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Revitalizing Aging Skin through Diet

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Mechanisms underlying aging of the skin dermis are poorly understood. Now, two studies (Marsh et al., 2018; Salzer et al., 2018) describe complementary approaches to this question: Salzer et al. show that aging dermal fibroblasts lose defined identity in a diet-influenced fashion, and Marsh et al. reveal that fibroblast loss over time is compensated by membrane expansion rather than proliferation, resulting in decreased cellular density.

Changes in skin appearance are some of the most evident signs of aging. These result in part from loss of cellularity and extracellular matrix in the skin's stromal compartment, known as the dermis (Demaria et al., 2015). However, the cellular and molecular mechanisms that maintain dermal functions in homeostasis, and how these are affected during organismal aging, are poorly understood. Now, Holger Heyn, Salvador Benitah, and co-workers use transcriptomics and single-cell analyses to show that aging upper dermal fibroblasts gradually acquire characteristics of the lower dermis, including pro-adipogenic traits. Interestingly, these are partially prevented by caloric restriction and are enhanced by a high-fat diet (Salzer et al., 2018, this issue of *Cell*). In parallel, Valentina Greco and co-workers use innovative intravital imaging approaches to reveal that clusters of fibroblasts are lost following focal damage and in aging skin; these spaces are filled by membrane expansion of neighboring cells rather than by proliferation, accounting for loss of cellularity (Marsh et al., 2018, this issue of *Cell*). Consistent with this, genes identified by Salzer et al. (2018) as upregulated in aging fibroblasts include those involved in promoting cytoskeletal extensions and cell contacts.

Elegant FACS analyses and genetic lineage tracing studies in mice have previously shown that the dermis arises from a mesenchymal progenitor population at approximately E12.5 of mouse embryogenesis. By E16.5, the descendants of these cells begin to assume distinct lineages, and four fibroblast types can be distinguished in newborn dermis: papillary fibroblasts that lie adjacent to the epidermis, participate in hair follicle morphogenesis, and are marked by expression of leucine-rich repeat protein 1 (Lrig1) and CD26; reticular, or lower dermal, fibroblasts that express delta-like homolog 1 (Dlk1); and two pro-adipogenic lineages that express stem cells antigen-1 (Sca1) and are, respectively, positive or negative for Dlk1 (Driskell et al., 2013). Dermal functions vary in different regions of the body: dermis of the mouse plantar foot does not induce hair follicle development; ear skin dermis supports formation of small, poorly regenerative hair follicles; and dorsal dermis induces formation of relatively large regenerative hair follicles (Millar, 2018). These regional differences depend in part on differential expression of Hox family transcription factors (Rinn et al., 2008; Yu et al., 2018).

Salzer et al. find that dermal fibroblasts in aged skin display decreased levels of extracellular matrix proteins, including collagens, and enhanced expression of inflammatory and innate immunity markers and genes related to adipogenesis, lipid metabolism, and fat cell differentiation compared with their young counterparts. In line with this, the master regulator of adipogenesis, peroxisome proliferator-activated receptor (PPAR) γ (Lefterova et al., 2014) is upregulated throughout the dermis in aged animals, whereas in young skin, it is largely restricted to sebocytes and subcutaneous adipocytes. Transcriptional profiling of FACS-isolated papillary and lower dermis in newborn mice showed that papillary fibroblasts express cell junction and Wnt pathway genes, while reticular and pro-adipogenic fibroblasts are more similar to each other than to papillary cells and express higher levels of extracellular matrix and innate immune transcripts. Comparison of Dlk1$^+$ reticular and Sca1$^+$/Dlk1$^-$ pro-adipogenic fibroblasts reveals that reticular fibroblasts are relatively enriched for extracellular matrix genes, while pro-adipogenic fibroblasts are enhanced for lipid metabolism. Interestingly, aged fibroblasts most resemble the newborn Sca1$^+$/Dlk1$^-$ population, suggesting that fibroblasts might acquire pro-adipogenic traits during aging (Figure 1).

To determine whether the altered transcriptional profile of aged fibroblasts is due to loss of the upper dermal papillary lineage, the authors marked papillary cells by tamoxifen-treating newborn mice carrying *Lrig1-Cre^{ERT2}* and the *ROSA26-STOPflox-tdTomato* Cre reporter allele. They observed that tdTomato-marked descendants of papillary fibroblasts populated the entire dermis in aged mice, and occasionally formed adipocytes. Single-cell RNA sequencing of newborn, young, and aged fibroblasts confirmed that each of these populations clusters into two main groups according to their transcriptional profiles, but these are less well separated in aged than in newborn and young fibroblasts. Furthermore, the transcriptional profiles of old fibroblasts are more variable than those observed in young skin and show similarity to those of newborn pro-adipogenic fibroblasts. Thus, papillary fibroblasts are maintained in aging, but lose their unique identity.

