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Cilia control fat deposition in tissue repair

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Fibro/adipogenic progenitors (FAPs) are emerging as crucial regulators of fibrous and fat deposits during skeletal muscle regeneration. In this issue of Cell, Kopinke et al. (2017) report that primary cilia restrict the adipogenic fate of FAPs in injured and diseased muscle through Hedgehog signaling.

Skeletal muscle is the most abundant tissue in vertebrates. After acute injury, healthy muscle can engage a strong regenerative response that usually leads to the complete repair of the tissue. This remarkable capacity relies on the presence of a population of adult stem cells named satellite cells but also in the coordinated intervention of inflammatory cells and other muscle resident cells, and their secreted factors, including a large amount of cytokines, growth factors and matrix metalloproteinases (MMPs) (Mann et al., 2011). Among the muscle resident cells, a population of PDGFRα+ mesenchymal cells have been identified as progenitors of fibroblasts and adipocytes (fibro/adipogenic progenitors, FAPs) (Joe et al., 2010; Uezumi et al., 2010). During normal regeneration, FAPs provide a transient extracellular matrix (ECM), as well as pro-myogenic signals for SCs. However, in situations of chronic tissue damage, such as in muscular dystrophies, activated FAPs persist in the tissue, leading to excessive ECM accumulation and fatty infiltration. Which signals drive FAPs transformation into adipocytes, and their persistence in faulty regenerating muscle are not well known.

In this issue of Cell, Kopinke et al (2017) provide an answer for this question uncovering a previously uncharacterized player in the muscle regeneration process: the ciliary Hedgehog (Hh) signaling (Kopinke, 2017). Primary (non-motile) cilia are microtubule-based organelles that emanate from the plasma membrane of most mammalian cell types and function as cell signaling hubs for many extracellular signaling cascades, especially for the Hh pathway (Malicki and Johnson, 2017; Rohatgi et al., 2007). Interestingly, Hh signaling has been shown to induce adipogenesis in vitro, although no evidence of such requirement has been reported in vivo (Dalbay et al., 2015).

Kopinke et al (2017) first determined which cellular populations implicated in muscle regeneration contained primary cilia and could be responsible for Hh signaling. Interestingly, they found that PDGFRα+ cells (FAPs) included most of the ciliated cells, despite that about half of the FAP population has no cilia. To assess the relevance of cilia-mediated signaling
in FAPs for fat accumulation in regenerating muscle, the authors generated mutant mice lacking primary cilia in FAPs (Pdgfrα-CreERT-Ift88<sup>−/−</sup> mice; FAP<sup>no cilia</sup>). These mice displayed reduced adipocyte accumulation after injury resulting in improved muscle regeneration. These results were validated crossing FAP<sup>no cilia</sup> with a mouse model of Duchenne muscular dystrophy (DMD). Since substitution of muscle fibers by fat is one of the hallmarks of DMD, cilia-mediated adipogenesis may be a new target for this disease.

Consistent with the close association of primary cilia and Hh, FAPs lacking cilia displayed increased expression of Hh target genes, supporting a role of Hh signaling in the adipogenic differentiation of FAPs. Accordingly, constitutive Hh pathway activation in FAPs blocked adipogenesis during muscle regeneration. The authors went on and determined that FAP cilia regulate specifically adipocyte differentiation through a mechanism involving the repression of CCAAT/enhancer-binding protein family (C/EBPα and C/EBPβ) and PPARγ transcription factors. Whether these adipogenic regulators are direct targets of Hh signaling remains unknown. Overall, these experiments demonstrate that FAP cilia promote adipogenesis by restraining Hh signaling.

In an elegant set of experiments using genetic and pharmacological gain-and-loss of function, the authors showed that cilia induce adipogenesis in FAPs via a cell non-autonomous mechanism, supporting the function of secreted factors as drivers of this differentiation process in damaged muscle. Through transcriptomic analysis between ciliated and non-ciliated FAPs, the expression of the tissue inhibitor of metalloproteinase 3 (TIMP3) (a secreted inhibitor of members of MMP and ADAM families) appeared under the control of cilia-Hh signaling. Since TIMP3 has been associated with the regulation of adipogenesis, these results suggested that the TIMP3/MMP balance might regulate FAP adipogenic fate through ciliary-Hh signaling. In agreement, downregulation of TIMP3 in a pre-adipocyte cellular model promoted adipogenesis in vitro. The transcriptomic analysis also uncovered the pro-adipogenic MMPs, MMP2 and MMP14, deregulated between ciliated and non-ciliated FAPs. Downregulation or inhibition of MMP14 (but not MMP2) or pharmacological mimicking of TIMP3 blunted adipogenic differentiation and blocked fat accumulation after muscle injury. Whether TIMP3 is a direct target of cilia-Hh signaling is not yet established, neither is it known if the ciliated FAPs are the sole source of TIMP3, as MMPs and their inhibitors are produced by many different cell types including those infiltrating the injured muscle.

In conclusion, this study demonstrates that cilia are crucial for the transition of FAPs into mature adipocytes in degenerating muscle through Hh signaling. Yet, whether cilia also promote adipogenic (or fibrogenic) differentiation of other muscle resident cell types, particularly in the context of muscular dystrophy, is not known. This is a timely question as, in mdx dystrophic muscle, cell types other than FAPs, such as muscle stem cells or endothelial cells, contribute moderately to fibrosis development through TGFβ-driven fibrogenic conversion (Biressi et al., 2014; Pessina et al., 2015). Whether the cilia-Hh signaling is operative in other tissues/organs and underlie, at least in part, fat and fibrous deposits, remains to be discovered.

Which is the cellular source of the endogenous Hh ligand in injured or dystrophic muscle? Using distinct methods of muscle injury with differential adipogenic-inducing potential, Kopinke et al (2017) identify Schwann cells as the major cell type producing Dhh (Desert hedgehog) in skeletal muscle after injury. However, a systematic analysis of potential non-
neural sources of Hh ligand in degenerating muscle should be performed. The neural source of Hh ligands is nevertheless consistent with the known fibro-fatty accumulation within muscle after denervation. In line with this interpretation, FAPs have recently been shown to increase in number in amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease coursing with muscle wasting and paralysis (Gonzalez et al., 2017). Yet, whether failure of the nerve to supply Hh ligand accounts for unrestrained adipogenic conversion of FAPs and persistent intramuscular fat accumulation in this disease is unknown. Future experiments should investigate this hypothesis based on its therapeutic relevance.

This study opens several biomedical venues. The fact that pharmacological inhibition of MMP14 activity with batimastat represses intramuscular adipogenesis paves the way to interventions in muscular dystrophies. Furthermore, new therapeutic strategies based on cilia-Hh regulation may possibly emerge for the treatment of other diseases coursing with adipogenesis.

References


