MONASH University

MONASH INNOVATION

SKELETAL MUSCLE PRECURSORS

Cell differentiation and isolation method

A method using specific small-molecule inhibitors and growth factors to derive skeletal muscle precursor cells from hPSCs. These cells provide a valuable *in vitro* and *in vivo* research tool for studying early human development and disease, and could support cell-based therapies to treat muscle diseases such as dystrophies.

- **Capacity to derive human skeletal muscle precursors from hPSCs without genetic modification/transgene expression**
- **Creation of a valuable source of human skeletal muscle precursors for research applications**
- **Capacity to isolate high purity human skeletal muscle precursors**
- **Potential methods for production of a therapeutic composition.**

THE CHALLENGE

Human pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced PSCs (iPSCs) provide an extraordinary research tool. In vitro, these cells display extensive proliferation and the ability to differentiate into derivatives of all three germ layers. Such characteristics give these cells a remarkable potential for use as an in vitro model of early human development and in cell-based therapies.

PSC differentiation protocols are currently available for a vast number of cell types. However, little progress has been made regarding differentiation of PSCs into derivatives of paraxial mesoderm, such as skeletal muscle, in the absence of forced transgene expression.

Appropriate combination of markers for efficient isolation of skeletal muscle precursors also remains to be determined.

There is a clear need for transgene free methods of differentiating and isolating pure populations of skeletal muscle precursors from PSCs.

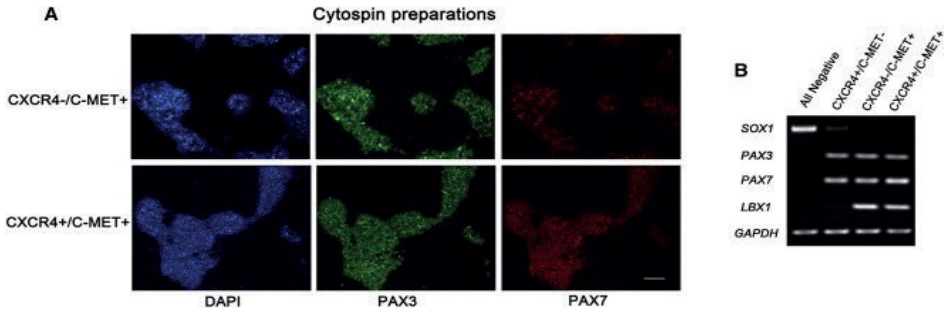

Figure 1: (A) Cytospin preparations of muscle progenitor cell populations CXCR4-/C-MET+ (top) and CXCR4+/C-MET+ (bottom) sorted at day 35 of human ESC (HES3) differentiation. Cells were cytospun on glass slides and analyzed by immunocytochemistry for myogenic stem cell markers PAX3 (green) and PAX7 (red) immediately follow- ing sorting. Each dot represents one nucleus as confirmed by DAPI counterstaining. (B) RT-PCR analysis of skeletal muscle progenitor genes (PAX3, PAX7, and LBX1) and neural gene (SOX1) in all sorted populations (from DPL-iPS) derived under treatment conditions. Myog, myogenin; MF20, sarcomeric myosin. Scale bars, 50mm.

THE TECHNOLOGY

Researchers from the Australian Institute of Regenerative Medicine have developed a simple two-step differentiation protocol to create skeletal muscle precursors, by treating hPSCs with a glycogen synthase kinase-3 inhibitor and then addition of fibroblast growth factor 21.

To isolate skeletal muscle cells generated in the system, the team also established a stringent cell sorting strategy using the muscle-specific markers known for embryonic hypaxial migratory precursors and mature myocytes.

The protocol has been successfully tested on several PSC lines and provides an invaluable standardised tool for directed differentiation of transgene-free myogenic cells for *in vivo* preclinical studies and in vitro functional and drug screening assays. Future applications of this method to patient-specific iPSC lines will also aid in the study of muscle diseases.

The transplantation of highly purified skeletal muscle precursors has been considered a possible option for the treatment of degenerative muscle disorders, such as muscular dystrophy.

Intellectual property: Two applications filed:US 14/601142 and AU 2014268197 on a method for producing skeletal muscle precursors.

THE OPPORTUNITY

Monash seeks a licensee/partner to develop this technology for research or clinical use.

Reference

1. Borchin B et al., (2013). Derivation and FACS mediated purification of PAX3+/PAX7+ skeletal muscle precursors from human pluripotent stem cells. Stem Cell Reports. 2013 Nov 27;1(6):620-31.

KEY CONTACT

Dr Kathy Nielsen
Senior Commercialisation Manager
Monash Innovation
T: +61 3 9905 5918
E: katherine.nielsen@monash.edu

ARMI
AUSTRALIAN REGENERATIVE MEDICINE INSTITUTE

CRICOS provider: Monash University 00008C
Produced by Monash Innovation: June 2016