PRIZE ESSAY

FINALIST
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Shruti Naik received her B.S. in cell and molecular biology from the University of Maryland and her Ph.D. in immunology from the University of Pennsylvania–National Institutes of Health Graduate Partnership Program. During her graduate training, she discovered that normal bacteria living on our skin educate the immune system and help protect us from harmful pathogens, opening the door for microbiota-based therapies in the skin. She is currently at Rockefeller University, where she is studying the interactions between immune cells and stem cells in an effort to develop stem cell–based therapies for inflammatory disorders. She is also a strong advocate for women in science. **www.sciencemag.org/content/359/6380/1113.1**

REGENERATIVE MEDICINE

The healing power of painful memories

Epidermal stem cells "remember" inflammation, accelerating subsequent wound repair

By **Shruti Naik**

Our body's epithelia are barriers that interface with the terrestrial environment and routinely experience inflammation. Although a vast majority of these inflammatory reactions resolve, they imprint the tissue with a memory. Cells of the immune system are traditionally thought to be the bearers of this memory, allowing them to react faster to subsequent inflammatory pressures (*1*, *2*). Yet, barrier tissues are composites of epithelial, mesenchymal, nervous, vascular, and immunological networks working in unison to sustain optimal function in health and disease. The question of whether tissue-resident cells, distinct from the immune system, are entrained in response to a perturbation remains to be addressed.

TRACKING TISSUE STEM CELLS IN INFLAMMATION

Somatic stem cells are responsible for maintaining tissues throughout an organism's lifetime (*3*). Thus, I teamed up with a group of tissue stem cell and chromatin biologists to understand the enduring consequences of inflammation on stem cells of the epidermis. Throughout the course of our research, we discovered that stem cells have a remarkable capacity to "remember" inflammation, which ultimately and consequently enhances their regenerative potential.

To induce an acute response, we employed a well-defined and self-resolving model of inflammation by applying a TLR7 agonist, imiquimod (IMQ), to mouse skin (*4*). Inducible-marker–based fate mapping (*5*) of skin epithelial stem cells and differentiated progeny revealed that basal skin epithelial stem cells (EpSCs) not only expanded during inflammation but also persisted for more than 180 days. At the peak of the response, EpSCs expressed all the hallmarks of IMQ inflammation, including hyperproliferation, activated STAT3, and increased cell death, but nevertheless returned to baseline upon resolution.

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INFLAMMATION-EXPERIENCED SKIN HEALS FASTER

Tissue repair is a cardinal function of stem cells (*6*). Therefore, we sought to determine how a previous immune response would alter stem cells' ability to cope with a secondary challenge and subjected post-inflamed skin to wounds.

Remarkably, wounds in post-inflamed epidermis healed 2.5 times as fast as wounds in naïve controls (*7*). Accelerated wound repair was also observed 180 days after the initial challenge and in response to a variety of acute stimuli, including a primary wound, a fungal infection, a model of atopic dermatitis, and another model of hyperplasia, all of which underscore the generality of our findings and the long-lasting duration of the effect. Although EpSC proliferation was comparable between control and post-inflamed wounds, inflammation-experienced EpSCs exhibited a significant increase in their migratory capacity.

We next sought to determine the upstream regulators of the observed enhancement. To this end, we excluded a role for circulating factors in controlling wound repair post-inflammation, because wounds distal to the initial inflammatory site did not exhibit any boost in healing. Additionally, we established that enhanced wound repair after inflammation occurred independently of skin-resident macrophages and lingering Rorc^{+} immune cells. These results directed our focus to the possibility of a sustained change within the EpSC compartment that may occur as a result of inflammation.

CHROMATIN CHANGES ARE THE CRUX OF MEMORY

Sustained epigenetic changes in innate immune cells were recently shown to alter

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their responsiveness to subsequent stimuli (*1*). To determine whether EpSCs similarly encoded a memory of previous inflammation, we evaluated alterations in chromatin accessibility of epidermal stem cells during and after inflammation.

Although the initial inflammatory response resulted in dramatic alterations to chromatin accessibility within EpSCs (>40,000 regions), a vast majority of inflammation-induced accessible regions reverted upon restoration of homeostasis (*7*). Remarkably, however, a small subset of open chromatin domains were retained (9561 peaks), ~2000 of which had been acquired during the primary response. Genes associated with these acquired peaks were enriched for apoptosis signaling, interleukin signaling, the oxidative stress response, Ras, and PI3 kinase pathways.

Intriguingly, transcription factor MOTIF analysis revealed that inflammation-instilled chromatin domains were enriched for EpSC homeostatic transcription factors such as p63, KLF5, and AP-1, as well as inflammatory transcription factors such as STAT1/3 and NF-κB. STAT3, a major mediator of IMQ response (*8*), was phosphorylated and activated specifically during both the primary (IMQ) and secondary (wounding) stimulus, but not in the interim (*7*).

Our data thus revealed a model in which memory chromatin domains serve as accessible platforms for accelerated reactivation after a secondary challenge. Indeed, using fluorescent reporters driven by memory chromatin domains, we found that these sustained open regions were functional in sensing inflammatory stress.

INFLAMMATION-TRAINED STEM CELLS EXPRESS TISSUE DAMAGE SENSORS

If these accessible chromatin elements were in fact conferring memory, we would expect that a secondary insult would result in augmented transcription of their associated genes. Strikingly, transcriptional profiles of inflammation-experienced EpSCs shortly after secondary wounding revealed that 140 genes were rapidly up-regulated. More than 50% of these genes were associated with sustained open chromatin domains post-inflammation (*7*).

Enriched among the transcriptional changes was the inflammasome signaling pathway, including the double-stranded DNA sensor, *Absence in Melanoma 2* (AIM2), which is known to detect tissue damage (*9*). Remarkably, *Aim2*-deficient stem cells lost the wound repair advantage endowed by inflammation, and elevating *Aim2* expression in EpSCs was sufficient to recapitulate the phenotype (*7*). Further probing revealed that a downstream effector of the AIM2 inflammasome, IL-1β, mediated this effect. In summary, inflammatory cues were able to tune EpSC function, enabling their adaptation to subsequent stressors.

MANIPULATING MEMORY

These findings reveal that inflammatory memory is not exclusive to the immune system but also exists in long-lived tissue stem cells. However, it remains to be determined whether memory occurs only in response to inflammation or whether a stem cell can remember all of its past stressors. Moreover, it is unclear whether these mechanisms of adaptation are always beneficial or if they may exert detrimental effects such as recurrent inflammatory disease, tumor formation, or premature aging.

Intriguingly, impaired wound responses in aging are associated with dysregulated skin immunity (*10*). Accumulating epigenetic alterations (*11*) resulting from recurrent stressors could contribute to the diminished regenerative capacity of aged stem cells. Thus, understanding how memory is established within an EpSC—whether it is passed on to differentiating progeny and whether it can be reversed—will be important for fine-tuning the remarkable ability of stem cells to regenerate tissues.

Our work indicates that inflammatory reprogramming can have a lasting impact on the tissue's healing capacity. Understanding the factors that rewire stem cells to remember inflammation may ultimately enable the development of therapies aimed at honing the stem cells' regenerative potential.

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