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RESEARCH HIGHLIGHT

# Regeneration: making muscle from hPSCs

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**In recent years, researchers worldwide have developed protocols to efficiently differentiate skeletal myogenic cells from human pluripotent stem cells through either ectopic gene expression or the use of small molecules. These stem cell-derived myogenic cells provide new avenues for the study of muscle-related diseases, drug screening and are potentially a new tool for cell therapy against muscular dystrophies.**

Muscular dystrophies (MDs) are heterogeneous genetic diseases that progressively affect the growth and regeneration of skeletal muscle. In the past decade, significant advancements to promote the efficient regeneration of various human tissues, including skeletal muscle, have been achieved. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have unique properties of infinite self-renewal and the potential of differentiating into every cell type in the human body. Theoretically, skeletal myogenic cells differentiated from hPSCs may represent a promising alternative for restoring muscle regeneration potential in MDs.

Satellite cells, the adult stem cell pool in skeletal muscle, are often compromised in MD patients. Therefore, supplementation of functional myogenic progenitors to repair damaged tissues through transplantation may represent an effective treatment against MDs. Successful generation of myogenic progenitors from hPSCs has been achieved by exogenous expression of transcription factors PAX7 or MYOD1 [1-5] (Table 1). Upon ectopic expression of PAX7 in hPSCs-derived embryoid bodies (EBs), the derived myogenic progenitors can differentiate to multinucleated myofibers *in vitro*, and develop into myocytes after transplantation into *mdx* mice (a mouse model of MDs) [2]. Moreover, PAX7+ progenitors can improve muscle contractility in the *mdx* mouse and supplement satellite cells. As an alternative approach, exogenous expression of MYOD1 in hPSCs differentiates them into terminal multinucleated myogenic cells [1, 3-5]. Myogenic cells generated using MYOD1 usually fail to maintain a progenitor status under current culture conditions, and thus are not an ideal cell type for transplantation. Nevertheless, terminally differentiated myocytes from patients may represent a favorable disease model for drug screening and pathological studies. In this line, Abujarour *et al*. [1] found that myotubes differentiated from Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) patients-specific iPSCs can respond to the stimuli of hypertrophy-inducing factors, Wnt7a and IGF-1. Similarly, mature myocytes differentiated *in vitro* from patient iPSCs with carnitine palmitoyltransferase (CP) II deficiency recapitulated some disease phenotypes, which were ameliorated by treatment with bezafibrate, an agonist of peroxisome proliferator-activated receptor. Due to the fact that mouse models do not always recapitulate the clinical manifestations of human diseases, human hPSC disease models are in high demand as platforms for basic research and drug discovery.

Even though myogenic differentiation by exogenous gene expression has been shown to be efficient, the use of integrative viral vectors incurs uncertain risks for potential cell-based therapeutic applications. Therefore, myogenic progenitors obtained from spontaneous cell differentiation [6-8] or lineage-specific differentiation achieved by manipulation of signaling pathways [9, 10] (Table 1) without genetic modification would be more preferable for cell transplantation. For example, free-floating spherical culture in the presence of high concentrations of FGF2 and EGF can induce differentiation of hESCs and hiPSCs (wild-type or BMD patient-specific) into PAX7+ myogenic progenitors [8]. However, the myogenic progenitors cannot be stably maintained as undifferentiated cells in culture, and contain mixed populations of neural and myogenic progenitors. In contrast, using a monolayer culture system and a two-step cell sorting process, Tiziano and colleagues obtained purified CD73+/NCAM+ myoblasts [7]. These myoblasts can be expanded in culture and subsequently differentiated into mature myocytes both *in vitro* and *in vivo*. Besides myogenic progenitors, a specific subpopulation of mesenchymal stem cells (positive for CD56, CD73, CD105, CD166, CD29) derived from EBs cultured under certain conditions has also demonstrated potential for myogenic differentiation both *in vitro* and *in vivo*, as well as been able to contribute to the satellite cell pool [6]. Nevertheless, spontaneous differentiation approaches share common disadvantages including low efficiency and low specificity.

