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Recapitulating human myogenesis *ex vivo* using human pluripotent stem cells

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Abstract

Human pluripotent stem cells (hPSCs) provide a human model for developmental myogenesis, disease modeling and development of therapeutics. Differentiation of hPSCs into muscle stem cells has the potential to provide a cell-based therapy for many skeletal muscle wasting diseases. This review describes the current state of hPSCs towards recapitulating human myogenesis *ex vivo*, considerations of stem cell and progenitor cell state as well as function for future use of hPSC-derived muscle cells in regenerative medicine.

Keywords

human myogenesis; human pluripotent stem cells; muscle stem and progenitor cells; development; cell differentiation

1. Introduction

Skeletal muscle is endowed with a remarkable regenerative capacity due in large part to the endogenous muscle stem cells called the satellite cells (SCs) that arise from skeletal muscle progenitor cells (SMPCs) during human myogenesis. In this review, we start by briefly summarizing key features of mouse myogenesis that have been used as the foundation to our understanding of human myogenesis. Next, we focus on skeletal muscle ontogeny in humans and highlight critical differences in SMPC and SC states across distinct stages of human development through adulthood. Building upon the *in vivo* knowledge, we summarize current progress and challenges in generating myogenic cells *in vitro* from human pluripotent stem cells (hPSCs) (depiction in Fig. 1 and summary of key findings in Table 1). Due to space limitations, we limit this review to certain aspects of the field and regretfully are not able to include all references.

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P.·C, **H.X**, and **A.D.P** conceived of the ideas, generated the outline, figures, manuscript writing and final editing of the review article.

Declaration of competing interest

The authors declare no conflict of interests.

2. Myogenesis in mice

All trunk and limb skeletal muscles are derived from somites, transient structures present during early embryonic development that are responsible for body axis elongation and give rise to a variety of tissues in addition to muscles including axial skeleton, brown fat, and dermis of the back [1]. Somites are formed through the periodic segmentation of the most anterior part of the developing presomitic mesoderm (PSM), which originates from the primitive streak during early gastrulation [2, 3]. Compelling evidence suggests the existence of a bipotent neuromesodermal progenitor population residing in the anterior primitive streak in all vertebrates, which can give rise to either PSM cells or the neural cells in the spinal cord under the control of WNT signaling [4]. In the posterior region of PSM, WNT/β-catenin and FGF signaling are highly active and mutually regulate each other to sustain cell proliferation and expansion of the posterior PSM. Toward the anterior region of PSM, activities of the WNT/β-catenin and FGF signaling pathways are gradually decreased. When the cells located in the anterior PSM reach a subthreshold WNT/FGF activity while simultaneously possessing high periodic NOTCH activation, they segment from the anterior PSM to form the nascent somite. An increasing gradient of retinoic acid signaling from posterior to anterior alongside the developing body axis also contributes to somite formation. The process of PSM segmentation and somite formation takes place in a cyclic manner as the embryo elongates [2,5–7].

Shortly after the somite forms, it develops into the dorsal dermomyotome (DM) and ventral sclerotome [1,8]. Skeletal myogenesis initiates when the $Pax3^+$ cells located at the dorsomedial lip of DM begin to express the myogenic regulatory factor Myf5. These cells migrate and settle in between the dorsal DM and ventral sclerotome and further commit to form the first terminally differentiated skeletal muscle structure called myotome [9–12]. In mice, the cells in the central domain of DM start to express Pax7 after the myotome forms and these $Pax3^+Pax7^+$ myogenic cells expand and delaminate ventrally into the myotome to serve as the progenitor pool for embryonic and fetal myogenesis [13,14]. At the limb levels, the cells at the ventral lateral lip of the DM migrate to the developing limb buds before they start to express Pax7 and establish the limb skeletal muscles [15–18]. During late fetal development in mice (E16.5), a subset of $Pax7^+$ myogenic cells start to localize in a specialized niche adjacent to the myofibers under the basal lamina [13,14,19], and these cells exit the cell cycle, fully establish quiescence, and become adult skeletal muscle stem cells (also called satellite cells or SCs) 3–8 weeks after birth [20–22].

