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Repeal and replace: adipocyte regeneration in wound repair

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Abstract: Adipocyte precursor cells generate lipid-filled mature adipocytes in multiple tissues including traditional adipose tissue during high fat diet and skin during hair follicle growth. In *Science*, Plikus et al. report that myofibroblasts can generate lipid-filled adipocytes in large skin wounds that regenerate hair follicles, suggesting a new source of adipocyte precursor cells.

Main text:

Skin regeneration after injury requires the coordination of multiple cell types to regenerate epidermal keratinocytes and dermal cells. The epidermis provides an essential barrier against external pathogens and is supported structurally by fibroblasts that produce a rich extracellular matrix (ECM) within the dermis. The dermis also contains many other cell types including immune cells, neurons and lipid-filled adipocytes. While fibroblasts and adipocytes both develop from the mesenchyme (Driskell et al., 2013), the cellular and molecular mechanisms by which mesenchymal cells regenerate after injury is not well understood.

In recent years, a growing area of inquiry has been the role of dermal adipose tissue in skin regeneration. Adipocyte precursor cells (APCs) and/or mature adipocytes have been implicated in several regenerative and pathological processes in the skin including hair follicle regeneration (Festa et al., 2011; Donati et al., 2014) and fibroblast regeneration after injury (Schmidt and Horsley, 2013). Despite the emerging importance of both APCs and their lipid-filled mature adipocyte progeny in skin regeneration, the lineages and molecular machinery that control the development and homeostasis of adipogenic cells *in vivo* remain poorly understood.

In traditional models of wound repair in mice and in adult human patients, hair follicles and mature adipocytes do not regenerate. However, hair follicles can regenerate de novo (called wound induced neogenesis) in the center of mouse wounds if the injury is sufficiently large (see references within Plikus et al., 2017). In a recent study, Plikus and colleagues note that when newly regenerated hair follicles form in sizeable wounds, mature adipocytes are present (Plikus et al., 2017). To identify the cellular origins of adipocytes in these wounds, the authors examine the possibility that adipocytes are regenerated by myofibroblasts, which repopulate the dermis of skin wounds after injury (Driskell et al., 2013; Rinkevich et al., 2015; Schmidt and Horsley, 2013) (Figure 1), and have been shown to generate mature adipocytes in other adipose depots (Jiang et al., 2014). Using genetic lineage tracing with SMA-CreER and SM22-Cre mice crossed to lacZ reporter mice, the authors identify lacZ+ cells adjacent to the regenerated adipose tissue in their large wound model, and conclude that myofibroblasts are reprogrammed to generate adipocytes in the skin (Plikus et al., 2017).

Transcriptional profiling of fibroblasts identifies several molecular changes during wound repair, including BMP2 and BMP4 expression. Mice overexpressing the BMP inhibitor, Noggin in keratinocytes, mice treated with a BMP antagonist, LDN-193189, or mice with a deletion of BMPR1A in myofibroblasts do not regenerate adipocytes, even when hair follicles regenerate. Thus, activation of BMP signaling in myofibroblasts is essential for the formation of mature adipocytes after repair of large wounds.

By identifying myofibroblasts as adipogenic cells in the skin, the present study adds to our current understanding of dermal mesenchymal cell heterogeneity. Distinct fibroblast cell populations in the skin have been highlighted by several recent studies (Figure 1). Lineage tracing studies in mice reveal that early in development, a common mesenchymal precursor present at E12.5 generates fibroblasts and APCs later in development and that as the skin matures, these two lineages maintain their own precursors (Driskell et al., 2013; Festa et al., 2011). Furthermore, recent work reveals that myofibroblasts in wounds and fibrotic skin are derived from embryonic precursors that express *Engrailed* (Rinkevich et al., 2015). CD26 is dynamically expressed on fibroblast subpopulations (Driskell et al., 2013) and the Rinkevich study highlights the expression of CD26 on myofibroblasts that contribute to ECM production after injury. These studies use standard wound models that fail to regenerate hair follicles during healing, and it will be interesting to define whether CD26+ and/or engrailed + cells form adipocytes in the large wound model.

The ability of cells that express myofibroblast markers to generate adipocytes seems contrary to the fibrotic events that reduce adipogenesis in the skin and other adipose depots. Defined as cells that express smooth muscle actin, myofibroblasts have been proposed to derive from fibroblast differentiation and/or the acquisition of contractile phenotypes by existing fibroblasts (Figure 1). Myofibroblast numbers increase within wound beds during skin regeneration and during fibrosis (Driskell et al., 2013; Rinkevich et al., 2015; Schmidt and Horsley, 2013). Activation of β-catenin in the lower dermal fibroblast lineage leads to conversion of the adipocyte layer into fibrotic dermis (Mastrogiannaki et al., 2016). Since myofibroblasts may be derived from current mesenchymal populations in the skin and/or from specific myofibroblast progenitor cells, whether the adipogenic capacity of myofibroblasts is a true “reprogramming” event is unclear. Importantly, induction of Sma-CreER activity prior to creation of the large wounds labels only vascular smooth muscle cells within the wounds (Plikus et al., 2017), suggesting that pre-existing myofibroblasts are not capable of adipogenesis in large wounds.

A standard method in the field to identify APCs involves the analysis of cell surface markers on mesenchymal cells by flow cytometry (Festa et al., 2011). APCs express several markers including PDGFRα, CD29, CD34, and Sca1 that when used in combination can purify APCs (Driskell et al., 2013; Festa et al., 2011). Analysis of APCs in the skin has revealed that they proliferate during hair cycle

associated adipogenesis (Donati et al., 2014; Festa et al., 2011; Rivera-Gonzalez et al., 2016) and following standard injury paradigms (Schmidt and Horsley, 2013). It is unknown whether APCs are activated in the authors' large wound model or whether APCs express *SMA* or *SM22*, the promoters used to lineage trace myofibroblasts in the Plikus et al. study, thus raising the possibility that APCs can generate myofibroblasts during wound healing.

The large wound model in which hair follicle neogenesis occurs (Plikus et al., 2017) highlights an emerging theme that hair follicles can influence differentiation and/or maturation of adipocyte lineage cells. Others have shown that activation of hair cycling by hair plucking or keratinocyte-driven expression of activated β-catenin or Shh can induce dermal adipogenesis (Donati et al., 2014; Zhang et al., 2016). While mesenchymal derived signals such as Pdgfa can also regulate APC proliferation (Rivera-Gonzalez et al., 2016), the study by Plikus et al. further highlights the ability of dermal adipocyte lineage cells to respond to signals derived from hair follicles. Further investigation into the source of BMP signals and how hair follicle cells impinge on adipocyte lineage decisions will have implications for adipose regeneration in the skin and regulation in other adipose depots.

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Figure 1. Model of the mesenchymal cell heterogeneity and lineages in the skin. CD24+, Sca1+ adipocyte stem cells and Sca1+ adipocyte precursor cells generate mature adipocytes in non-wounded skin. Fibroblast lineages that express CD26 and Engrailed1 can generate myofibroblasts in skin wounds. Plikus et al. reveals that myofibroblasts generate mature adipocytes in large skin wounds. Whether a myofibroblast progenitor, adipocyte stem or precursor cells can generate myofibroblasts is not known.