Stem Cell Reports
Report
OPEN ACCESS

Development of Bipotent Cardiac/Skeletal Myogenic Progenitors from MESP1+ Mesoderm

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http://dx.doi.org/10.1016/j.stemcr.2015.12.003

SUMMARY

The branchiomeric skeletal muscles co-evolved with new chambers of the heart to enable predatory feeding in chordates. These co-evolved tissues develop from a common population in anterior splanchnic mesoderm, referred to as cardiopharyngeal mesoderm (CPM). The regulation and development of CPM are poorly understood. We describe an embryonic stem cell-based system in which MESP1 drives a PDGFRA+ population with dual cardiac and skeletal muscle differentiation potential, and gene expression resembling CPM. Using this system, we investigate the regulation of these bipotent progenitors, and find that cardiac specification is governed by an antagonistic TGFβ-BMP axis, while skeletal muscle specification is enhanced by Rho kinase inhibition. We define transcriptional signatures of the first committed CPM-derived cardiac and skeletal myogenic progenitors, and discover surface markers to distinguish cardiac (PODXL+) from the skeletal muscle (CDH4+) CPM derivatives. These tools open an accessible window on this developmentally and evolutionarily important population.

INTRODUCTION

Cardiac and skeletal myogenesis both generate striated muscle, but are traditionally thought of as two separate processes with distinct developmental anlagen. However, recent work suggests the existence of at least one bipotent anlage for cardiac and skeletal muscle: the common progenitor population that leads to the formation of both the head muscles and the second heart field (Lescroart et al., 2010; Nathan et al., 2008; Tirosh-Finkel et al., 2006), and which is unique to chordates (Diogo et al., 2015). Somite-derived myogenesis depends on PAX3 to initiate the core myogenic network (Tajbakhsh et al., 1997), but the head muscles do not derive from a PAX3+ progenitor, instead requiring TBX1 and PITX2 (Sambasivan et al., 2009). These genetic differences between head and trunk muscles may have functional consequences, as several muscular dystrophies have differential severity in craniofacial skeletal muscles compared with trunk muscles (e.g., facioscapulohumeral muscular dystrophy shows greater involvement of facial muscles, while Duchenne muscular dystrophy shows less). Cranial myogenesis has been much less investigated than somite-derived myogenesis.

Cranial mesoderm can be divided into three regions: prechordal mesoderm, cranial paraxial mesoderm, and cardiopharyngeal mesoderm (CPM). CPM, also known as lateral splanchnic mesoderm, is the only embryonic population with both cardiac and skeletal myogenic potential, giving rise to second heart field and distal facial skeletal muscles (e.g., digastric, mylohyoid, and stylohyoid) (Lescroart et al., 2010; Tirosh-Finkel et al., 2006). This developmental intimacy may explain why cardiac and craniofacial congenital defects are often linked, with DiGeorge syndrome being a prominent example (Hutson and Kirby, 2003).

The transcription factor MESP1 is essential to mesoderm patterning (Saga et al., 1999). In the ascidian *Ciona*, cells expressing the homolog *Mesp* give rise to the trunk ventral cells, which subsequently develop into cardiac and skeletal progenitors (Satou et al., 2004). The *Ciona* trunk ventral cells thus act as the ascidian CPM (Razy-Krajka et al., 2014). In the mouse, MESP1 is the earliest acting factor in heart development (Saga et al., 1999). We recently reported that MESP1 also promotes other mesoderm lineages including hematopoietic and skeletal myogenic (Chan et al., 2013). Lineage-tracing studies further revealed that *Mesp1*+ cells contribute to the muscle stem cell pool of the head (Chan et al., 2013; Harel et al., 2009). While the cardiac effects of MESP1 are well studied (Bondue et al., 2008, 2011; David et al., 2008; Lindsley et al., 2008), the mechanisms governing the differentiation of these *Mesp1*-marked bipotent CPM progenitors remain poorly understood.