Myogenic specification and lineage progression are tightly regulated by signals emanating from the surrounding embryonic tissues [11,23]. WNTs secreted by the dorsal neural tube and surface ectoderm are essential for the specification and maintenance of the DM [24,25]. WNT signaling is also important for myogenic regulatory factor expression and the myogenic fate commitment [26–30]. However, stabilized β-catenin and constant WNT signaling drives the DM cells to a dermal fate while limiting the myogenic program [31]. BMP from the lateral plate mesoderm prevents the myogenic progenitors from precocious terminal differentiation to ensure the expansion of the progenitor pool [32,33]. Interestingly, while SHH produced by the notochord and floor plate promotes the sclerotome fate and limits DM specification of the newly formed somites [1,34,35], the same signaling pathway

has been shown to promote myogenesis in the already established DM [36]. These studies point to the importance of the temporal, spatial, and quantitative coordination of the diverse signaling pathways in the proper ontogeny of skeletal muscles *in vivo* and have key implications of myogenesis *in vitro* which we will discuss in more detail below.

3. Myogenesis in humans

Compared to the wealth of information on skeletal myogenesis in model organisms, our knowledge of human muscle ontogeny is much limited. Nevertheless, studies on human skeletal muscle development confirm that myogenesis is grossly conserved in humans compared to other mammals [37–42]. For example, similar to mice, myofibers appear in successive waves during human development, where in limbs primary fibers arise at embryonic stage (starting from week 7), secondary fibers emerge at early fetal period (around week 10) and tertiary fibers start to form during later fetal development (sparse at week 16–17 and more evident after week 20) [43–45]. Moreover, myofibers at different developmental stages possess unique expression patterns of various myosin isoforms in humans as well as in other mammals [46–48]. For example, *MYH3* and *MYH8* are expressed in embryonic and fetal muscles whereas *MYH4*, which encodes the myosin heavy chain (MyHC) isoform in type 2 B fibers, is not expressed until after birth.

In addition to anatomical and histological characterizations, a few early studies investigated the *ex vivo* properties and behaviors of myogenic cells isolated from human embryos and fetuses. Through clonal expansion and subsequent terminal differentiation, various subtypes of myogenic cells were found to exist both within the same and across different developmental periods [49–53]. These subtypes of cells exhibit distinct myogenic differentiation and fusion capacity *in vitro* and produce myotubes expressing different MyHC isoforms. The unique phenotypes associated with each myogenic subtype are heritable through multiple passages in culture, suggesting the observed heterogeneity is at least partially due to the intrinsic properties of human embryonic and fetal myogenic cells, rather than a mere artifact of prolonged *in vitro* culture. The types and properties of the myogenic subtypes are not exactly the same across different studies, possibly resulting from different isolation procedures, culture conditions, and assessment methods employed. For example, Edom-Vovard et al. showed the presence of four distinct myoblast subtypes giving rise to myotubes with different morphologies and MyHC isoform expression patterns from the beginning of primary myogenesis, and this heterogeneity persists across development and in newborn infants with only the relative proportions of the different myoblast subtypes changing along with development [53]. On the other hand, other studies found that heterogeneous myoblast subtypes arise sequentially during development, and they do not completely co-exist at different developmental stages. Nevertheless, a common observation is that embryonic and fetal myogenic progenitors form different myotubes with distinct sizes and morphologies. Notably, these myotubes possess MyHC isoform expression patterns reminiscent of primary or secondary myofibers *in vivo*, respectively, suggesting that human embryonic and fetal myogenic progenitors are encoded with different myogenic programs and differentially contribute to distinct waves of myogenesis during development.

With the technical advancement of fluorescence-activated cell sorting, several groups have identified cell surface markers that enable purification of myogenic cells arising during different stages of human development as well as SCs in adults [54–60]. The ability to isolate relatively pure human myogenic populations has enabled more detailed molecular and functional characterizations of these cells. For example, Castiglioni et al. found that the myogenic cells purified from human fetal skeletal muscles possess myogenic-osteogenic bipotential when differentiated *in vitro* under permissive conditions [61]. Using CD82 as a positive surface marker, Alexander et al. isolated myogenic cells and SCs from human fetal and adult muscles, respectively, and found that this surface protein is involved in myogenic proliferation and differentiation in culture and likely contributes to the pathogenesis of Duchenne muscular dystrophy [58]. When purified and differentiated *in vitro*, human fetal myogenic cells are less prone to fuse, make smaller myotubes, and express different MyHC isoforms compared to their adult counterparts [60], which is reminiscent of the behaviors of mouse myogenic cells from different developmental stages [62,63]. Furthermore, multiple groups have found that human SCs are heterogeneous [57,64,65], and unlike the mouse, some human SCs were not stained positive for PAX7 *in vivo* but did express key cell surface markers [57], which could be a technical artifact or differences in mouse and human PAX7 regulation and control of stem cell behavior. While most of these studies are focused on mid-to-late fetal period and adulthood, it would be interesting to develop strategies to explore the features of myogenic cells present during human embryonic development to determine similarities and differences of embryonic and fetal progenitors to adult SCs.

