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Review

Myogenic progenitor specification from pluripotent stem cells

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ABSTRACT

Pluripotent stem cells represent important tools for both basic and translational science as they enable to study mechanisms of development, model diseases in vitro and provide a potential source of tissue-specific progenitors for cell therapy. Concomitantly with the increasing knowledge of the molecular mechanisms behind activation of the skeletal myogenic program during embryonic development, novel findings in the stem cell field provided the opportunity to begin recapitulating in vitro the events occurring during specification of the myogenic lineage. In this review, we will provide a perspective of the molecular mechanisms responsible for skeletal myogenic commitment in the embryo and how this knowledge was instrumental for specifying this lineage from pluripotent stem cells. In addition, we will discuss the current limitations for properly recapitulating skeletal myogenesis in the petri dish, and we will provide insights about future applications of pluripotent stem cell-derived myogenic cells.

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Since the discovery that 5-azacytidine treatment of fibroblasts was capable of inducing the activation of the myogenic program [1], it became clear that somatic cells could be forced to switch identity and, importantly, this paved the way for the future cloning of the first master regulator transcription factor: MyoD [2,3]. As a matter of fact, myogenic conversion of fibroblasts using MyoD represented the first example of controlled cellular reprogramming, a concept that has seen its groundbreaking application with the advent of reprogramming somatic cells into induced pluripotent stem (iPS) cells by Yamanaka and colleagues [4]. The iPS cell technology represented an exceptional achievement that opened the possibility to generate patient-specific iPS cell lines which, in turn, broadened the application of pluripotent stem (PS) cells from primarily basic research to potentially regenerative medicine [5]. Throughout the text, we will use pluripotent stem (PS) cells for referring to studies involving both iPS and/or ES cells.

PS cells are capable of differentiating into virtually any cell type and over the years they became important tools for studying mechanisms of development or pathogenesis, and an attractive source of progenitors for the development of cell-based therapies in regenerative medicine [6]. In addition, PS cells are more permissive to genome editing through homologous recombination and this property has further enhanced their potential application in personalized medicine [7]. However, the generation of tissue-specific stem/progenitor cells with proven in vivo regenerative potential from PS cells is evidently a challenging goal, due mostly to the complex cell signaling processes occurring during embryonic development, which are difficult to recapitulate in the culture dish.

In the specific case of the skeletal muscle, signals from adjacent structures, including the neural tube and the notochord, and from migrating neural crest cells are essential for somite patterning toward the dermomyotome and ultimately the skeletal muscle commitment [8–10]. Although early attempts to induce the myogenic program from differentiating PS cells were inefficient, significant progress has been achieved in the last decade. In this review we will provide an historical perspective and an update of the recent findings in this field (Fig. 1).

1. Skeletal myogenesis in the embryo

1.1. Skeletal muscle cell identity

Skeletal muscle cells are defined by the expression of Muscle Regulatory Factors (MRFs), the transcription factors regulating activation of muscle-specific genes. This family of proteins include 4 members, MyoD, Myf5, MyoG and Myf6 (also called Mrf4), which were identified based on their ability to convert fibroblasts into muscle cells, sequence similarity to MyoD, and/or subtractive hybridization [2,11–14]. These transcription factors, which are highly conserved across mammals, result from the duplication of an ancestral gene present also in non-vertebrates (e.g. *nautilus* in the case of *Drosophila melanogaster*) [15]. Subsequent studies demonstrated that MRFs can bind the promoters of several skeletal muscle-specific genes and modulate their transcription through collaboration with multiple proteins, including other transcription factors and chromatin remodeling complexes (reviewed by [16]). The myogenic transcriptional activity of MRFs is also not equivalent, as showed by the role of Myogenin and Myf6 in the later stages of myocyte differentiation [17–19], while Myf5 and MyoD play a major role in myoblasts determination [20]. Interestingly, Myf5 and MyoD also differ on their capability to remodel chromatin and recruit RNA polymerase II [21]. The complexity of the skeletal myogenic program is also highlighted by the complex regulatory regions driving the expression of these transcription factors, with the Myf5-Myf6 and MyoD loci representing the most well characterized [22]. In the case of Myf5-Myf6, enhancer elements are dispersed in genomic regions spanning about 150Kb [23–25], only a few of which have been well characterized using transgenic animals [26,27]. The diversity in regulatory elements responsible for the activation of a single MRF likely reflects the need to control their expression through a mechanism involving different transcription factors restricted to specific subpopulations of myogenic progenitors.

1.2. Embryonic origin of skeletal muscles

All the skeletal muscles of the body originate from the commitment of mesodermal progenitors, which through distinct mechanisms, lead to the expression of the MRFs (reviewed by [28]). Thanks to lineage tracing studies, we now know that, except for the head muscles, all the other muscles of the body derive from the somites, aggregates of paraxial mesoderm that forms on both sides of the neural tube through segmentation of the presomitic mesoderm [28]. Mesoderm patterning represents a key step during embryogenesis, as multiple lineages arise from these uncommitted mesodermal progenitors. In mouse embryos, time and position of entry of mesodermal progenitors into the primitive streak result in the acquisition of a specific fate, and this phenomenon has been attributed to the exposure to different signals from the adjacent structures [29]. Understanding the signals responsible for the specification of paraxial mesoderm has represented a key step for the generation of myogenic cells from pluripotent stem cell cultures in vitro.

Presomitic mesoderm specification occurs in the tail bud of the mouse embryo, an important region containing neuromesodermal progenitors (NMPs) capable of generating both neural and paraxial mesoderm cells [30]. NMPs are characterized by the expression of both Sox2 and Brachyury (T) transcription factors, which define their neural and mesodermal fate, and represent a transient but extremely important population for the formation of the vertebrate trunk (reviewed by [31]). Differentiation of NMPs involves WNT, FGF and RA signals, and interference with any of these signaling pathways is associated with a truncated body axis due to depletion of NMPs [31–33]. Acquisition of the presomitic mesoderm fate involves Wnt3a and Fgf8, which exert a posteriorizing effect on NMPs by inducing expression of the Cdx transcription factors [31]. In NMPs undergoing mesoderm differentiation, Cdx proteins are important for Sox2 repression in favor of T expression [34]. As cells become presomitic mesoderm, T is downregulated while Msgn1 and Tbx6 are upregulated [35]. The expression of these

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