OPEN ACCESS Freely available online

ISSN: 2157-7633
Journal of
Stem Cell Research
& Therapy

Commentary

Generation and Maintenance of Murine Primary and PSC-Derived Basal Cells *In Vitro*

Eugenio Ditadi*

Institute for Biomedicine, University of Lubeck, Eurac Research, Bolzano, Italy

DESCRIPTION

Basal cells are a critical component of the epithelial lining in various tissues, including the respiratory tract, where they serve as progenitors capable of differentiating into multiple cell types. The generation and maintenance of murine primary and Pluripotent Stem Cell (PSC)-derived basal cells in vitro have become pivotal for advancing respiratory research, modeling diseases, and exploring regenerative therapies. This article search into the methodologies and considerations essential for cultivating these cells in vitro.

The murine model is extensively used in biomedical research due to its genetic and physiological similarities to humans. Basal cells, identified by markers such as *p63* and *KRT5*, are essential for tissue homeostasis and regeneration. Establishing strong in vitro cultures of murine primary and PSC-derived basal cells allows for detailed studies of cell behavior, gene function, and responses to various stimuli. This article outlines the procedures for generating and maintaining these cells in vitro, highlighting the importance of specific culture conditions and medium components.

Generation of murine primary basal cells

Isolation of primary basal cells:

Tissue harvesting: The first step involves harvesting the epithelial tissue from murine models. Typically, tracheal or nasal tissues are used due to their high basal cell content.

Enzymatic digestion: The harvested tissue is subjected to enzymatic digestion using dispase or collagenase to dissociate the epithelial cells from the underlying mesenchyme. This step is important for isolating a pure population of basal cells.

Cell sorting: Following digestion, the cell suspension is filtered to remove debris and then subjected to Fluorescence-Activated Cell Sorting (FACS) or Magnetic-Activated Cell Sorting (MACS) to isolate basal cells based on specific surface markers like NGFR (Nerve Growth Factor Receptor).

Culturing primary basal cells:

Culture medium: Primary basal cells are cultured in a defined medium that supports their growth and maintenance. This typically includes a basal medium such as DMEM/F12 supplemented with growth factors like EGF (Epidermal Growth Factor) and FGF2 (Fibroblast Growth Factor 2), along with other additives like insulin, transferrin, and selenium.

Coating and substrate: To enhance attachment and growth, culture plates are often coated with extracellular matrix proteins such as collagen, laminin, or fibronectin. These substrates represent the natural basement membrane environment.

Maintaining cell health: Regular monitoring of cell morphology and medium pH is essential. Media should be changed every 2-3 days to ensure an optimal environment for cell proliferation and maintenance.

Generation of PSC-derived basal cells

Differentiation protocols:

Induction of pluripotency: PSCs, including Embryonic Stem Cells (ESCs) or induced Pluripotent Stem Cells (iPSCs), are first maintained in an undifferentiated state using media supplemented with factors like LIF (Leukemia Inhibitory Factor) for mouse ESCs.

Directed differentiation: The differentiation into basal cells involves a stepwise protocol.

Mesoderm induction: Initial differentiation towards mesodermal lineage using factors such as BMP4 (Bone Morphogenetic Protein 4) and Activin A.

Epithelial induction: Further differentiation is guided by factors like retinoic acid and WNT signaling modulators to induce epithelial characteristics.

Correspondence to: Eugenio Ditadi, Institute for Biomedicine, University of Lubeck, Eurac Research, Bolzano, Italy, E-mail: ditadi.euge@gmail.com

Received: 06-May-2024; Manuscript No. JSCRT-24-26212; **Editor assigned:** 08-May-2024; PreQC. No. JSCRT-24-26212 (PQ); **Reviewed:** 22-May-2024; QC. No. JSCRT-24-26212; **Revised:** 29-May-2024; Manuscript No. JSCRT-24-26212 (R); **Published:** 06-Jun-2024, DOI: 10.35248/2157-7633.24.14.640

Citation: Ditadi E (2024) Generation and Maintenance of Murine Primary and PSC-Derived Basal Cells *In Vitro*. J Stem Cell Res Ther. 14:640.

Basal cell specification: Finally, the addition of specific growth factors (e.g., EGF, FGF2) and signaling inhibitors (e.g., Rho kinase inhibitor) directs the differentiation towards a basal cell phenotype.

Verification and expansion:

Marker analysis: The presence of basal cell markers such as *p63*, *KRT5*, and *NGFR* is confirmed using immunocytochemistry or flow cytometry.

Expansion: PSC-derived basal cells are expanded under similar culture conditions as primary basal cells, with careful attention to maintaining an undifferentiated state and preventing overconfluence.

Maintenance of basal cells *in vitro*

Long-term culture: Long-term maintenance of basal cells requires periodic passaging to prevent differentiation and senescence. Passaging is typically performed using trypsin or other enzymatic dissociation methods when cells reach 70%-80%confluence.

Cryopreservation: For long-term storage, basal cells can be cryopreserved in freezing media containing DMSO (Dimethyl Sulfoxide) and Fetal Bovine Serum (FBS). Proper cryopreservation techniques are important to maintaining cell viability and functionality upon thawing.

Quality control: Regular quality control measures, including mycoplasma testing, karyotyping, and functional assays, ensure the integrity and consistency of basal cell cultures.

Applications and future directions

The ability to generate and maintain murine primary and PSC-derived basal cells *in vitro* opens numerous paths for research and therapeutic applications. These cells can be used to model respiratory diseases, study cellular responses to environmental toxins, and test regenerative therapies. Future advancements may focus on refining differentiation protocols, enhancing the scalability of cultures, and translating findings to human models.

The generation and maintenance of murine primary and PSC-derived basal cells *in vitro* are important for advancing our understanding of epithelial biology and developing novel therapeutic strategies. Through careful isolation, culture, and differentiation techniques, researchers can control the potential of these cells to uncover new insights and drive innovation in biomedical research.