Recently, Xi et al. performed a single cell RNA-sequencing (scRNA-seq) study of developing human limb and skeletal muscle tissues from embryonic, fetal, to adult stages [66]. The authors unbiasedly identified various myogenic as well as non-myogenic cell types at different stages. Furthermore, this work provided transcriptional evidence of extensive heterogeneity within the skeletal muscle populations regarding myogenic commitment as well as developmental progression, which supports the phenotypic and biochemical data of different myogenic waves during human development *in vivo* and distinct behaviors of isolated myogenic cells *in vitro*. Interestingly, a myogenic subpopulation was found to be uniquely present during embryonic stage and gradually decreases in proportion as development progresses (still present at fetal week 17–19 but absent in adults). These cells express both skeletal muscle and mesenchymal markers and exhibit myogenic-osteogenic bipotent lineage specification upon purification and *in vitro* differentiation. Future studies are warranted to explore whether a similar population exists in other species and the functional role of this population in development. Leveraging the valuable data from this and other high throughput single cell studies [65,67–71], it will be possible to uncover novel subtype markers based on the bulk myogenic population markers described above to enable detailed downstream analyses of heterogeneous myogenic subpopulations during human development. These analyses could provide novel insights on many open questions regarding human myogenesis, such as the decision of progenitor cell expansion *vs.* commitment during muscle establishment and the emergence of postnatal SCs from prenatal progenitors, to name a few.

Due to the scarcity of human samples and technical limitations, human pre-myogenic somite development has been relatively unexplored until recent work of transcriptional profiling of

developing PSM and somites in early human embryos of 4–5 weeks of gestation [72]. Many of the key signaling pathways involved in mouse somitogenesis are found to be conserved in human embryos but a few seem to be human specific. For example, while TGFβ signaling is upregulated in mouse nascent somites compared to PSM, it is downregulated in human newly formed somites. Accordingly, inhibition of TGFβ signaling was effective in enhancing the transition of a PSM to somite fate of human pluripotent stem cells (hPSCs) during *in vitro* directed differentiation.

Although technological advancement has enabled us to gain a better understanding of human skeletal myogenesis *in vivo*, it is ethically impossible to apply most of the sophisticated genetic approaches routinely used in model organisms to finely dissect the cellular and molecular mechanisms underlying myogenesis during human development. However, thanks to the rapidly growing hPSC technology, it is now possible to recapitulate human myogenesis to a certain degree in a dish for basic mechanistic studies, disease modeling, drug screening, and regenerative medicine applications as described in more detail below.

4. hPSC directed differentiation

hPSCs, which include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are a valuable tool that can be used to understand core aspects of the biology of human myogenic development that otherwise would be difficult or unethical to investigate in humans. Considering that differences exist between model organisms and humans, hPSCs provide a unique system to model human myogenesis in a dish. Various protocols have been developed to differentiate hPSCs into cells of myogenic lineage *in vitro* through two primary approaches. The first approach is overexpression of myogenic transcription factors, primarily PAX7 and MYOD, in pluripotent stem cells to reprogram them into myogenic cells [73–83]. This method promotes quick myogenic specification and the generation of relatively homogeneous cell populations of large cell numbers. However, this approach is not particularly informative for understanding human myogenic developmental biology, and so we will not focus on this approach in this review. The second approach is directed differentiation, which can more tightly control the cell lineages made from hPSCs by recapitulating early developmental signaling patterns that are understood to occur during early skeletal muscle formation in the embryonic and fetal developmental stages [84]. Directed differentiation is achieved by introducing signaling molecules at specific timepoints in the culture medium throughout the directed differentiation timeline. Continuous optimization of this approach would enable human myogenic development to be closely mimicked in the dish and thereby generate *in vitro* hPSC-derived myogenic cells that are similar to those found *in vivo*.

