Immune Cell–Stem Cell Cooperation

Understanding interactions between the immune system and stem cells could pave the way for successful stem cell–based regenerative therapies.

By Waleed Rahmani, Sarthak Sinha and Jeff Biernaskie | July 1, 2016

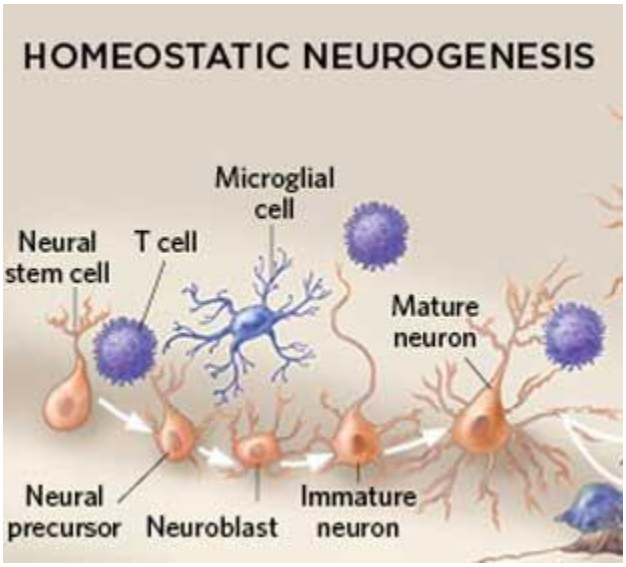
We may perceive ourselves as static beings, but the cells of our bodies are in constant flux. The outer layers of our skin and intestinal tract are replaced every few weeks; red blood cells circulate in our bodies for about 100 days before they are replaced; cells in our liver and fat are longer lived—more than a year for a liver cell, 10 years on average for a fat cell—but still turn over repeatedly during our lifetimes. More slowly, up to half our heart cells may be replaced during a normal lifespan. And, of course, when healthy tissue is lost due to injury, new cells are made to patch up the damage. What are the biological processes responsible for normal cell turnover and organ homeostasis? What controls proper repair after injury? What allows organisms like the salamander to regenerate an amputated limb while humans form scars and struggle to regrow much simpler structures, such as hair?

These and other questions are the target of ongoing research in the field of regenerative medicine. But what we do know, and have known for nearly half a century, is that stem cells are crucial players. Stem cells self-renew to maintain their numbers and differentiate into the specialized cell types that make up our tissues and organs—a function that becomes especially important after stress or injury. The ultimate goal of regenerative medicine is to harness stem cells' regenerative potential to treat and even cure many of the diseases besetting society today. Despite progress in understanding the potential of these multipotent cells, the unfortunate reality is that we remain far from cures. One possible reason for this is scientists' failure to sufficiently consider what goes on within the biological environment surrounding the stem cell.

For years, stem cell biologists have focused their attention on the intrinsic properties of stem cells to understand what gives them the ability to self-renew and differentiate into a range of cell types. While these investigations have uncovered a collection of genes and proteins responsible for a cell's "stemness," the role of the microenvironment, also known as the stem cell niche, was largely ignored. But neighboring cells, secreted proteins, the extracellular matrix, circulating metabolic signals such as oxygen and glucose, and diverse physical parameters, such as shear stress and tissue stiffness, can all affect the behavior of stem cells.

Diverse immune cells have been caught in the act of manipulating stem cell behavior.

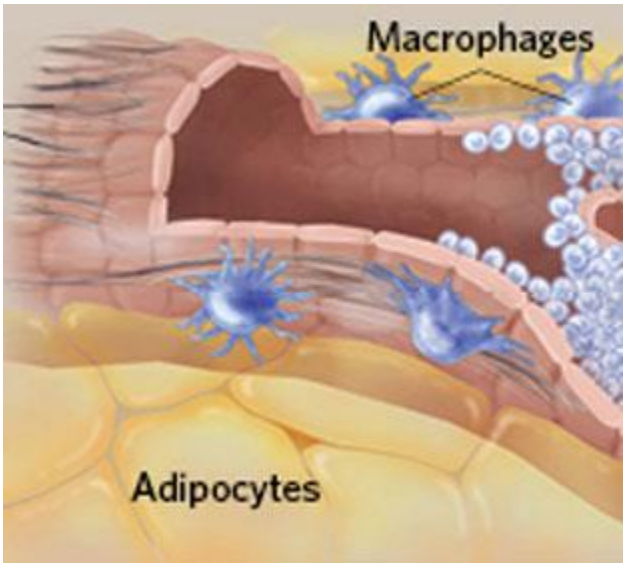
One of the best-studied examples of mammalian stem cell environments is the intestinal stem cell (ISC) niche. The small intestine's epithelium is the fastest self-renewing tissue in the body due to ISCs' exceptionally rapid rates of cell division and the rapid migration of their differentiated progeny out of the stem cell niche. But the system would not work without the help of Paneth cells, one of four differentiated cell types produced by ISCs, which remain in the niche and secrete essential proteins that are critical for ISC survival. Indeed, the genetic inactivation of Paneth cells results in a near-total loss of ISCs.[1]



