Commentary

iMedPub Journals
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Cellular & Molecular Medicine: Open access
ISSN 2573-5365

2015
Vol.1 No.1:9

DOI: 10.21767/2573-5365.100009

In vitro Differentiation of ESCs/ iPSCs to Myogenic Lineage: Moving towards Cell Therapy

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Received: September 28, 2015, **Accepted:** December 11, 2015, **Published:** December 16, 2015

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Citation: Gasparri J, Ping Hu. In vitro Differentiation of ESCs/iPSCs to Myogenic Lineage: Moving towards Cell Therapy. Cell Mol Med. 2015, 1:1.

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Directed cell lineage differentiation *in vitro* combined with genetic modification approaches provides an enormous range of possibilities to treat muscular degenerative diseases such as muscular dystrophies (MDs). Currently, no effective therapy is available to treat any form of MDs, which are characterized by progressive muscle wasting and weakness [1]. The limited efficacy of conventional drugs slow down the progress of the disease to certain level, yet the long-term course cannot be changed. Hence, cell-based therapies represent a promising strategy to treat MDs.

Enormous efforts have been invested in finding the appropriate method to obtain muscle progenitors suitable for cell therapy by *in vitro* differentiation of pluripotent stem cells. Human induced pluripotent stem cells (iPSCs) are one of the favorite sources to generate muscle progenitor cells [2-8]. These cells can propagate indefinitely and have potential to differentiate to all cell types originated from the three germ layers [9]. Furthermore, obtaining iPSCs do not involve manipulation of human embryos, allowing the production of autologous pluripotent cells while avoiding the ethical issues raised by the use of human embryonic stem cells (ESCs). However, the efficiency of the directed differentiation to functional myogenic population from iPSCs is fairly low. Therefore, increasing the efficiency of directed differentiation to myogenic lineage cells from iPSCs is a key step towards the development of cell therapies. Early protocols to obtain myogenic lineage cells were based on the embryoid body (EB) formation method. The progenitors obtained by this method displayed long-term engraftment ability in cardiotoxin pre-injured muscle tissue of immunocompromised mice. Moreover, they showed evidence of replenishing the satellite cell pool [10-12]. However, the differentiation efficiency was relatively low and the population obtained was very heterogeneous. Consequently, muscle lineage progenitors were hard to be separated from other lineages [12]. Difficulties in the isolation step and utilization of medium containing fetal bovine or calf serum made these cells unsuitable for cell therapy. Later studies succeeded in obtaining large amounts of myogenic lineage cells with high efficiency by overexpression of myogenic transcription factors Pax7 [5], Pax3 [13] or MyoD1 [14]. The engraftment efficiency of muscle lineage cells obtained by this method could reach 53% [15], and transplantation of these cells improved muscle functions [5]. However, these methods required the utilization of viral vectors that are also incompatible with human cell therapies. Several new protocols have been developed lately to overcome the limitations of the above methods. These new protocols are mainly based on addition of soluble factors to precisely manipulate BMP, WNT and FGF signaling pathways to increase myogenic differentiation efficiency in monolayer cultures [16-18]. In contrast to the EB formation method, these stepwise monolayer cell differentiation systems, are developed using serum free medium. In addition, cells with myogenic differentiation potentials such as mesoderm cells can be specifically enriched to improve the differentiation efficiency, and progenitors differentiated from these systems are easier to isolate to the purity required for cell therapy. Shelton and coworkers described a step by step protocol to differentiate ES cells first to mesoderm, then to myogenic lineage cells. To achieve that, GSK3 inhibitor was first added to activate WNT signaling for mesoderm induction. Next, FGF2 was added to expand the differentiated muscle progenitors and N2 supplemented medium was used to induce further muscle differentiation. Up to 90% of myogenic lineage cells were obtained with this method [16]. Recently, Chal *et al* described a protocol to differentiate iPSCs to myogenic progenitors and mature muscle. In this case, after the presomitic mesoderm induction by WNT activation, BMP inhibitor was added and then several growth factors, including FGF2, IGF and HGF, were used to induce the final maturation of muscle

fibers [17]. These strategies enable us to specifically generate myogenic lineage progenitors, recapitulating the entire myogenic developmental program in xeno-free conditions, without ectopic expression of transcription factors. Nevertheless, additional *in vivo* studies are needed to analyze the engraftment efficiency, functionality and safety of these myogenic progenitor cells. Combined with genetic modification techniques such as CRISPR/CAS9 and TALEN [19], the myogenic lineage cells obtained from the *in vitro* differentiation system have great potential to evolve in cell based therapies. Yet this is just the beginning of the journey. In order to translate the research to clinical treatment of muscle degenerative diseases, several questions remain to be answered. What is the best way to deliver the myogenic cells to patients? Are they safe? Would they trigger unexpected immune responses? Further investigations will provide us more knowledge to answer the above questions and pave the road towards the development of cell therapies for MDs.

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