Several groups developed *in vitro* myogenic directed differentiation protocols in which PSM cells are generated first from hPSCs by activating WNT/β-catenin signaling [72,85–91]. PSM cells can then be transitioned to somitic cells by inhibiting BMP and TGFβ signaling, and then transitioned to DM by activating WNT/β-catenin signaling again [72,87,92]. Current protocols then promote the transition from the DM stage towards myogenic lineage using HGF, IGF1, and/or FGF2 during which $PAX7^+$ myogenic progenitors arise [72,86,87,92]. However, these signaling molecules dictating this transition are not specific

because myogenic specification and commitment are currently not well characterized and understood in early human development.

Underexplored and perhaps underappreciated differences in the biology between humans and model organisms could be one reason underlying the inefficiency in hPSC myogenesis, when the manipulation of signaling pathways and culture conditions is almost exclusively dependent on the knowledge from animal development. Similar to mouse somitogenesis, retinoic acid and NOTCH signaling were found to be upregulated in nascent somites than PSM in human embryos. Nevertheless, activation of either of these pathways was not shown to enhance *in vitro* somite specification from hPSCs [72]. Actually, high doses of retinoic acid even shift the culture toward a more neural fate. Similarly, when FGF or MEK-ERK signaling was blocked in hPSC directed differentiation cultures to mimic their low activity near the site of somite formation seen in mice, no significant increase was found in hPSC somite specification efficiencies [72]. Despite the critical roles of WNT and SHH signaling in model organism myogenesis, we could not observe enhanced myogenic specification after activating these pathways in hPSC-derived somite-like cells *in vitro* (unpublished data). These observations point to the potential unique mechanisms underlying human myogenesis compared to model organisms, as well as the complex signaling requirements of finely balanced pathway activities in the right cells and at the right timing.

To make things even more complicated, a seemingly overlooked factor is the impurity of all currently available hPSC myogenic protocols. As a comparison between two timepoints of the directed differentiation protocol by Xi et al., more than 80% of cells after initial activation of WNT/β-catenin signaling (after 2 days) become the posterior PSM cells as directed, but only 20–40% of mononucleated cells are $PAX7^+$ myogenic progenitors after the myogenic specification step (between 4 and 5 weeks), thereby illuminating the inefficiency of myogenic directed differentiation at the latter step [72]. Culturing for multiple weeks for the myogenic cells to arise may also contribute to this inefficiency, and in development, $PAX7^+$ myogenic progenitors arise earlier than in directed differentiation [66]. It is difficult to control all the cell lineages that arise at this stage of directed differentiation as a considerable number of the existing cells do not adopt a myogenic fate [66]. As non-myogenic cell types are inevitably present in the culture, the net effect of myogenic efficiencies upon manipulation of certain developmental signaling pathways will be determined by all the cells, myogenic as well as non-myogenic, that are responsive to the signaling manipulation. Imagine a situation where activation of a certain pathway increases myogenic fate commitment while at the same time greatly augments the growth advantage of a “contaminating” non-myogenic population. In this case, a net decrease of myogenesis could occur. In fact, different directed differentiation protocols produce different populations of non-myogenic cells; Xi et al. showed how different protocols produce different proportions of neural progenitor cells, mesenchymal stromal cells, epithelial cells, and skeletal cells [66]. While some of the non-myogenic cell types in the culture may support the survival of myogenic lineage cells, others may do the opposite and repress it. For example, neural crest cells support developmental myogenesis via NOTCH signaling [93], and neural cells may support myogenesis by promoting formation of functional neuromuscular junctions [94]. On the other hand, some mesenchymal/fibrogenic cell populations arising during directed differentiation may produce extracellular matrix that may

either support or interfere with myogenesis [95]. Furthermore, the percentage of cells that become myogenic vary not only between protocols, but also between different batches of performing the same protocol, influenced by factors such as distinct hPSC lines, various cell passage numbers, and different researchers performing the protocols, among others. This again highlights the difficulty of controlling myogenic differentiation *in vitro*. In order to improve the efficiency of myogenic directed differentiation, it is thereby critical to better optimize this period of myogenic directed differentiation by more precisely identifying the specific signaling pathways that promote skeletal muscle formation, particularly $PAX7^+$ progenitor and satellite cells, as well as the specific non-myogenic cell types that may be needed to support myogenic cells in the human microenvironment.