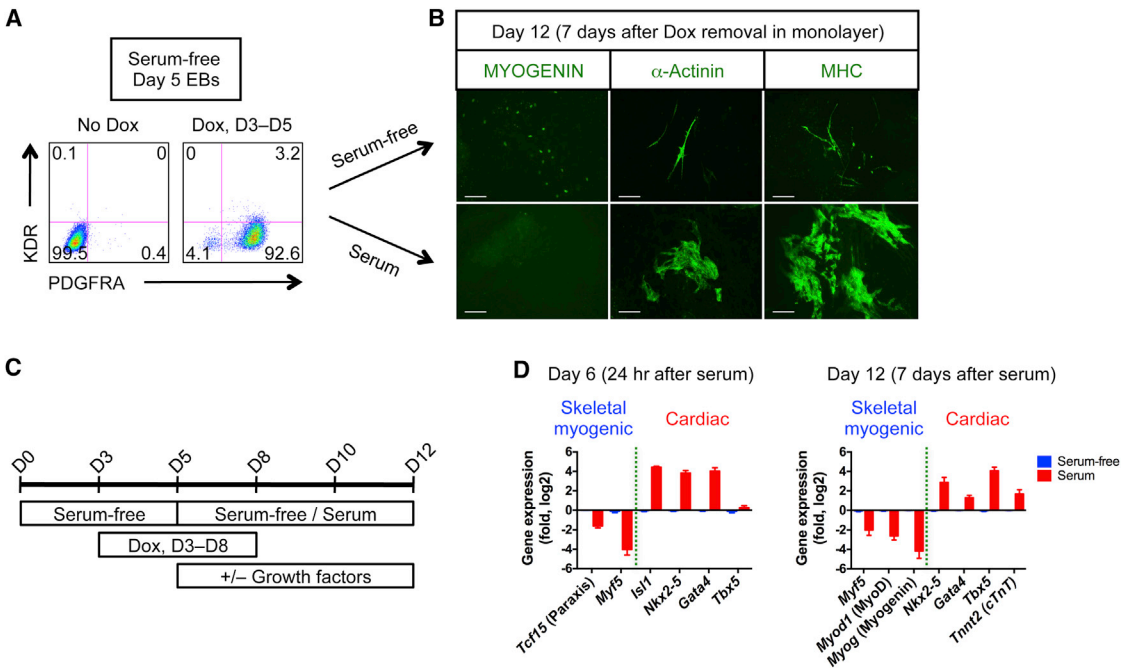

Figure 1. MESP1 Promotes Cardiac or Skeletal Myogenic Differentiation Depending on Serum-Factor Signaling

(A) MESP1 induced a KDR− PDGFRA+ population in day-5 EBs cultured in serum-free condition.

(B) These putative paraxial mesoderm cells gradually acquired a skeletal myogenic fate that was tubular, MYOGENIN+, α-actinin+, and myosin heavy chain (MHC)+ by day 12 (top row). Serum supplement from day 5 produced cardiac cells that were planar, MYOGENIN−, α-actinin+, and MHC+ (bottom row). Images are representative of five independent experiments. Scale bar represents 100 μm.

(C) Scheme depicting the protocol used to evaluate the effects of various treatments on MESP1-induced cardiac versus skeletal myogenic differentiation.

(D) Quantitative RT-PCR analysis showing that the addition of serum downregulated skeletal myogenic genes (*Tcf15*, *Myf5*, *Myod1*, *Myog*) and upregulated cardiac genes (*Isl1*, *Nkx2-5*, *Gata4*, *Tbx5*, *Tnnt2*) as early as 24 hr (left), and also after 7 days (right) (n = 12, from four independent experiments). Mean ± SEM is shown.

See also Figure S1 and Tables S1 and S2.

Here we report that under serum-free conditions, MESP1 induction in embryonic stem (ES) cells produces PDGFRA+ progenitors with dual cardiac/skeletal myogenic potential, functionally resembling CPM. We further demonstrate the robustness of this MESP1-induced system for identifying pathways that enhance the cardiac/skeletal myogenic bifurcation and discovering cell-surface proteins as markers to distinguish these two lineages.

RESULTS AND DISCUSSION

MESP1 Induces Cardiac or Skeletal Myogenic Differentiation Depending on Serum-Factor Signaling

We have previously developed a mouse ES cell line in which MESP1 expression can be tightly regulated by the addition of doxycycline (Dox) (Chan et al., 2013). In embryoid bodies (EBs) cultured in serum-free medium, mesoderm does not form, as evidenced by the absence of KDR (Flk-1) and PDGFRA (PDGFRα) (Figure 1A, No Dox). In contrast, MESP1 induction from days 3–5 of serum-free culture generated a KDR− PDGFRA+ population (Figure 1A, Dox). When attached and continued in serum-free culture, skeletal myocytes emerged by day 12 (Figure 1B, top row). If serum was included from the start, MESP1 induction led to cardiac differentiation instead (Chan et al., 2013). We next performed differentiation in serum-free conditions, but added back serum at day 5. Surprisingly, this converted differentiation toward cardiac instead of skeletal myogenic (Figure 1B, bottom row). We tested different windows of MESP1 induction to map the window of maximal skeletal myogenic activity and found that the day 3–8 window was optimal (Figure S1A); thus this window was used for subsequent studies (Figure 1C).

Gene expression analysis revealed that within 24 hr of serum addition, paraxial mesoderm (*Tcf15*, paraxis) and skeletal myogenic genes (*Myf5*) were downregulated while cardiac genes (*Isl1*, *Nkx2-5*, and *Gata4*) were upregulated