A method for studying mouse mammary tumorigenesis

Metastasis is the last step of primary breast cancer progression in which cells from the primary tumor leave and go to other organs. Breast cancer cells often metastasize to the lung, among other organs. Each step in metastasis is tightly regulated by genes and it is very important to understand which genes are responsible for this phenomenon. Due to ethical reasons, we cannot manipulate tumor gene profiles in humans and therefore mouse models are very helpful in mimicking what disease progression would be for a human. The goal of this protocol is to study breast cancer progression in mice. Cells isolated from mouse breast tumors and mouse embryos can be grown in a culture dish and their DNA, RNA and protein profiles can be analyzed. We have also included a method to visualize cells that have metastasized to the lung.

Removing cells from mouse breast tumors:

Develop mice with genes that allow them to develop genes.
Sacrifice the mouse with Isoflurane.
Open the mouse by cutting it with sterile scissors. Pin the skin down to allow easy access to the organs.
Remove the breast tumor and place it in a salt solution for washing.
Under a sterile hood, place the tumor on a tissue culture plate and chop it with a sterile razor blade.
Add a collagen degrading solution (Collagenase) to the tissue and let it dissolve for 2 hours in a 37°C incubator. After incubation, spin the tissues in a centrifuge machine for 3 minutes so that the tissue pieces can collect on the bottom of the tube.
Remove the Collagenase solution and wash 3 times with a salt solution. Aspirate the remaining saline.
Add a protein degrading solution to the tissue to break it down more and let incubate for 5 minutes at 37° Wash again with the salt solution 3 times.
Add cell growing medium to the tissue and plate on a tissue culture plate. Media must be changed every day for the next 3 days.

Isolating a mouse lung to visualize cancer spreading:

Develop mice with genes that allow them to develop genes.
Sacrifice the mouse with Isoflurane.
Open the mouse by cutting it with sterile scissors. Pin the skin down to allow easy access to the organs.
With sterile scissors, open the upper part of the trachea.
Inject a dye (ex. India Ink) through the upper part of the trachea, down into the lungs to fill them.
Measure tumor nodules.

Removing cells from a mouse embryo:

Sacrifice a pregnant mouse with Isoflurane.
Open the mouse by cutting it with sterile scissors and isolate the embryos from the uterus.
Separate each individual embryo from the placenta and place in a salt solution.
Remove the head, gut and liver from the embryo and place the rest of the organs in a tissue culture dish with the salt solution.
After a brief washing, transfer the embryo body to another tissue culture dish with a protein degrading solution and chop with a sterile razor blade. To allow the body to break down, incubate the chopped material for 5 minutes at 37°
After incubation, spin the tissues in a centrifuge machine for 3 minutes so that the tissue pieces can collect on the bottom of the tube.
Add cell growing medium to the tissue and plate on a tissue culture plate. Media must be changed every day for the next 2 days.

Understanding the genes responsible for metastasis will help find a cure for cancer.

Publication

Primary Tumor and MEF Cell Isolation to Study Lung Metastasis.
Dong S, Maziveyi M, Alahari SK
J Vis Exp. 2015 May 20