Editorial

Sonic hedgehog-regulated myotome formation relies on the myomiR, miR-133

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Over the past two decades a great deal of information has been provided concerning myogenesis—the process of skeletal muscle formation. Much is known about the cellular and molecular mechanisms of adult myogenesis—the process through which adult muscle regenerates following acute injury and embryonic myogenesis—the process of skeletal muscle tissue development (1,2); however, the picture of the molecular mechanisms governing myogenesis is far from being complete. A recent paper by Mok *et al.* (3) adds an important piece of information to the mosaic of molecules/factors driving the ordered sequence of muscle-related events leading to cell fate decisions in developing embryos.

Skeletal muscle is the most abundant tissue and largest endocrine organ of the vertebrate body playing vital roles in breathing, posture maintenance, locomotion, whole-body metabolism, and reservoir of glucose and amino acids that can support energy demand during extreme metabolic perturbation (4). Skeletal muscles form early during embryonic development in a highly regulated manner (5,6). The process of skeletal muscle development requires a number of extracellular signals that induce the expression of intracellular effectors such as the myogenic regulatory factors (MRFs) which become expressed in a hierarchical fashion, and noncoding RNAs as posttranscriptional regulators (5,6). In vertebrate embryos, the somites transient paired segments that form in a regular sequence on either side of the neural tube and notochord give rise to skeletal muscles of the trunk and limbs (7). Extracellular signals including Wnt, Sonic hedgehog (Shh) and Notch (8-13), promote morphogenetic changes in the somites that differentiate from essentially epithelial structures into the sclerotome and the dermomyotome. Cells dissociating from the ventral side of somites form the sclerotome, while those dissociating from the dorsal (i.e., dermomyotomal) side originate the myotome by providing myoblast progenitors. Myotome formation initiates at the epaxial lip of the dermomyotome, along the lateral border of the neural tube (14). Migrating neural crest cells promote the dorso-ventral migration of dermomyotome-derived progenitors to form the myotome, where they begin to differentiate into myoblasts, the precursors of muscle fibers (13).

In this scenario, the notochord- and floor plate-derived extracellular signal, Shh, activates myogenesis in combination with Wnt proteins (12), and is required for the activation of the myogenic determination gene, *Myf5*, in epaxial and/or hypaxial muscle progenitor cells in avian and mouse embryo (10,15,16). *Myf5* activation requires the activity of the activator proteins, Gli1 and Gli2 (5,6). Gli proteins are a family of zinc-finger proteins (Gli1, Gli2, and Gli3) of which Gli1 and Gli2 are transcription activators whereas Gli3 is a transcriptional repressor (17). The net result of the activity of Gli proteins depends on the relative abundance of individual proteins. Shh signaling changes the amounts of the three Gli family members by regulating their post-translational proteolytic processing. Absence of Shh leads to Gli3 repressor-induced inhibition of *Myf5* transcription (18) resulting in defective muscle

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development. Also, in both avian and mouse embryos, Shh signaling is required for the transition from proliferating (Pax7-positive/MyoD-positive/myogenin-negative) myoblasts to terminally differentiating (Pax7-negative/MyoD-positive/myogenin-positive) myocytes (16).

The muscle-specific (myomiR) miR-133 is now reported to mediate Shh-induced myotome formation in chick embryo via a negative regulation of Gli3 repressor (3). MicroRNAs (miRs) are short noncoding RNAs that bind to target sites located in 3'UTRs of mRNAs leading to inhibition of translation, mRNA cleavage and transcript degradation via deadenylation (19). By affecting target gene expression, miRNAs regulate developmental timing and support cell fate decisions (20). The miR-133 family comprises two members, miR-133a and miR-133b, of which miR-133a is expressed in cardiac and skeletal muscles, whereas miR-133b is skeletal muscle-specific (5,6,21). miR-133 is transcriptionally controlled by some of the major regulators of muscle differentiation such as serum response factor, MyoD, myogenin, and Mef2 (5,6). During embryogenesis, miR-133 can silence non-muscle (i.e., endoderm and neural) genes during cell lineage commitment (5,6). Also, miR-133 regulates myoblast proliferation and differentiation (5,6) and prevents myoblast-brown adipocyte transition by repressing the expression of brown adipose determination gene, *Prdm16* (5). Thus, miR-133 serves several functions in *Myf5*$^{+}$ progenitors repressing endoderm and neural differentiation in embryonic cells and brown fat formation and promoting skeletal muscle formation.