In addition to niche-specific cells, stem cells regularly interact with the body's mobile and diffuse army of immune cells. Traditionally regarded as the primary line of defense against pathogenic invaders, the immune system is now also recognized as essential for tissue homeostasis and healing, even in the absence of infection. Diverse immune cells have even been caught in the act of manipulating stem cell behavior.

The precise roles that immune cells play in the stem cell niche is context dependent. Whether macrophages and T cells ensure homeostasis, promote regeneration (e.g., regrowth of liver tissue after a partial hepatectomy), or mediate scar-forming tissue repair depends on the species, its developmental stage, the organ or tissue in question, the severity of injury, and the availability of a stem cell pool. Which molecules immune cells secrete, and the effect the cells have on regeneration, can also change drastically depending on the organism and tissue. In some cases, immune cells may even work against the body, supporting the growth and spread of cancer. Understanding the immune system's role in stem cell biology may help clinicians and scientists better respond to injuries or homeostatic imbalances, as well as develop stem cell therapies to treat diverse ailments, from anemia to multiple sclerosis, muscular dystrophy, and heart failure.

Maintaining homeostasis



MAMMARY MATURATION; During puberty, as hormones trigger the maturation of the rudimentary mammary ducts, macrophages and other immune cells migrate to the ducts' tips, where they support rapid proliferation and duct branching.

Another organ that relies on immune cells to regulate normal cell turnover is the brain. Once believed to occur only during embryonic and late gestational stages in mammals, neurogenesis is now known to occur throughout adult life in the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricle, two locations where neural stem cells reside. (See "Brain Gain," *The Scientist*, October 2015.) And investigations of the cellular mechanisms regulating adult neurogenesis have revealed that immune cells play crucial roles in hippocampal-dependent learning and memory.

NEW NEURONS: As new neurons differentiate from neural stem cells in the hippocampus, T cells and microglia are recruited to the neurogenic site. Following injury, macrophages stimulate remyelination of neurons.

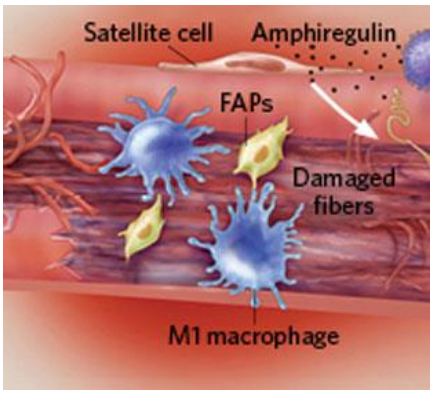
An integral part of homeostasis in diverse tissues is the continuous replacement of differentiated cell types. Research is now showing that the immune cells residing within the stem cell niche are essential to this process. For example, specialized macrophages in the bone marrow remain in direct contact with a

red blood stem cell called an erythroblast. Without this direct cell-cell contact, erythroblasts are not able to mature properly and repopulate the blood with new red blood cells, a deficiency that can lead to aplastic anemia.[2]

Immune cells are also critical for the development of mammary glands during puberty. At birth, mammary glands consist of fat pads with rudimentary ducts descending from the nipple. At the start of puberty, ovarian hormones trigger the bifurcation and elongation of the ductal structures towards the outer edges of the fat pad while diverse immune cells—mast cells, eosinophils, and macrophages—migrate to the region around the ducts' tips. Genetic or pharmacological disruption of mast cells and macrophages in mice has revealed that these immune cells are critical for rapid proliferation and normal duct branching during puberty. Mast cells secrete protein-degrading serine proteases, which are necessary for the breakdown and reorganization of collagen fibers surrounding the developing ducts, for example,[3] while macrophages phagocytize apoptotic cell debris and directly act on mammary stem cells through an unknown mechanism.[4,5]

Studies of the bone marrow, mammary gland, and brain reveal that stem cells' immune niches play an important role in maintaining homeostasis in our organs, ensuring a stable equilibrium between cell overpopulation and atrophy under normal conditions. But what about when homeostasis is disturbed?