Caloric restriction is known to expand lifespan in diverse organisms and retards aging phenotypes in many tissues. The underlying mechanisms include reduced inflammation, oxidative stress and energy expenditure, and enhanced metabolic

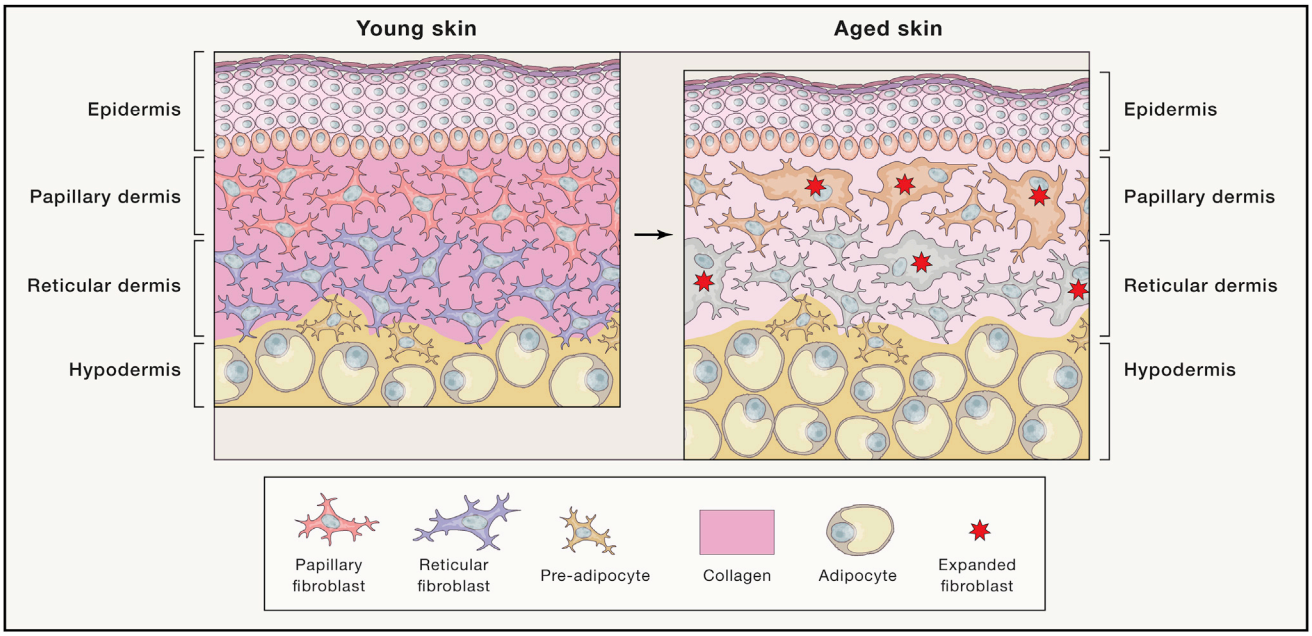

Figure 1. Effects of Skin Aging

In aged skin, the epidermis becomes thinner, the adipocyte layer expands, and dermal collagen (pink coloring) is produced at lower levels. Cell loss occurs in aged papillary and reticular dermis and is compensated by expansion of remaining fibroblasts (marked by asterisks). Aged dermal fibroblasts acquire markers of pro-adipogenic cells, and their identities become less well-defined.

flexibility (Cummings and Lamming, 2017). Interestingly, following caloric restriction, Salzer et al. (2018) find that while young and old fibroblast transcriptional profiles are still distinct, those of fibroblasts from old, calorie-restricted mice are less well correlated with age. In particular, the papillary signature is better maintained, and the pro-adipogenic signature is decreased, in fibroblasts from old calorie restricted animals compared with old mice on a normal diet. Conversely, the transcriptional profile of fibroblasts from adult mice that have been maintained since youth on a high fat diet correlates with that of aged fibroblasts. Some, but not all, of the "anti-aging" effects of calorie restriction on aged fibroblasts are maintained following resumption of a normal diet, while others are lost, suggesting that a subset of these features requires a constant low-calorie diet. These data identify the acquisition of transcriptional "noise," rendering papillary fibroblasts more similar to lower dermal and adipogenic cells, as a key effect of dermal fibroblast aging, and reveal interesting effects of diet on these traits.

