Heat shock protein 70 (Hsp70) overexpression drives myoblast fusion during C2C12 cell differentiation

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Skeletal muscle is the most abundant tissue in the human body and constantly exposed to mechanical forces and shear stresses. It is susceptible to injury particularly during forced lengthening. Fortunately, skeletal muscle has a high regenerative capacity due to its resident population of adult stem cells (MuSCs). MuSCs primarily exist in a quiescent state and express the paired homeobox protein, Pax7. In response to injury, MuSCs are activated, re-enter the cell cycle and become specified to the myogenic lineage (*via* expression of MyoD). They then proliferate rapidly, differentiate (marked by expression of myogenin; MyoG) and undergo fusion to form multinucleated myotubes (Yin *et al.*, 2013). During myogenesis there are dramatic changes in cell size, shape, metabolism and motility which cause cellular stress and alter proteostasis (Tang & Rando 2014).

The molecular chaperone heat shock protein 70 (Hsp70) maintains proteostasis by regulating protein biosynthesis and folding, facilitating transport of polypeptides across intracellular membranes and preventing stress-induced protein unfolding/aggregation (Clerico *et al.*, 2015). Interestingly, whole transcriptome analyses of quiescent and activated MuSCs revealed that the *Hspa1a* and *Hspa1b* genes encoding Hsp70 are significantly downregulated upon MuSC activation, suggesting potential involvement of Hsp70 in mediating myogenesis (Ryall *et al.*, 2015).

We first characterized the protein expression of Hsp70 in proliferating and differentiating myogenic C2C12 cells and observed a 2-fold enhancement in Hsp70 levels during early stages of differentiation relative to proliferating cells ($P < 0.05$). Based on this finding, we hypothesized that Hsp70 overexpression in proliferating muscle cells would promote myogenesis through enhanced cell fusion. To better understand the roles of Hsp70 in myogenesis, plasmid DNA encoding GFP alone or a GFP-Hsp70 fusion protein was transiently transfected into proliferating C2C12 myoblasts and effects on cell proliferation, differentiation and fusion were determined.

Hsp70 overexpression did not affect mean doubling time (Td) of proliferating C2C12 cells (Td ± 95% CI: GFP 28.0 ± 3.0 h *vs* GFP-Hsp70 28.3 ± 3.2 h) or the early differentiation process governed by MyoG expression at one day post-differentiation (mean % MyoG+ cells ± SEM: GFP 30.2 ± 0.8% *vs* GFP-Hsp70 30.1 ± 0.8%). However, GFP-Hsp70 overexpression resulted in an ~30% increase in both median number of myonuclei per myotube ($P < 0.01$) and median myotube width ($P < 0.0001$) relative to GFP three days after induction of differentiation. Similar increases were observed in median number of myonuclei per myotube ($P < 0.001$) and median myotube width ($P < 0.0001$) for GFP-Hsp70 transfected myotubes compared to GFP at four days post-differentiation.

These findings imply that enhanced HSP70 expression strongly promotes myoblast fusion with potential for treating muscle injuries and numerous disorders associated with muscle atrophy. Future studies will attempt to uncover the cellular mechanisms and identify fusion related molecules that interact with HSP70 to drive myoblast fusion.

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