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The Path from Pluripotency to Skeletal Muscle: Developmental Myogenesis Guides the Way

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Abstract

Following cues from mouse embryogenesis, Chal et al. (2015) identified key regulators of skeletal myogenesis from mouse and human pluripotent stem cells. Emerging myogenic progenitors were specified to form multinucleated fibers that enabled development of quiescent, satellite cell-like progenitors and a model for Duchenne Muscular Dystrophy.

Recapitulating the complexity of skeletal myogenesis in vitro from pluripotent stem cells (PSCs) has presented numerous challenges for the field. The longstanding protocols for generating skeletal muscle require viral-mediated overexpression of transcription factors such as Myogenic Differentiation 1 (MYOD) or Paired Box 7 (PAX7) that limit the generation of truly representative myogenic progenitors and the ability to accurately model muscle diseases in a dish. Skeletal myogenesis relies on tightly controlled spatial and temporal cues to ensure timely embryonic transition through the presomitic mesoderm, the somites, and dermomyotome to form the myotome. Skeletal muscle progenitors delaminate from the dermomyotome to seed individual muscles and eventually give rise to satellite cells, the endogenous stem cells in skeletal muscle responsible for adult homeostasis and repair. Harnessing the genetic instructions of this developmental process enabled Chal et al. to differentiate PSCs to skeletal muscle in vitro (Chal et al., 2015) (Figure 1).

To better understand the in vivo landscape they were aiming to recapitulate, Olivier Pourquié and colleagues developed a detailed expression profile of mouse presomitic mesoderm (PSM) (Chal et al., 2015). The PSM is dynamic. Somites are generated at the anterior end, while the posterior end is continually renewed with new cells entering from the tail bud. The formation of somites from the PSM gives rise to the axial skeleton and skeletal muscles. An evaluation of inducers of posterior PSM specification in vivo identified a 300-fold increase in expression of the secreted factor R-spondin3 (Rspo3), a Wingless-Type MMTV Integration Site Family (WNT) signaling molecule. Similar studies have also shown that WNT is a key inducer of early skeletal myogenic fate in both model organisms as well as PSCs (Borchin et al., 2013; Mendjan et al., 2014; Shelton et al., 2014; von Maltzahn et al., 2012; Xu et al., 2013). The prior central dogma of mesoderm specification from PSCs normally involved activation of Bone Morphogenetic Protein (BMP) signaling (Kattman et

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al., 2011; Mendjan et al., 2014), which traditionally activates the lateral plate mesoderm and cardiomyocyte differentiation. Chal et al. found that inhibition, not activation, of BMP is critical to coaxing the majority of mouse embryonic stem cells (mESCs) to adopt a PSM fate. Importantly, at each step, Chal et al. profiled mESC-derived Mesogenin 1 (Msgn1; posterior) and Paired Box 3 (Pax3; anterior) cells and compared them to endogenous mouse posterior and anterior PSM cells in vivo. By following developmental myogenic cues, they were able to develop a scalable protocol that recapitulates differentiation stages of PSM to generate Pax3-positive myogenic precursor cells, an important first step toward differentiating skeletal muscle in vitro from PSCs.

Skeletal muscle is generated in waves during embryogenesis when Pax3-positive cells undergoing primary (embryonic) myogenesis give rise to Pax7-positive myogenic precursors during secondary (fetal) myogenesis that fuse to form multinucleated muscle fibers (Buckingham et al., 2003). After identification of in vitro differentiation conditions to derive Pax3-positive cells, Chal et al. induced these cells toward secondary myogenesis by applying known developmental growth factors including hepatocyte growth factor (Hgf), insulin growth factor 1 (Igf-1), and fibroblast growth factor 2 (Fgf-2). Additional mechanistic studies will be required to determine the role of each factor during skeletal myogenesis from PSCs. Pax7 and Myogenin (Myog) reporters were both utilized to monitor efficiencies of differentiating muscle fibers and after just 2–3 weeks in culture, the Pax7-positive cells gave rise to several thousand multinucleated muscle fibers that express fast Myosin Heavy Chain (MyHC), a mature muscle marker. These myofibers contained a remarkable amount of myonuclei (approximately 25–50), which had not previously been feasibly generated from PSCs.

Pax7-positive satellite cells are the major source of regenerative cells in skeletal muscle and could be used as a cell-based therapy to repair or replace damaged myofibers. A holy grail for Duchenne Muscular Dystrophy (DMD) cell-based therapies is to obtain satellite cells capable of genetic modification ex vivo and continuous expansion while able to retain their stemness. The discovery that donor myoblasts could restore dystrophin expression in dystrophin-deficient mice set the precedent for a number of clinical trials in the 1990s to restore muscle function in DMD patients (Tremblay et al., 1993). Unfortunately, myoblasts cannot continuously repopulate the satellite cell pool, which led to their failure. Chal et al. provide tantalizing evidence that the developmentally guided protocol derives Pax7-positive cells residing inside the myofiber basal lamina that are Ki67 negative and potentially quiescent. When engrafted in vivo, the Pax7-positive cells were able to home to the satellite cell position and fuse with myofibers to restore expression of muscle proteins including dystrophin.

Chal et al. adapted their developmentally derived mESC protocol to human pluripotent stem cells (hPSCs) that included modifications to timing and concentrations of supplemented factors. The optimized hPSC differentiation method derived PAX7-positive cells in 3 weeks and resulted in 50,000–70,000 fast MyHC fibers starting from 75,000 cells in 30 days. The muscle fibers showed organized sarcomeres and exhibited spontaneous twitching. However, in contrast to the mESC-derived myofibers, the human myofibers were 3- to 4-fold smaller in diameter and had reduced multinucleation. Devising methods to further mature human

fibers in vitro, as well as engrafting the human PAX7-positive cells in vivo to demonstrate their functional potential, are important next steps in understanding the true myogenic potential of the hPSC-derived skeletal myogenic progenitor cells. Overall the developmentally derived protocol presents a significant step forward in the field by improving the efficiency and timing for the generation of human-derived MyHC-positive fibers.