After having confirmed miR-133 expression in nascent myoblasts, Mok *et al.* (3) documented partial loss of Myf5, MyoD and myogenin expression after 9 h of knockdown of miR-133 in HH14/15 embryos with accompanying loss of epithelial morphology in the dermomyotome and myotome and compromised myogenesis by 24 h. However, knockdown of miR-133 in HH20/20 embryos, when miR-133 is expressed in a more developed myotome, did not lead to myogenic defects, which points to a critical temporal window during which miR-133 function is essential in less mature somites. By gene ontology analysis, Mok *et al.* (3) found that following miR-133 knockdown, genes involved in cell proliferation and myogenic differentiation were significantly decreased as were genes operating downstream of the Shh pathway including patched-1 (Ptch1) and patched-2 (Ptch2) receptors, Hedgehog interacting protein (HHIP), Fgf8, and Gli1. On the other hand, in these conditions the transcriptional repressor, Gli3, turned out to be upregulated, suggesting that Gli3 might be a direct target gene for miR-133. Knockdown of miR-133 along with treatment with the Shh signaling activator, purmorphamine, or with Gli3 morpholino to knockdown Gli3 resulted in restoration of myogenesis, yet with reduced myotome size likely consequent to Shh-dependent de-repression of Gli3 and reduced miR-133-dependent cell proliferation. However, following miR-113 knockdown and boosting of Shh signaling with purmorphamine the epithelial nature of the dermomyotome was preserved in somites and basement membrane deposition was restored around the dermomyotome and myotome. These latter results suggest that while miR-113 activities might be restricted to muscle progenitor cell fate, effects of miR-113 knockdown during early embryonic myogenesis might extend beyond the myotome affecting the overall architecture of dermomyotomes. Importantly, Mok *et al.* (3) found that miR-133 directly target Gli3 as investigated by luciferase assay, and that silencing of Gli3 in myoblasts at an early stage of muscle formation promotes the stable activation of the skeletal muscle differentiation program in response to Gli activators, Gli1 and Gli2, a finding corroborated by the observation that miR-133 expression in somites is mutually exclusive with Gli3. Thus, Mok *et al.* (3) uncovered a novel Shh/MRF/miR-133/Gli3 axis that connects epithelial morphogenesis with myogenic fate specification.

Ptch1 and Ptch2 receptors, the transcriptional activator Gli1, the membrane glycoprotein HHIP, Fgf8, and the myogenic determination gene, Myf5, become expressed in the myotome in response to Shh [see (3,5,6,10,15,16)]. As these genes are negatively regulated by miR-133 knockdown in developing somites (3), they might be secondary targets of miR-133. Alternatively, they may be directly regulated by Gli3 repressor as suggested by the presence of potential Gli-binding sites within 2kb upstream of transcription start sites (3). In this context, the compromised cell proliferation following miR-133 knockdown in developing somites might be accounted for by the resultant Fgf8 downregulation. Also, the reduced Fgf8 signaling in these conditions might contribute to the disturbed dermomyotome organization and basement membrane deposition consequent to miR-133 knockdown (3), which points to long-range effects of miR-133.

Together, the results described by Mok *et al.* (3) point to a critical role of miR-133 in the orchestration of early embryonic myogenesis downstream of Shh signaling. However, Shh also has a role in adult myogenesis acting

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to promote myogenin-independent myoblast fusion in the deep myotome, myoblast proliferation in acute and chronic muscle injury, and angiogenesis (22-24), to inhibit adipogenesis in acutely and chronically injured muscle (25), and to exert a protective role during skeletal muscle ischemia/reperfusion injury (26). Future studies would hopefully establish whether the Shh/miR-133 axis has a role in these conditions. Recently, oxidative stress was linked to downregulation of miR-133 in myoblast cell lines via an S100B/NF-κB/YY1 axis that negatively regulates the promyogenic and anti-adipogenic miR-133 and promotes myoblast-brown adipocyte transdifferentiation (27). This same study documented that differently from their young counterparts, geriatric mouse muscles express elevated levels of S100B and show atrophic myofibers intermingled with interstitial brown adipocytes along with expression of the brown adipocyte marker, uncoupling protein 1, in myofibers (27). These results point to an inverse correlation between oxidative stress and miR-133 abundance in geriatric muscle tissue, which calls for an in-depth analysis of the Shh/miR-133 axis in the context of the disturbed redox status characterizing the aging muscle, with potential impact on our knowledge of the pathophysiology of primary sarcopenia and muscle wasting during the course of certain cancerous conditions, chronic cardiac, respiratory and renal failure, and chronic inflammatory diseases as well as on therapeutic interventions. In conclusion, the Shh/miR-133 axis described by Mok *et al.* (3) might represent a global player in the context of myogenesis from embryogenesis to the adulthood.

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