Dealing with injury



MUSCLE REPAIR: Following an acute injury to the skeletal muscle, local and infiltrating immune cells remove damaged tissue, while T cells help spur the generation of new muscle cells.

Researchers at the Weizmann Institute of Science in Israel have shown that hippocampal neurogenesis in rodents, induced by housing the animals in enriched environments, was associated with the recruitment of T cells and microglia (macrophages of the brain and spinal cord). Immune-deficient mice, on the other hand, exhibited impaired hippocampal neurogenesis that led to poor results in spatial learning and memory tasks.[6] It is still not clear how immune cells influence the neural stem cell niche during hippocampal neurogenesis. However, because only a small subset of newborn neurons integrate into the hippocampal circuitry, with the majority undergoing death by apoptosis, it is believed that microglia shape hippocampal neurogenesis by rapidly phagocytizing the apoptotic newborn neurons.[7]

Perhaps the best-understood example of immune- and stem-cell cooperation is in skeletal muscle following an acute injury. Tissue repair begins with the removal of damaged muscle fibers by local and infiltrating immune cells. Rare, circulating immune cells called eosinophils instruct resident progenitor cells known as fibro/adipogenic progenitors (FAPs) to generate the fibroblasts and fat cells that deposit collagen and secrete growth factors to support muscle fiber regeneration.[8] Concurrently, T cells secrete a protein called amphiregulin, which instructs resident muscle stem cells known as satellite cells to differentiate into new muscle cells and replace the lost muscle fibers.[9]

Such immune–stem cell interactions are not restricted to skeletal muscle, but have been observed across many organs in mice. During chronic liver injury, macrophages secrete a protein called Wnt3a, which drives the differentiation of local liver stem cells into mature liver cells.[10] In the colon, macrophages are recruited to activate intestinal stem cell proliferation and regenerate wounded intestinal epithelium.[11] And in the nervous system, recent work has shown that following injury, anti-inflammatory M2 macrophages are essential for efficient replacement of the myelin sheath, an insulating layer of fatty substance that facilitates the transmission of action potentials along the axons of neurons. Specifically, the macrophages secrete a protein called activin-A that triggers oligodendrocyte

progenitor cells (OPCs) to differentiate into oligodendrocytes, neural support cells that are responsible for myelination.[12]

It's becoming clear that immune cells are an important component of stem cell niches across the body, with crucial roles in injury-induced regeneration.

A particularly interesting system in which researchers have explored the relationship between stem cells and immune cells is the hair follicle, one of the few mammalian tissues capable of continuous regeneration throughout life. Last year, the University of Southern California's Cheng-Ming Chuong and his colleagues showed that macrophages are responsible for the regrowth of a new hair following plucking.[13] When researchers plucked hairs off the backs of mice, they found that damaged hair follicles beneath the skin's surface secrete, in unison, a protein called CCL2. In response to this distress signal, macrophages migrated up the CCL2 gradient and toward hair follicles, where they secreted a protein called tumor necrosis factor (TNF), which instructed hair follicle stem cells to produce new hair.

It's becoming clear that immune cells are an important component of stem cell niches across the body, with crucial roles in injury-induced regeneration. Theoretically, targeting certain immune cells should promote healing. However, the great diversity and heterogeneity found within each immune cell population have made it difficult to develop effective therapies. More research is needed to sufficiently discriminate among subpopulations of immune cells and to understand which cells must be targeted to elicit the desired effect in injured tissues.

Stem cells in disease

Communication between immune cells and stem cells does not always do the body good; at times, cell interactions can result in fibrosis and organ dysfunction. In mouse models of chronic muscle damage approximating Duchenne muscular dystrophy (DMD), immune cell infiltration and FAP activity are abnormally prolonged, while the reparative capacity of satellite stem cells is diminished. These abnormalities, a result of genetic defects in the dystrophin gene, lead to excessive and disorganized collagen deposition, ultimately causing fibrosis and loss of muscle function. Why does this happen? The answer may have to do with how infiltrating macrophages communicate with FAPs.

Last year, researchers at the University of British Columbia in Canada showed that, in healthy muscle regeneration, FAP numbers dramatically increase three days after an acute injury but quickly drop to pre-injury levels by day five.[14] It turns out that macrophages are directly responsible for the quick decline in FAP numbers; the immune cells secrete TNF, which binds to FAPs and signals them to undergo apoptosis. In the mouse model of DMD, however, macrophages increase the production of another protein called transforming growth factor b1 (TGFb1). Unlike TNF, TGFb1 instructs FAPs to survive longer and differentiate into the collagen-secreting cells that, when present in excess, cause muscle fibrosis and dysfunction. Treatment with nilotinib, a US Food and Drug Administration–approved therapy for the treatment of a drug-resistant form of leukemia, reduced muscle fibrosis in the mice by blocking the adverse effects of TGFb1.