There is a significant need in the field to develop protocols for obtaining physiologically relevant skeletal muscle cells in order to effectively model muscle diseases. By applying their skeletal muscle differentiation protocol to mESCs derived from mdx mice, a mouse model of DMD lacking dystrophin, Chal et al. were able to model aspects of DMD that have not previously been seen in vitro. They found that in vitro myotubes lacking dystrophin had increased lateral branching, consistent with myofiber splitting observed in some neuromuscular diseases. Previous work in the field (Pavlath, 2010) suggests that defective myoblast fusion may contribute to aberrant branching and thus dystrophin may play a role in regulating membrane fusion between adjacent myoblasts. A clearer understanding for the role of dystrophin in muscle development and its effects on the muscle membrane stability remains to be clarified.

In muscle wasting diseases such as DMD, the endogenous satellite cells become exhausted over time and get replaced by fat and fibrotic tissue. The ability to generate an efficient protocol for producing skeletal muscle from PSCs provides a unique in vitro model for improving our understanding of the consequences of dystrophin loss in human skeletal muscle. Optimization and further characterization of the muscle progenitor cells derived from hPSCs could provide a remarkable source of cells with repopulation potential to replace exhausted satellite cells in DMD.

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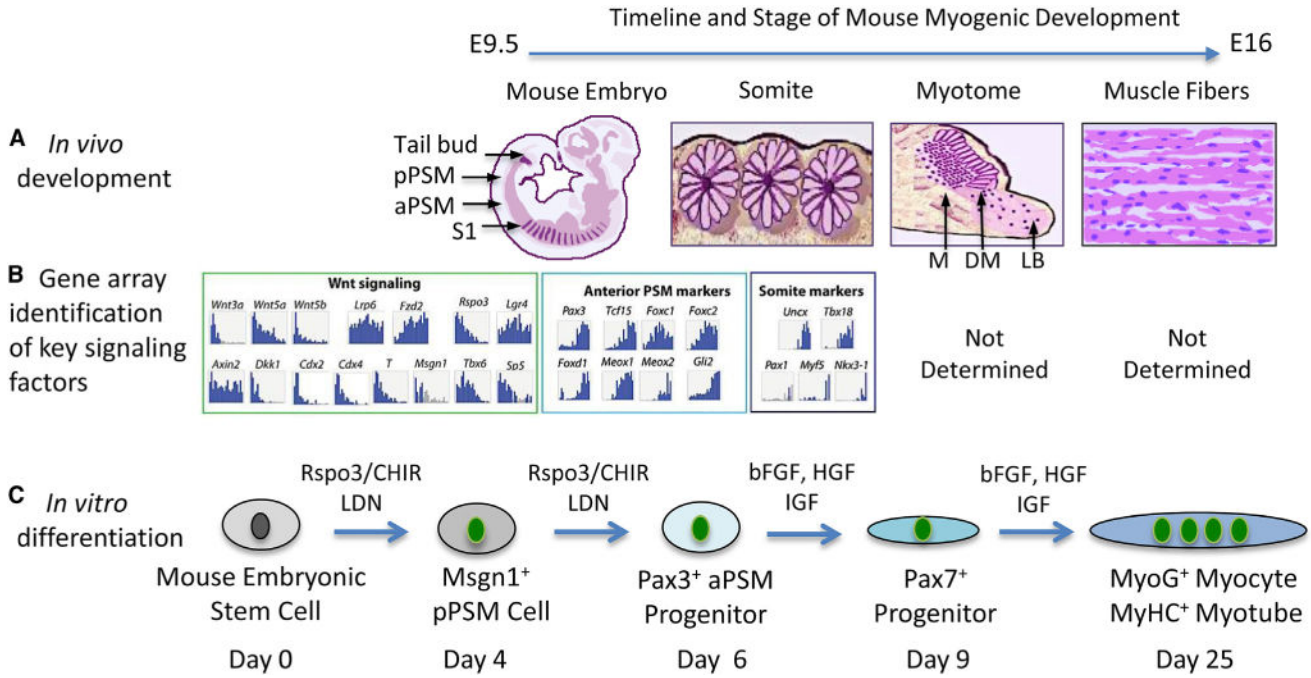

Figure 1. Developmental Myogenesis Guides In Vitro Differentiation

(A) Timeline of skeletal myogenesis from E9.5–E16 mouse embryo. Left to right: images depict a mid-sagittal cross section of a mouse embryo during development and longitudinal sections of three somites. The somites give rise to the dermomyotome/myotome and migratory muscle progenitor cells that seed the limbs and mature to multinucleated fibers. Arrows indicate specific regions in the developing embryo (black).

(B) Micro dissections of tissue regions between the first somite (S1) and tail bud were collected and profiled to aid in the identification of novel targets to drive in vitro myogenesis of mouse embryonic stem cells (mESCs). Not determined refers to the lack of profiling of the myotome and muscle fibers in this work.

(C) The corresponding in vitro cells differentiated from mESCs using developmentally identified growth factors and small molecules are shown. Green nuclei indicate that Chal et al. utilized a reporter to monitor differentiation efficiency at each stage. pPSM, posterior presomitic mesoderm; aPSM, anterior presomitic mesoderm; S1, newly formed somite; DM, dermomyotome; M, myotome; LB, limb bud.