DEVELOPMENTAL BIOLOGY

A scar is born: Origins of fibrotic skin tissue

A fibroblast lineage is characterized by an inherent ability to form scar tissue in skin

By Rachel Sennett[1,2] and Michael Rendl[1,2,3]

Tissues rely on fibroblasts to produce and distribute extracellular matrix (ECM) proteins that provide crucial structure and organization for other resident cells. Although their presence is imperative to normal tissue morphogenesis and maintenance, these mesenchymal cells are frequently overlooked as "merely" ubiquitous supportive cells or uniformly vilified because of their role in aberrant connective tissue deposition that can occur during wound healing or reactive fibrosis. Consequently, the study by Rinkevich *et al.* on page 302 of this issue (*1*), teasing apart the embryonic origins, molecular profiles, and functional capacities of discrete fibroblast lineages within adult skin, turns a thought-provoking spotlight on these unassuming cells and paves the way for future studies with potentially important clinical implications.

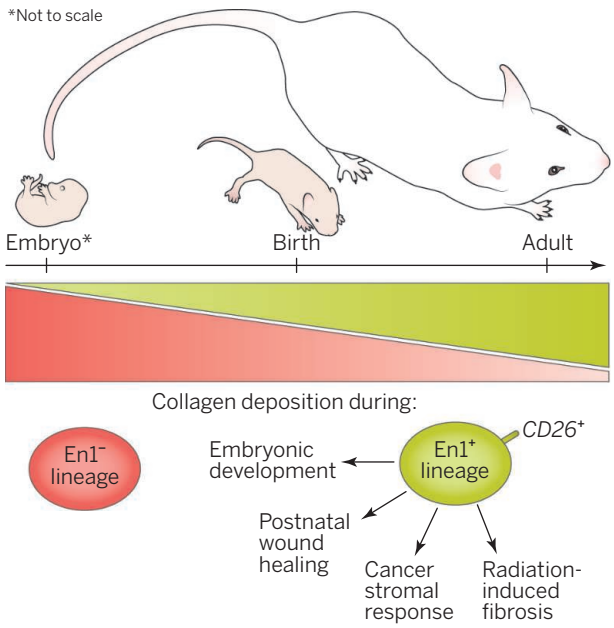
Historically, skin fibroblast heterogeneity has been solely described in terms of localization within the skin; papillary fibroblasts sit close to the epidermis, the outer epithelial layer of skin, as opposed to reticular fibroblasts residing deeper in the dermis, both distinct from hair follicle–associated mesenchymal cells (*2*–*4*). Each region has largely been defined by morphology and/or localization of specific protein markers. By a relatively early stage of development, postnatal skin fibroblasts have already acquired a positional identity that they maintain after transplantation (*5*). In a break from tradition, the two main fibroblast populations identified by Rinkevich *et al.* are initially defined on the basis of their embryonic ancestry and are peppered throughout the entire dermis.

The authors found these cells by taking an inclusive inventory of mainly connective tissue cells in a preparation of digested dermis from mice, with a twist: All cells originating from *Engrailed1* (*En1*)–expressing progenitors had been permanently labeled by recombination-mediated activation of a green fluorescent protein (GFP) reporter. *En1* is a homeobox transcription factor transiently expressed by cells of the central dermomyotome, a derivative of embryonic mesodermal segments or somites along the head-to-tail axis, and their progeny are known to contribute to the dorsal dermis in addition to other organs (*6*). Flow cytometry enabled Rinkevich *et al.* to account for and disregard hematopoietic, endothelial, and epithelial cells from their dermal preparations using a negative gating strategy also famously utilized in studies of hematopoietic stem cells (*7*) but not generally used for studies of heterogeneous skin cell populations. By weeding out cells that are not fibroblasts, the authors avoided preselecting for any fibroblast subset. Using single-cell and population-level gene analysis of freshly isolated cells, they determined that of the dermal cells remaining, both GFP-positive and -negative fractions are enriched for fibroblasts according to canonical marker expression.

Despite fundamental similarities between the two populations, the *En1*-lineage fate-mapped cells are distinguished by their unique expression of select genes and their proclivity to produce and deposit extra-cellular collagens during normal skin development. More remarkably, when the authors challenged their two fibroblast lineages by engineering situations that call for ECM expansion, including wound healing (*8*), a melanoma-associated stromal response (*9*), or radiation-induced fibrosis (*10*), they observed collagen production exclusively from *En1*-derived cells. It therefore appears that this discrete lineage of fibroblasts carries fibrogenic potential, which the authors confirmed when they genetically ablated these cells and observed a significant decrease in connective tissue deposition during wound healing and tumor response.

Concurrently with investigating heterogeneity between dorsal skin fibroblasts, Rinkevich *et al.* explored separate lineages from oral and cranial skin that appear to function similarly in that they are primarily responsible for ECM deposition in their respective regions. Isolation and genome-wide expression analyses revealed distinctions among scar-forming oral, cranial, ventral, and dorsal fibroblasts that were mirrored by functional studies. For example, in local wound-healing assays, the authors observed that fibrogenic cells within the oral cavity respond by patterning connective tissue with appreciably less collagen than scars observed in dorsal skin. In cross-transplantation assays, in which dorsal fibroblasts work to heal oral wounds and vice versa, the authors noted that donor cells manufacture scars that are structurally faithful to their original location. These results are reminiscent of studies describing the "positional memory" of fibroblasts native to distinct adult and fetal tissues with defined gene expression profiles and functional capacity (*11*).