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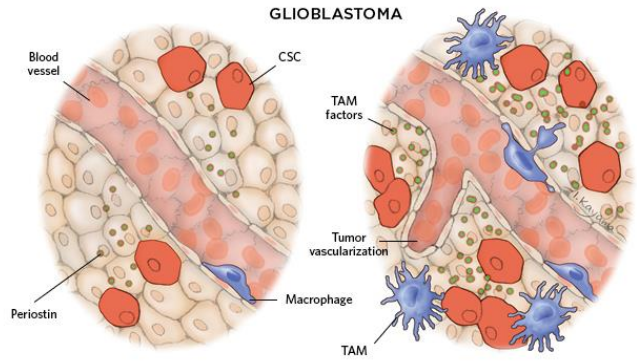
Immune cells can get especially dangerous when they start supporting the survival and metastasis of tumors by interacting with cancer stem cells (CSCs), a small subset of tumor cells that self-renew and generate the majority of cells within tumor masses. Many traditional cancer therapies discriminately kill actively dividing CSCs and their progeny, but slow-dividing CSCs remain untouched, enabling relapse and



even metastasis. Scientists are now racing to better understand and target CSCs. Intriguingly, the key to success may lie in our own immune system.

The most abundant immune cell within the tumor microenvironment is the macrophage. While biologists once suspected that macrophages provided anti-tumor immunity, we now know that the tumor microenvironment is enriched with signals that rewire these cells into tumor-associated macrophages (TAMs), which actually fuel the cancer's survival, malignancy, invasiveness, and drug resistance. Lactic acid, for example, supports lung cancer and melanoma growth by converting normal macrophages into TAMs that produce high levels of vascular endothelial growth factor (VEGF) to promote tumor vascularization, as well as enzymes that support nitrogen metabolism, increasing tumor cell proliferation.[15] Indeed, many clinical studies have demonstrated that increased macrophage density is strongly correlated with poor prognoses in thyroid, breast, lung, and liver cancers.

Recent research has suggested that some CSCs encourage the transformation of normal macrophages into TAMs. Last year, for example, a team led by researchers at the Cleveland Clinic found that CSCs in glioblastomas, a highly malignant brain cancer, secrete a potent chemoattractant called periostin that instructs blood-derived macrophages to migrate into the tumor, where they are converted into TAMs. In a mouse model of glioblastoma, genetically silencing periostin reduced the number of TAMs within the tumor, inhibited tumor growth, and extended the animals' survival.[16]

IMMUNE CELLS DO HARM: Sometimes, immune- and stem-cell interactions do not promote homeostasis or healing, but instead lead to further damage or disease. The most dramatic example is the cooperation of tumor-associated macrophages (TAMs) and cancer stem cells (CSCs) to drive cell proliferation and tumor malignancy and invasiveness, as well as drug resistance. In glioblastomas, a highly malignant brain cancer, CSCs secrete a potent chemoattractant called periostin that instructs blood-derived macrophages to migrate to the tumor, where they are converted into TAMs (left). TAMs then secrete factors to promote tumor growth and progression. (right) © IKUMI KAYAMA/STUDIO KAYAMA

Researchers are now exploring ways to more effectively prevent macrophages from infiltrating and acquiring this tumor-supportive identity, and to disrupt the ongoing crosstalk between CSCs and TAMs. A 2013 study of mouse pancreatic cancer showed that inhibiting CSF1R and CCR2, macrophage receptors key for migration and survival, decreased the total number of pancreatic CSCs, enhanced chemotherapeutic efficiency, and inhibited metastasis.[17] And when human patients were treated with a drug targeting CSF1R, patients had significantly fewer TAMs at tumor sites and improved clinical outcomes.[18]

Tissue-resident stem cells' remarkable ability to self-renew while also giving rise to diverse mature cell types is critical for our existence. In order to carry out their inherent roles in tissue maintenance and regeneration, these stem cells rely on signals provided by diverse cell types, including immune cells, within the local and systemic environments. We are at the dawn of understanding the complex and dynamic roles of the immune system's many cell types and their functional relationships with stem cells—a feat that will be critical to harnessing the power of stem cells to treat or cure disease.