Another area of myogenic directed differentiation that has yet to be explored is understanding what type of myogenic cells are being made in currently available protocols. It is not yet clear whether these protocols produce muscle cells more similar to those arising in the limb and trunk or those arising in the head [96]. These cells, although are all myogenic, arise through different lineages in early development and therefore have different cell identities. More thorough profiling of these cells would allow us to better understand these differences, which would provide additional insight on how to optimize *in vitro* myogenic specification.

To overcome these various challenges in myogenic directed differentiation, it is therefore critical to systemically test a variety of signaling perturbations at multiple time points in order to achieve the maximal efficiency for a specific hPSC myogenic protocol, which could be practically prohibitive using the traditional trial-and-error approach [97, 98]. To this end, incorporating Design-of-Experiments [99,100], artificial intelligence [101], and/or barcode-based high sample multiplexing scRNA-seq [102,103] into high throughput screening could offer a cost-effective strategy to accomplish this daunting task.

5. Myogenic $PAX7^+$ cell states

PAX7 is the master myogenic transcription factor expressed in skeletal muscle progenitor cells (SMPCs) during prenatal development and in SCs primarily seen in postnatal skeletal muscles. In the process of skeletal myogenesis, these myogenic PAX7-expressing cells expand, differentiate into precursor cells as myoblasts then myocytes then myotubes, which then fuse to generate myofibers that form skeletal muscle across all stages of human development, beginning at approximately embryonic week 6–7 in the limbs [66]. Yet despite this common role, SMPCs and SCs have many different molecular and functional properties. When isolated fetal SMPCs and adult SCs are plated *in vitro* in differentiation medium, fetal SMPCs do not fuse into myotubes as efficiently as adult SCs for both mice and humans [60,104]. *In vivo* mouse studies have shown that SMPCs continuously contribute to muscle growth during developmental myogenesis, but SCs are predominantly quiescent and enter the cell cycle upon injury to contribute to muscle homeostasis and regeneration [104]. Thus, SCs primarily reside in SC niches that maintain their quiescence whereas SMPC niches are molecularly and functionally different than adult niches (unpublished data). Furthermore, Tierney et al. has shown that following transplantation after injury, mouse SCs are more efficient than SMPCs in niche repopulation, and therefore SCs contribute

to repopulating the stem cell pool better than SMPCs [104]. On the other hand, Tierney et al. also showed that fetal SMPCs expand more efficiently during regeneration than adult SCs following transplantation [104]. Using scRNA-seq, Xi et al. also identified that $PAX7^+$ cell states at distinct stages of human myogenic development from embryonic to adulthood possess distinct gene expression profiles [66]. These data collectively emphasize that PAX7 expression alone does not distinguish between SMPCs and SCs from different developmental stages. However, although SMPCs and SCs have these distinct properties, the point in human development at which SMPCs transition into SCs is still uncertain.

Understanding differences in cell identities between SMPCs and SCs *in vivo* provides us with a benchmark in evaluating the maturity of hPSC-derived myogenic $PAX7^+$ cells. Xi et al. evaluated *in vitro* hPSC-derived $PAX7^+$ cells from myogenic directed differentiations reported by different groups and found that all cells across three protocols resemble a gene expression profile similar to those of *in vivo* SMPCs from a late embryonic to early fetal transitional stage [66]. hPSC-derived $PAX7^+$ cells, after enrichment and supplementation with a TGFβ inhibitor, can engraft and restore dystrophin in mdx-NSG mice (a mouse model of Duchenne muscular dystrophy) at a similar efficiency to fetal SMPCs after transplantation. However, unlike adult SCs, transplanted fetal or hPSC-derived SMPCs are rarely found to reside in the SC niche and they do not repopulate the skeletal muscles after reinjury (unpublished data). Furthermore, they do not differentiate and promote myotube fusion *in vitro* as efficiently as adult SCs [60]. Altogether, these studies illuminate that hPSC-derived $PAX7^+$ myogenic progenitors are molecularly and functionally immature in comparison to adult SCs.

