1. Equipment, reagents and consumables

1.1. Equipment: Biosafety cabinet, cell incubator, low temperature horizontal centrifuge, inverted microscope

1.2.Reagents:Matrigengel(Cat:082714/082716/082724/082726/082723/082721),Complete medium corresponding to the cell, 1×PBS, Trypsin digestion solution

1.3.Consumables: sterile pipette tips; 10cm cell culture dish; Sterile EP tube; Disposable syringes（Or be adjusted according to the experimental design）

2. Experimental contents and methods

2.1.Put the Matrigengel in the ice box and put it in the refrigerator at 4℃ so that the Matrigengel can slowly melt overnight;(Note：Do not allow this product to warm up above 4° C.)

2.2. Digestion the cells which in good condition during the logarithmic growth phase by trypsin digestion solution (Note: Add 1 mL 0.25% trypsin cell digestion solution into the petri dish, let it stand for 10 seconds, discard the trypsin, and continue digestion at room temperature for 1~3 minutes with the residual trypsin.),when the cells become round (keep in mind that they cannot be digested until the cell edge is clear), add 1 mL medium containing 10% fetal bovine serum (FBS) to terminate digestion. Collect the cell suspension into a plastic centrifuge tube.

2.3. Centrifuge at 1000 rpm for 3 minutes and discard the supernatant (You should choose centrifugal force that is appropriate for your cells).Then washing the cells with PBS,after that the cells were re-suspended by adding serum-free basal medium, and take 10 μL of cell suspension for cell count.

2.4. Successful subcutaneous tumor-formation of different tumor cells strains may require the selection of mice of different genders and strains, as well as different cell injection dosages.

We provide three types of cell injection quantities.

$HepG_2$:Each mice should be vaccinated 5×10^6 cells. The injection volume is 100 μL

HCT-116:Each mice should be vaccinated 1×10^6 cells. The injection volume is 100 μL

MIA-PaCa-2: Each mice should be vaccinated 1×10^7 cells. The injection volume is 200 μL.

2.5. Insert the prepared cells and Matrigengel in ice, and transfer to the laboratory animal room as soon as possibly.

2.6. Mixing of the Matrigengel: Cell suspension and Matrigengel are mixed in a 1:1 ratio at 4℃.

2.7.Subcutaneous injection: The nude mice were fixed with the one hand and injected subcutaneously into the right back of the BALB/c-nu/nu. During inoculation, the needle was inserted a little deeper into the subcutaneously, about 1 cm deep, to reduce the overflow of cell suspension after injection.

2.8. Recording data: The nude mice were put back into the cage for further feeding, the tumor volume was measured regularly according to the different experimental requirements, the data was recorded to make a curve, and the nude mice were euthanized before the tumor volume was more than 2000 mm^3, and the tumor was removed and photographed.