cell growth in the animal model of the present invention.

Another aspect of the present invention provides a use of the animal model of the present invention in screening anti-tumor drugs.

Drawing

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 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 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.

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~~Inulin~~;

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 $2*10^6$/mL~~mol/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.

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

The concentration of Y-27632 in the composition of step (1) can be 1-30 μmol/L, in particular about 2-20μmol/L, about 4-18μmol/L, about 6-16μmol/L, about 8-12μmol/L, about 9-11μmol/L, about 9.5-10.5μmol/L, about 2μmol/L, about 4μmol/L, about 6μmol/L, about 7μmol/L, about 8μmol/L, about 9μmol/L, about 10μmol/L, about 11μmol/L, about 12μmol/L, about 14μmol/L, about 16μmol/L, about 18μmol/L, about 20μmol/L, about 25μmol/L, about 30μmol/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%, 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:

a) 0.3-0.5 mg/L, preferably 0.4 mg/L Hydrocortisone;
b) 4-6 mg/L, preferably 5 mg/L insulin~~Inulin~~;
c) 7-9μg/L, preferably 8.3 μg/L Cholera toxin;
d) 20-30 mg/L, preferably 24.2 mg/L Adenine;
e) 8-12μg/L, preferably 10 μg/L EGF;
f) 8-12μmol/L, preferably 10 μmol/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

(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*10^6/mL~~mol/L~~, less than about 1.9*10^6/mL~~mol/L~~, less than about 1.8*10^6/mL~~mol/L~~, less than about 1.7*10^6/mL~~mol/L~~, less than about 1.6*10^6/mL~~mol/L~~, less than about 1.5*10^6/mL~~mol/L~~, less than about 1.4 *10^6/mL~~mol/L~~, less than about 1.3 *10^6/mL~~mol/L~~, less than about 1.2 *10^6/mL~~mol/L~~, less than about 1.1 *10^6/mL~~mol/L~~, less than about 1.0 *10^6/mL~~mol/L~~, less than about 0.9 *10^6/mL~~mol/L~~, less than about 0.8 *10^6/mL~~mol/L~~, less than about 0,7 *10^6/mL~~mol/L~~, less than about 0.6 *10^6/mL~~mol/L~~, less than about 0.5 *10^6/mL~~mol/L~~, less than about 0.4 *10^6/mL~~mol/L~~, less than about 0.3 *10^6/mL~~mol/L~~, less than about 0.2 *10^6/mL~~mol/L~~, less than about 0.1 *10^6/mL ~~mol/L~~ or less, such as less than about 2 *10^6/mL~~mol/L~~, less than about 1.7 *10^6/mL~~mol/L~~, less than about 0.8 *10^6/mL~~mol/L~~, less than about 0.64 *10^6/mL~~mol/L~~, less than about 0.59 *10^6/mL~~mol/L~~. 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 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.

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.

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.

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 ~~twelve~~ colorectal cancer and lung cancer.

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

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.

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 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 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 candidate drug is administered to the animal model of the present invention by oral administration or ~~intramuscular~~ 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.

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.

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

~~intramuscular~~ 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

Examples

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

Conditional reprograming 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).

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)~~HBES~~ 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 containing1-mg cholera toxin (commercially available), obtaining a 10 µM solution. Dilute 50 µL of said 10 µM solution into 50 mL HBSS ~~HBES~~ 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 µg/L.

- 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 ~~HBES~~ containing 0.1% bovine serum albumin. Filter sterilize and store at –20°C in 10 mL aliquots. The final concentration of EGF is 10 μg/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 (10μg/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 reprograming

Conditional reprograming 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 ~~tnesoshelioma~~ 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

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 tumor cell concentration in the suspension. M: *10^6/mL~~mol/L~~.

	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 ~~tnesoshelioma~~ 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 ~~biopsy~~ sample of a patient in a composition comprising:

a) 0.01-1.0 mg/L Hydrocortisone;
b) 1.0 -10.0 mg/L ~~Inulin~~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.

2. The method of claim 1, wherein the composition in step (1) comprises

a) 0.3-0.5 mg/L, preferably 0.4 mg/L Hydrocortisone;
b) 4-6mg/L, preferably 5 mg/L ~~Inulin~~Insulin;
c) 7-9μg/L, preferably 8.3 μg/L Cholera toxin;
d) 20-30 mg/L, preferably 24.2 mg/L Adenine;
e) 8-12μg/L, preferably 10 μg/L EGF;
f) 8-12μmol/L, preferably 10 μmol/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.

3. The method of claim 1, wherein the tumor ~~biopsy~~ sample is a ~~needle~~ surgical sample or a biopsy sample.

4. 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~~mol/L~~, less than about $1.9*10^6$/mL~~mol/L~~, less than about $1.8*10^6$/mL~~mol/L~~, less than about $1.7*10^6$/mL~~mol/L~~, less than about $1.6*10^6$/mL~~mol/L~~, less than about $1.5*10^6$/mL~~mol/L~~,

less than about 1.4 *10^6/mL~~mol/L~~, less than about 1.3 *10^6/mL~~mol/L~~, less than about 1.2 *10^6/mL~~mol/L~~, less than about 1.1 *10^6/mL~~mol/L~~, less than about 1.0 *10^6/mL~~mol/L~~, less than about 0.9 *10^6/mL~~mol/L~~, less than about 0.8 *10^6/mL~~mol/L~~, less than about 0.7 *10^6/mL~~mol/L~~, less than about 0.6 *10^6/mL~~mol/L~~, less than about 0.5 *10^6/mL~~mol/L~~, less than about 0.4 *10^6/mL~~mol/L~~, less than about 0.3 *10^6/mL~~mol/L~~, less than about 0.2 *10^6/mL~~mol/L~~, less than about 0.1 *10^6/mL~~mol/L~~, such as less than about 2 *10^6/mL~~mol/L~~, less than about 1.7 *10^6/mL~~mol/L~~, less than about 0.8 *10^6/mL~~mol/L~~, less than about 0.64 *10^6/mL~~mol/L~~, less than about 0.59 *10^6/mL~~mol/L~~.

5. The method of claim 1, wherein the animal is a mouse or a rat, in particular an immuodeficient mouse.
6. The method of claim 1, wherein 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, more particularly the MEF cells have been treated with mitomycin C.
7. The method of claim 1, wherein the primary tumor cell obtained in step (1) was 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.
8. The method of claim ~~1~~7, wherein the hollow fiber was implanted subcutaneously into the animal.
9. An animal model for screening anti-tumor drugs obtained by the method of one of claims 1-8, wherein the animal is a mouse or a rat, in particular an immunodeficient mouse.
10. A method for screening anti-tumor drugs, comprising administering a candidate drug to the animal model of claim 9.
11. The method of claim 10, further comprising measuring tumor cell growth in the animal model of claim 9.
12. Use of the animal model of claim 9 in screening anti-tumor drugs.