Video Article

ES Cell-derived Neuroepithelial Cell Cultures

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Abstract

ES cells have the potential to differentiate into cells from all germ layers, which makes them an attractive tool for the development of new therapies. In general, the differentiation of ES cells follows the concept to first generate immature progenitor cells, which then can be propagated and differentiated into mature cellular phenotypes. This also applies for ES cell-derived neurogenesis, in which the development of neural cells follows two major steps: First, the derivation and expansion of immature neuroepithelial precursors and second, their differentiation into mature neural cells. A common method to produce neural progenitors from ES cells is based on embryoid body (EB) formation, which reveals the differentiation of cells from all germ layers including neuroectoderm. An alternative and more efficient method to induce neuroepithelial cell development uses stromal cell-derived inducing activity (SDIA), which can be achieved by co-culturing ES cells with skull bone marrow-derived stromal cells (1). Both, EB formation and SDIA, reveal the development of rosette-like structures, which are thought to resemble neural tube- and/or neural crest-like progenitors. The neural precursors can be isolated, expanded and further differentiated into specific neurons and glia cells using defined culture conditions. Here, we describe the generation and isolation of such rosettes in co-culture experiments with the stromal cell line MS5 (2-5).

Video Link

The video component of this article can be found at https://www.jove.com/video/118/

Protocol

Step 1

1. Plate mitomycin-C ggrowth-inhibited (10 µg/ml for 2.5 hours) MS5 cells at a density of 70,000/cm2 on gelatin-coated (0.01 % for 30 minutes) 6 well plates in α-MEM media.
2. When cells are attached and have formed a monolayer (over night growth), switch to SRM.
3. Manually isolate ES cell colonies from the ES cell cultures using a syringe with a 27 ½ G needle.
4. Tritrurate the colonies carefully with a 1 ml blue tip and plate at low density on the MS5 cells (usually 2-3 colonies per 6 well plate).

Note: The ES cells can also be taken from enzymatic propagation using collagenase or trypsin.

Step 2

1. Grow the ES cells on the MS5 feeders for 14-21 days until rosette-containing colonies form.

Note: Change media often. The rosettes are usually at the outer edges of the colonies and their formation can be greatly enhanced if 300-500 ng/ml Noggin is added to the SRM (3). In some differentiation protocols, SRM can be exchanged with N2-A media and supplemented with growth or other factors to promote cell specification (2-5).

Step 3

1. Isolate and pick the rosettes with a 27 ½ G needle under a microscope.

Note: Avoid cutting and picking the MS5 stromal cells. If colonies are packed with rosettes, one can also isolate the entire colony.

Step 4

1. Aspirate the clusters of rosettes, tritrurate them carefully with a 1 ml blue tip, and plate them on poly-L-ornithine (0.001%) and fibronectin (1 µg/ml) or laminin (1 µg/ml) -coated 6 wells in N2-A media usually supplemented with bFGF (20 ng/ml). The rosettes will reform and expand.

Note: To enhance cell survival, one can plate the small clusters in droplets to increase cell density. This step can also be modified by supplementing the N2-A media with additional growth or other factors to promote cell specification (2-5).

Discussion

This protocol demonstrates the different steps in generating and isolating neuroepithelial cells from human ES cells using SDIA. The application of this method is manifold and has been used in many protocols to produce specified neurons (e.g. 1, 2, 5-9). The rosettes are thought to resemble neural tube cells with an anterior phenotype (2, 5, 10) and also contain neural crest progenitors (11, 12). In addition, they retain a certain level of plasticity, since they can be patterned by specific factors in defined culture conditions. Thus, the SDIA-derived neural progenitors can give rise to many cell types from the central and peripheral nerve system making them a useful tool for the derivation of different neural cell populations in ES cell differentiation paradigms.

Disclosures

The authors have nothing to disclose.

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