Video Article

Mouse Epidermal Neural Crest Stem Cell (EPI-NCSC) Cultures

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Abstract

EPI-NCSC are remnants of the embryonic neural crest in an adult location, the bulge of hair follicles. They are multipotent stem cells that have the physiological property to generate a wide array of differentiated cell types, including neurons, nerve supporting cells, smooth muscle cells, bone/cartilage cells and melanocytes. EPI-NCSC are easily accessible in the hairy skin and can be isolated as a highly pure population of stem cells. This video provides a detailed protocol for preparing mouse EPI-NCSC cultures from whisker follicles. The whisker pad of an adult mouse is removed, and whisker follicles dissected. The follicles are then cut longitudinally and subsequently transversely above and below the bulge region. The bulge is removed from the collagen capsule and placed in a culture plate. EPI-NCSC start to emigrate from the bulge explants 3 to 4 days later.

Video Link

The video component of this article can be found at https://www.jove.com/video/772/

Protocol

Dissection of the Bulge from Adult Mouse Whisker Follicles

1. We use 10 weeks to 6 months old mice. Younger mice often yield more cells. Euthanize mouse and submerge entire animal into 1:1 mixture of betadine (iodine solution) and hydrogen peroxide (available from pharmacy) for about 3 minutes.
2. Squirt entire mouse, especially the facial region, with 75% ethanol, and carry mouse to dissection microscope in laminar flow hood.
3. Dissect whisker pads, carefully avoiding cutting into hair bulbs, and pool in HBSS.
4. Dissect whisker follicles and pool in HBSS at room temperature. Do this by holding skin next to hair follicle with a forceps, and then cutting around the follicle with the straight scissors. Cut deeply to avoid injuring hair bulbs.
5. Lift whisker follicle out of whisker pad and put into new plate with fresh HBSS.
6. Flush loose adipous and dermal tissue with squirts of buffer, until whisker follicles are clean. If necessary, cut nerve to whisker follicle and remove adherent tissue by scraping and repeated flushes of HBSS.
7. Pin clean hair follilce onto Sylgard-coated glass Petri dish using sharpened tungsten needles, using microforceps for holding onto the skin part next to the follicle.
8. Cut whisker follicle longitudically with a microblade. Avoid cutting too deeply, as this will injure the bulge. Remove blood with repeated squirts of HBSS until gone. Appearance of blood is a good indication that the cut was deep enough. If the longitudial cut is not sufficiently long, it may be lengthened with bent microscissors.
9. Make a transverse cut above the level of the cavernous sinus and subsequently a second transverse cut at the level within the ring sinus, close to the skin.
10. You will now see the bulge inside the capsule.
11. Grab an end of to collagen capsule with the forceps and roll out the bulge with a bent tungsten needle. You will now see the empty capsule and the isolated bulge.
12. Pool isolated bulges in a separate culture plate in HBSS at room temperature. Make sure no other tissue is contaminating the pooled bulges.

Culture of bulge Explants

1. Coat 35 mm culture plates with collagen by placing 50 µl collagen and 10 µl sterile 6% NaCl next to each other. Mix well with a double-bent Pasteyr pipet and push towards the edge of the culture plate. Incubate overnight in a clean dessicator, but do not let dry. Before use, rinse the plates with saline.
2. Pre-incubate collagen-coated and rinsed culture plates for approximately 3 hr with culture medium. Culture medium consists of 85% Alpha-modified MEM, 10% fetal bovine serum and 5% day 11 chick embryo extract.
3. After re-incubation, remove culture medium from plates and add several bulges with as little medium as possible. Remove excess culture medium.
4. Incubate for 1 hr, but not longer, in cell incubator in a humidified atmosphere with 10% oxygen and 5% CO_2.
5. After 1 hr, gently add 1.5 ml of culture medium. Bulge explants should adhere to the collagen substratum. Replace 50% of the culture medium daily.

6. Within 3 - 4 days, highly migratory cells will emigrate from the bulge explants. Note their stellate morphology, motility, and predominant absence of cell-cell contacts. Over the next few days, more cells will emigrate, and emigrated cells will proliferate rapidly.
7. Remove bulge 2 - 3 days after onset of EPI-NCSC emigration with a sharpened tungsten needle. If the bulges are left longer, they tend to flatten and make it impossible to obtain pure EPI-NCSC cultures.
8. Important: Rare cells with flattened morphology, which become sometimes apparent several days later than EPI-NCSC, and which are less motile are NOT EPI-NCSC, but putative epidermal stem cells/progenitors. Cultures consisting of these cells, or mixed cultures containing EPI-NCSC and putative epidermal stem cells/progenitors need to be discarded.
9. Hint: Do NOT keep the cells for too long in primary explant medium, as they tend to differentiate rapidly at high cell density, putatively due to autocrine/paracrine growth factor signaling.

Discussion

By virtue of their migratory ability, EPI-NCSC can be isolated as a highly pure population of stem cells, which can be expanded in vitro. As embryonic remnants in an adult location, EPI-NCSC are potentially attractive candidates for future cell replacement therapies, biomedical engineering and/or regenerative medicine. We have tested EPI-NCSC in a mouse model of spinal cord injury, where they show desirable traits. Through gene expression profiling by LongSAGE (www.ncbi.nlm.nih.gov/geo, series number GSE4680) we showed that, as expected, embryonic neural crest cells and EPI-NCSC share a similar gene expression pattern that differs from that of neighboring epidermal stem cells and other skin resident stem cells/progenitors.

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