Video Article

Isolation of Early Hematopoietic Stem Cells from Murine Yolk Sac and AGM

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Abstract

In the mouse embryo, early hematopoiesis occurs simultaneously in multiple organs, which includes the yolk sac and aorta-gonad-mesonephros region. These regions are crucial in establishing the blood system in the embryos and leads to the eventual movement of stem cells into the fetal liver and then development of adult stem cells in the bonemarrow. Early hematopoietic stem cells can be isolated from these organs through microdissection of the embryo followed by flow cytometric sorting to obtain a more pure population. It remains unclear how these stem cell populations contribute to the fetal and adult stem cell pool. Also, our lab investigates how early stem cells functionally differ from fetal and adult hematopoietic stem cells. Furthermore, our lab sorts different populations of hematopoietic stem cells and test their functional role in the context of a variety of genetic models. In this video, we demonstrate the micro-dissection procedure we commonly use and also show the results of a typical FACS plotfter isolating these rare populations, it is possible to perform a variety of functional assays including: colony assays and bone marrow transplants.

Video Link

The video component of this article can be found at https://www.jove.com/video/789/

Protocol

Dissection and isolation of early hematopoietic stem cells from murine yolk sac and aorta-gonad-mesonephros region

1. Before starting, it is important to have all the reagents and tools necessary for the procedure:
   - Scissors
   - Forceps, size 4, and surgical grade bent-tipped forceps, size 5/45 (optional)
   - 30 gauge needles with syringe
   - 35mm and 100mm Petri dishes
   - PBS with 10% and 20% fetal bovine serum
   - and lastly, Collagenase
2. To begin the dissection, we need to harvest the uterine horns from a pregnant female at 10.5 days post conception.
3. Animals are euthanized according to approved procedures. We use carbon dioxide asphyxiation, followed by cervical dislocation.
4. The female's abdomen is wet with ethanol and the skin is opened with scissors.
5. Holding the skin with forceps, additional cuts are made to open the peritoneum.
6. The abdomen is opened and the uterine horns can be visualized. Using forceps to hold the uterine horns, cut each distal end and the center to excise both horns.
7. Uterine horns are then placed in a Petri dish containing cold PBS with 10% FBS to remove uterine tissues.
8. Using size 4 forceps and working under the microscope, gently remove the endometrial tissue from around each embryo conceptus, being careful not to puncture the placenta.
9. Once removed, the embryos are placed in a fresh dish with cold PBS and 10% FBS.
10. Carefully remove the placental tissue to reveal the embryo encased in the yolk sac by using the forceps to cut the embryo-encased yolk sac away from the placenta at their juncture. The yolk sac containing vitelline arteries, and a portion of umbilical cord, can be gently teased away from the embryo and set aside on ice for dissociation.
11. The embryo is moved to a small Petri dish with minimal levels of PBS with 10% FBS -- just enough to wet the embryo but not too much. Optimal levels keep the embryo stationary while the plate is slowly agitated, keeping the embryo securely in place during dissection.
12. Switch to the 30 gauge needles, which are used to remove the upper and lower portions of the embryo by cutting above the forelimbs and below the hindlimbs. Becoming familiar with using the needles as cutting instruments may take some practice. A method of using repeated

small cuts by crossing the needles will dissect the tissue, with the needle held in the dominant hand to act as a cutting guide for the needle that is held in the non-dominant hand.

13. Remove the dorsal portion of the embryo. This is the tissue that contains the somites. Gently cut away the dorsal-most tissue while remaining a sufficient distance from the aorta (red line) to prevent any knicks. Cutting the aorta would result in loss of blood and the inability to easily visualize its location. Again, making small cuts with the needles is vital to maintaining a precise cutting location.
14. Push the extra tissue away out of view and turn the embryo around to cut the ventral tissue, again cutting as close to the aorta as possible without knicking it. Cuts made too far from the optimal location would result in abdominal tissues being present, which can be removed once the AGM is isolated.
15. Position the embryo with its dorsal side down or its back against the plate. Gently splay the embryo open by pushing the sides down and visualizing the location of the gonadal ridges. These are located slightly off-center as tube-like structures on either side of the aorta.
16. Complete the dissection by removing the excess side tissues using the same cutting techniques as before. Once the side tissue of the embryo is removed, then the embryo is turned upside down to remove the remaining side tissue.
17. At this point, the AGM is inspected for any excess tissue that can be removed easily with the needles without injury to the aorta or gonads. Removing as much excess as possible results in better isolation of the stem cell population since we rely on only a few cell surface markers for purification.
18. Using the bent-tipped forceps, gently scoop up each AGM and place in cold PBS with 10% FBS until ready for dissociation.

Dissociation and FACS Analysis

1. To dissociate the tissue, first place yolk sacs and AGMs in separate tubes.
2. Spin briefly to pellet tissues and discard PBS with 10% FBS.
3. Resuspend tissues in 0.25% collagenase in PBS with 20% FBS, with enough volume to adequately cover tissues.
4. Incubate the tissues at 37 degrees. 25-30mins for AGMs and 35-40mins for yolk sacs.
5. At the end of the incubation, gently pipette tissues up and down to dissociation into single cell suspensions.
6. Wash the tissues with excess PBS with 10% FBS, pellet the cells and resuspend in fresh sterile PBS with 10% FBS.
7. For FACS analysis, cells are stained according to manufacturer's recommendations. We use a FACSAria from BD Biosciences to perform flow cytometry. Due to the size and fragility of the embryonic cells, the sorting aperture is adjusted for a larger diameter than for adult hematopoietic cells, which we also study in the lab. Likewise, the pressure is adjusted to be less than the conditions required by adult hematopoietic cells. For example, sorting on the FACSAria, the nozzle size would be 100 microns and the sort pressure would be 30 psi.
8. For stem cell analyses, triple positive cells expressing the markers cKit, CD34, and CD41 are isolated and sorted from the yolk sac and double positives (cKit, CD34) are isolated from the AGM via FACS. A typical plot would look like this: cKit positive cells are analyzed for CD34 and CD41 simultaneously or individually. Usually cells display a gradation of positivity for each marker with the stem cell population found as a subset of the positive populations. These plots show us that we have achieved a good cell yield with 100-300 cells per yolk sac and per AGM.