Considering how engraftment ability, replenishment of the stem cell pool, and fusion efficiency correlate to developmental timing, it is therefore necessary to develop methods that can mature hPSC-derived SMPCs (hPSC-SMPCs) to improve their regenerative capacity for therapeutic uses. For developing long-term cures, hPSC-SMPCs must be matured into an ideal cell state that has an optimized balance between high self-renewal both *ex vivo* and *in vivo* and high stem cell niche repopulation. One option is to mature hPSC-SMPCs into postnatal-like hPSC-derived SCs (hPSC-SCs) that can repopulate and repair muscle lifelong. However, one challenge with using hPSC-SCs is that a quiescent SC population may be difficult to expand *ex vivo*. Strategies will need to be developed to either support quiescence *ex vivo* or to be able to support proliferating SCs that enable expansion and engraftment in large numbers without losing stemness properties. The second option to consider is a cell state that may instead exist somewhere between a fetal SMPC state and a postnatal SC state, though the exact developmental stage is still unknown since these properties have yet to be evaluated in SMPCs and SCs at those developmental stages, especially in humans. Developing methods to mature hPSC-derived cell types into the optimal stage of development is a pervasive challenge across many cell types in the regenerative medicine field. For example, hPSC-derived dopamine neurons are promising for use in cell replacement therapies for neurodegenerative diseases such as Parkinson's disease, but perhaps similar to transplantation for muscle regeneration, transplanted mature neurons may not survive or provide sufficient function even if they are transplanted into a brain of the same developmental age [105]. Developing a hPSC-SMPC maturation strategy to the most regenerative cell state is one of the key challenges in the path to generating

stem cell therapies for muscle diseases. Recent exciting studies have shown generation of skeletal muscle in three-dimensional (3D) culture systems, but the maturation status and functional potential of these SMPCs or SCs compared to adult SCs or two-dimensional (2D) cultured SMPCs will require additional evaluation in functional assays and animal models [82,106,107]. Additionally, generation of a stem cell state capable of migrating and extravasating will also be important for reaching multiple muscles but will likely require cell engineering in combination with maturation and may be independent of the developmental state. Identifying the optimal state may require driving hPSC-SMPCs to different levels of maturity or cell states and may depend on the intended application in disease modeling or cell-based therapies across different neuromuscular diseases.

6. Conclusions

Significant progress has already been made to develop methods to recapitulate human myogenesis in the dish, informed by the cell types and signaling pathways that arise throughout animal and human myogenic development. Nonetheless, there is still much more to understand regarding how to more efficiently generate $PAX7^+$ myogenic cells during directed differentiation as well as how to manipulate $PAX7^+$ myogenic progenitors to resemble SMPCs and SCs at different levels of maturation. Understanding the optimal cell state with the most regenerative potential still requires an improved understanding of these states in human development through adult. Advances made to overcome these challenges will be critical in the development of regenerative medicine therapies for neuromuscular diseases and muscular dystrophies.

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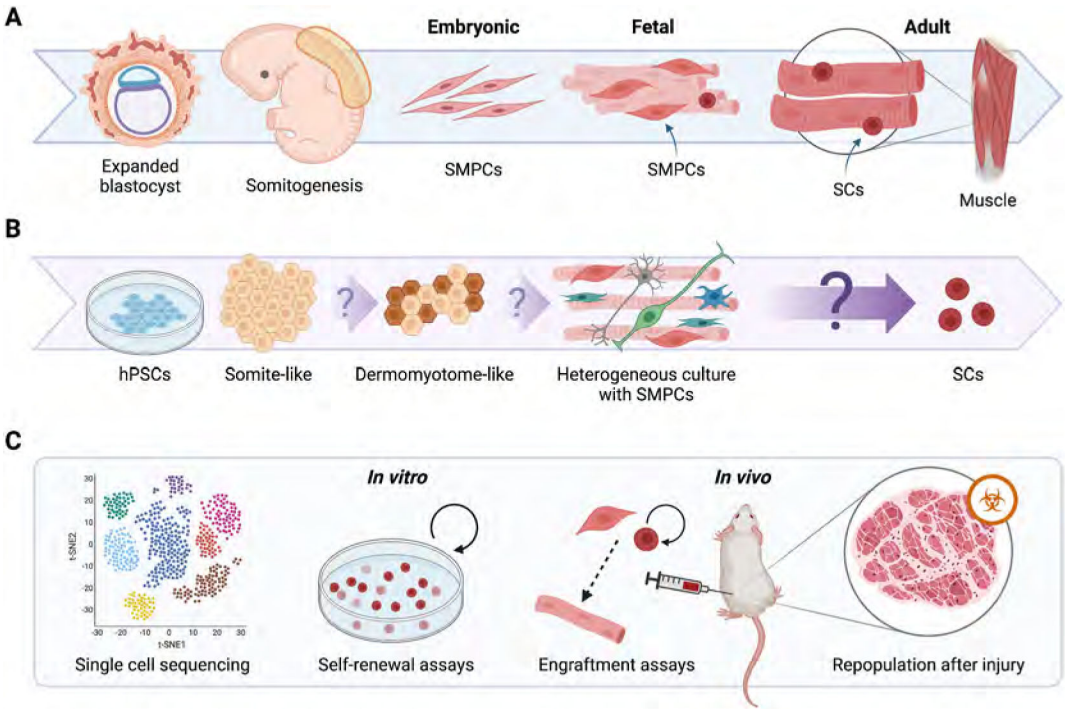