In an accompanying paper, Marsh et al. (2018) use two-photon intravital imaging of adult mouse plantar skin to examine the behaviors of genetically labeled dermal fibroblasts in homeostasis and following localized or more broad damage of dermal tissue. By revisiting the same labeled fibroblasts over time in hairless plantar skin and in ear skin, where the hair follicles are quiescent, the authors find that the positions of fibroblast nuclei remain essentially unchanged in homeostasis. However, the fibroblast membranes are in constant motion, extending and retracting, presumably to sense the environment. By contrast, in dorsal skin where hair follicles are actively remodeling, there is extensive movement of fibroblasts within the dermis. Laser ablation or Cre-mediated induction of diphtheria toxin expression in limited numbers of dermal cells in hairless or non-remodeling skin did not result in movements or proliferation of neighboring fibroblasts; instead the membranes of undamaged fibroblasts extended to fill the spaces left by cell ablation. The membrane extensions seen in homeostasis and following focal dermal ablation were greatly reduced in the skin of Rac1 null mice, identifying Rac1 as a key regulator of these behaviors. More extensive damage, caused by increased intensity of laser ablation or broader induction of diphtheria toxin expression, caused proliferation of undamaged fibroblasts; however, membrane expansion behaviors were still observed and occurred both in the plane of the dermis and vertically, such that cells expanded to fill space above them.

By imaging mice as they age, the authors demonstrate that, even in the absence of laser or diphtheria toxin-induced damage, patches of dermis lacking nuclei, which appear as "holes" in nuclear imaging experiments, accumulate progressively. Interestingly, just as in the focal damage experiments, these are filled by membrane projections from nearby cells rather than by fibroblast proliferation or migration (Figure 1). These findings help to explain the decrease in dermal density observed in skin aging.

As with any truly novel work, these two papers raise even more questions than they answer. Chief among these is how, or whether, loss of papillary dermal cell identity and acquisition of pro-adipogenic traits in aging papillary dermis is related to the dermal "holes" observed by Marsh et al. Presumably these focal areas of nuclear loss result from cell senescence and elimination. The tools developed by Marsh et al. (2018) should allow investigators to re-image dermal fibroblasts at

more frequent intervals to observe these processes and determine when and how cells are eliminated. Salzer et al.'s interesting observation that calorie restriction helps to prevent transcriptional noise and maintain cellular identity of aging papillary fibroblasts raises the question of whether a low-calorie diet can also maintain cellular density of the papillary dermis and prevent the appearance of "holes" within the tissue. Given the acquisition of a pro-adipogenic transcriptional profile in aged papillary fibroblasts, it will also be interesting to test whether this trait is reversed by genetic deletion or pharmacological inhibition of PPARγ. If so, topical treatment with PPARγ inhibitors could be an attractive method to prevent some aspects of dermal aging. Finally, the precise mechanisms by which a low-calorie diet influences dermal fibroblast identity are unclear. As caloric restriction is known to prevent certain aging-related phenotypes in epidermal stem cells (Solanas et al., 2017), some features of retarded dermal aging in calorie-restricted mice could be due to maintenance of youthful epithelial signals, while others could be facilitated by reduced systemic inflammation and/or prevention of local metabolic changes. Unravelling these complex effects in the context of the whole organism will be a fruitful area for future investigation.

In summary, these two innovative studies explore the fascinating and clinically relevant subject of fibroblast aging *in vivo*. The skin provides a remarkably accessible and easily manipulatable system to address these questions. It is likely that many of the paradigms established here will be applicable to understanding aging mechanisms in other stromal tissues.

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Trained Immunity and Local Innate Immune Memory in the Lung

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Trained innate immunity mediates protection against heterologous infections and is mediated by epigenetic and functional reprogramming of myeloid cells and their progenitors. Now, Yao et al. describe trained immunity induced locally in alveolar macrophages by a viral infection, with IFNγ release from effector CD8$^+$ lymphocytes initiating this process.

Host immune responses are classically divided into innate and adaptive immune responses, with the latter displaying antigen-specific immunological memory after an infection or vaccination. However, a growing body of evidence in recent years argues that innate immune responses also exhibit adaptive characteristics, a *de facto* innate immune memory that has