Although the findings of Rinkevich *et al.* are intriguing, perhaps equally exciting are the areas of future research they suggest. First, because ~70% of adult dermal fibroblasts are *En1*-derived, it is tempting



Scar potential. A specific fibroblast lineage in dorsal skin increasingly populates skin with age and is responsible for extracellular matrix production in multiple developmental and pathophysiological scenarios.

ILLUSTRATION: ADAPTED BY P. HUEY/*SCIENCE*

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to speculate that such a broadly represented population contains further subsets of specialized fibroblasts. Furthermore, it is not entirely clear how this large population originates from a very small pool of precursors in skin at early embryonic time points, nor where other fibroblast-type, mesenchymal cell subsets fit into their lineage partition, such as hair follicle–associated dermal papilla and sheath cells and vascular smooth muscle cells. In addition, the results of this study focus primarily on the fibrogenic activity of En1-lineage–positive cells, leaving us to wonder about the origins and purpose of the lineage-negative cells that constitute at least 30% of adult skin fibroblasts.

Finally, the authors used a cell surface marker screen to identify *CD26/Dpp4* as a unique label for En1 fate-mapped fibrogenic fibroblasts in adult skin. Subsequent experiments revealed that prospectively isolated adult $CD26^+$ mesenchymal cells primarily contributed ECM components during wound-healing and tumor response assays. More intriguingly, the authors observed a slight but significant difference in fibrogenic gene expression between $CD26^+$ and $CD26^-$ fibroblasts that was greatly exacerbated by a wounding assay stimulus. Thus, although fibroblasts from separate lineages are intrinsically similar at baseline, they are unique in their individual transcriptional and functional responses to signals from within the greater milieu of the skin.

Not only is *CD26/Dpp4* useful for isolating or identifying fibrogenic cells in situ, but the discovery is also clinically relevant. *Dpp4* signaling itself appears to coordinate fibrogenic activity, and the authors could mitigate scar size during healing by applying a specific *Dpp4* inhibitor to wound sites. This discovery has far-reaching implications for drug development, provided the observation proves relevant to wound healing in human skin as well. Furthermore, the utility of *Dpp4* as a marker or druggable target might also be applicable to studying or treating fibrosis in organs outside of the skin. ■

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NEUROSCIENCE

Systemically treating spinal cord injury

A drug that crosses the blood-brain barrier has therapeutic potential for central nervous system trauma

By **Amanda P. Tran** *and* **Jerry Silver**

Spinal cord injury is a debilitating condition. Axons of nerve cells are severed, resulting in a range of deficits, including the loss of voluntary movements and sensation. Failure of axonal regeneration after such an injury may be partly explained by a decreased intrinsic capacity for neuron growth, especially at the lesion site (*1*). On page 347 in this issue, Ruschel *et al.* (*2*) show that this inhibition can be overcome with a small molecule that can be injected into the body cavity, cross the blood-brain barrier, and reach the central nervous system. The drug, epothilone B, stabilizes microtubules in extending axons, thereby promoting spinal cord regeneration.

Upon approaching the glial scar at the lesion site of the spinal cord, the tips of regenerating axons form swollen dystrophic growth cones (*3*). These were first described as "sterile clubs" by the neuroscientist and Nobel laureate Ramón y Cajal, who also believed that they persisted only briefly in a quiescent state before the axon "died back" to a sustaining collateral (now defined as a branch off the main axon that feeds back onto the neuron's cell body) (*4*). By contrast, Ruschel *et al.* describe how dystrophic growth cones remain in the injured human spinal cord for a remarkable 42 years after injury. Advances in in vivo imaging have also revealed that, for a time, dystrophic growth cones are dynamic and can regenerate in a more accommodating environment (*5*). Moreover, dystrophic growth cones eventually form synaptic-like relationships with oligodendrocyte precursor cells in the lesion core, enabling them to persist for long periods (*6*, *7*).

Electron micrographs of adult rats with spinal cord injury illustrated that dystrophic growth cones are bloated with disorganized microtubules arranged in nonparallel networks (*8*). To better understand the internal machinery of dystrophic growth cones and determine whether they are malleable, earlier studies assessed the effects of the anticancer drug paclitaxel (Taxol). Paclitaxel belongs to the taxane family of drugs that targets tubulin. It stabilizes microtubule polymers and protects them from disassembly. Suppression of microtubule dynamics thus interferes with cellular processes such as cell division and cell motility. Indeed, it was shown that caged Taxol (which could be activated in a restricted area) specifically stabilized the dystrophic growth cone microtubule cytoskeleton in cultured rat neurons, and that this was sufficient for axon formation (*9*). This effect was reversed with nocodazole, a microtubule-destabilizing drug (*8*, *9*). Intrathecal delivery (injection into the spinal fluid) of Taxol following a dorsal hemisection of the rat spinal cord also promoted microtubule stabilization, allowing increased axonal penetration through the glial scar (*10*). Taxol additionally decreased the ability of transforming growth factor–β1 to adversely affect rearrangement of the cytoskeleton in astrocytes, which reduced scarring induced by spinal cord injury (*10*).

> ***"...there are currently no drugs approved...to treat this traumatic injury that allow for functional recovery."***

Using a more clinically relevant microtubule stabilization strategy as a putative spinal cord injury therapy, Ruschel *et al.* tested epothilone B in different rat models of spinal cord injury. Epothilone B also targets tubulin, but is a member of a different family of drugs. Unlike Taxol, it penetrates the blood-brain barrier, as seen through mass spectrometry analysis of rat spinal cord tissue after intraperitoneal delivery (injection into the body cavity). Ruschel *et al.* found that by stabilizing microtubules, epothilone B enhanced axon regeneration and ultimately improved sensorimotor function in an injured rat, boosting intrinsic axonal growth while reducing axon-inhibitory scarring after injury.

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