Fig. 1. Schematic of Recapitulating and Evaluating Human Myogenesis Ex Vivo.

A. In human myogenesis, somites generate embryonic, fetal, and adult SMPCs and SCs. **B.** Directed differentiation of hPSCs through generation of somite- and dermomyotome-like cells which give rise to embryonic- and fetal-like SMPCs and then eventually to adult-like SCs. Arrows with question marks represent still unknown timing and regulators controlling each step. **C.** SMPC and SC states can be evaluated using sequencing assays as well as *in vitro* self-renewal and *in vivo* functional assays.

Table 1

Key recent literature on studies of human myogenesis.

Topic	Key Finding	Reference
Characteristics of human developmental myogenesis	Primary, secondary, and tertiary myotube formation in human myogenesis	Draeger et al., J Neurol Sci [44]
	Developmental myosins isoforms and expression	Schiaffino et al., Skelet Muscle [48]
	Presence of four myoblast subtypes in human limb development	Edom-Vovard et al., J Cell Sci [53]
Identification of cell surface markers to isolate myogenic PAX7$^+$ cells	CD34$^-$, CD56^{int}, ITGA7^{hi}	Castiglioni et al., Stem Cell Rep [55]
	CD56$^+$, CD29$^+$	Xu et al., Stem Cell Rep [57]
	CD82$^+$	Alexander et al., Cell Stem Cell [58]
	CD82$^+$, CD318$^+$	Uezumi et al., Stem Cell Rep [59]
	ERBB3$^+$, NGFR$^+$	Hicks et al, Nature Cell Bio [60]
Single cell RNA-seq studies of human skeletal muscle	Atlas of embryonic, fetal, to adult skeletal muscle tissues	Xi et al., Cell Stem Cell [66]
	Analysis of skeletal muscle cell types	Rubenstein et al., Sci Rep [70]
	Analysis of muscle stem cell populations	De Micheli et al., Skelet Muscle [71]
	Identification of subpopulations of PAX7$^+$ satellite cells	Barruet et al., Elife [65]
hPSC directed differentiation to myogenic culture	Specification of PSM-like cells from hPSCs via WNT activation, BMP inhibition, and Nodal signaling	Umeda et al., Sci Rep [88]
	Induction of skeletal muscle from hPSCs via WNT activation and bFGF	Borchin et al., Stem Cell Rep [85]
	Induction of skeletal muscle from hPSCs via bFGF, forskolin, and WNT activation	Xu et al., Cell [89]
	Induction of skeletal muscle from hPSCs via WNT activation, bFGF, and N2	Shelton et al., Stem Cell Rep [86]
	Differentiation of hPSCs to PSM-like cells to primary and secondary skeletal myogenesis	Chal et al., Nature Biotechnol [87]
	Derivation of somite cells from hPSCs via WNT, BMP, and TGFβ signaling	Xi et al., Cell Reports [72]
	Development of a segmentation clock model from hPSCs via NOTCH and WNT signaling	Chu et al., Cell Reports [91]
	Recapitulation of segmentation clock from hPSC-derived PSM-like cells via FGF, WNT, NOTCH, and YAP signaling	Diaz-Cuadros et al., Nature [90]
	Derivation of fetal SMPCs from hPSCs	Zhao et al., Stem Cell Rep [92]