Alternatively, the use of small molecules targeting specific signaling pathways results in more efficient

and specific myogenic differentiation. Through a chemical screening, Xu *et al*. [10] found that simultaneous inhibition of GSK3β, activation of adenylyl cyclase and stimulation of FGF2 signaling during EB formation could specifically promote skeletal muscle differentiation. The derived myogenic progenitors not only undergo terminal differentiation in culture, but also act as satellite cells upon transplantation, thereby supporting muscle regeneration. Moreover, after a combined treatment of hPSCs with a selective inhibitor of GSK3β and FGF2, Borchin *et al*. [9] obtained PAX3+/PAX7+ muscle precursors in culture after isolation of two populations: HNK1–/CXCR4+/C-MET+ and HNK1–/CXCR4–/C-MET+ hypaxial migratory muscle precursors. Treatment with FGF2 in both studies exhibited the capacity to promote proliferation of myogenic progenitors. Considering the important roles of these signaling pathways in regulating development and cell fate determination, future development of myogenic differentiation strategies in the absence of genetic modifications should focus more on the coordinated regulation of these signaling networks.

In the future, in order to fulfill the hopes posed on the field of muscle regenerative medicine, efficient myogenic progenitor differentiation methods should be established. However, this goal currently hindered by the lack of a comprehensive understanding of embryonic myogenesis and the crosstalks between different signaling pathways. Therefore, studies of *in vitro* myogenic differentiation may conversely help us to better understand the process of

**Table 1** Summary of current myogenic differentiation strategies

| Year | Initial cells | Intermediate stage | | Terminal differentiation | | Ref. |
|---|---|---|---|---|---|---|
| | | Methods | Intermediate state cells | Methods | Final cells | |
| 2014 | hiPSC | Doxycycline induced exogenous MYOD1 overexpression in hiPSCs by lentiviral vector | --- | Optimized culture conditions | Myotubes | [1] |
| 2012 | hESC/ hiPSC | Doxycycline induced exogenous PAX7 overexpression in EBs derived from hESCs/hiPSCs by lentiviral vector | PAX7+ myogenic progenitors | Low glucose DMEM and horse serum | Myocytes | [2] |
| 2012 | hESC | Doxycycline induced exogenous MYOD1 overexpression in hESCs by lentiviral vector | --- | Doxycycline treatment in hESC conditional medium | Myotubes | [3] |
| 2013 | hiPSC | Doxycycline induced exogenous MYOD1 overexpression in hiPSCs by transposon vector | --- | Doxycycline treatment and adjusted culture conditions | Myocytes | [4] |
| 2014 | hiPSC | Doxycycline induced exogenous MYOD1 overexpression in hiPSCs by transposon vector | --- | Doxycycline treatment and low glucose medium culture | Myocytes | [5] |
| 2012 | hESC/ hiPSC | Selective expansion of myogenic mesenchymal cells from embryoid bodies through stepwise culture conditions | Embryoid bodies | Serum-free ITS medium | Myofibers | [6] |
| 2007 | hESC | Feeder-free monolayer culture system together with selective culture condition and FACS-mediated isolation | CD73+/NCAM+ skeletal myoblasts | N2 medium | Myocytes | [7] |
| 2014 | hESC/ hiPSC | "Stemline medium, S-3194" supplemented with bFGF, EGF, heparin sulfate | Free-floating spherical culture (EZ sphere) | DMEM with B27 | Myotubes | [8] |
| 2013 | hESC/ hiPSC | Two-step differentiation method by using CHIR99021 and FGF2 | PAX3+/PAX7+ skeletal muscle precursors | FACS isolation based on muscle-specific nicotinic AChR antigen | AChR+ Myocytes | [9] |
| 2013 | hiPSC | Embryoid bodies formed in STEMDDiff Apel medium containing bFGF, BIO and forskolin | Embryoid bodies | DMEM with horse serum | Myotubes | [10] |

myogenesis during development. Along this line, one study has previously reported that inhibition of GSK3β by CHIR99021 in hPSC differentiation led to a sequential increase in the expression of genes in the paraxial mesoderm, somitic mesoderm, migratory muscle progenitor and myogenic commitment [9]. These findings demonstrate that *in vitro* differentiation may recapitulate key events of *in vivo* myogenesis.

Recent advances on the differentiation of hPSCs to skeletal muscle may lead to an unlimited source of myocytes for cell-based therapy or drug screening, and serve as an ideal model to study developmental myogenesis. Myogenic cells differentiated from patient-specific stem cells provide a previously inaccessible tool for the study of MDs, which may lead to a better understanding of these devastating diseases. For instance, mutations in the lamin A gene (*LMNA*) results in several degenerative diseases referred to as laminopathies, which include MDs. Recently, iPSCs have been successfully derived from patients with laminopathies, and targeted correction of the mutated *LMNA* was achieved in patient iPSCs by a HDAdV-based genome editing strategy [11]. As novel myogenic differentiation strategies are established and our knowledge about MDs continues to expand, we may well expect new therapies for MDs to be developed in the near future.

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