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Adult stem cells and regenerative medicine—a symposium report

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Abstract

Adult stem cells are rare, undifferentiated cells found in all tissues of the body. Although normally kept in a quiescent, nondividing state, these cells can proliferate and differentiate to replace naturally dying cells within their tissue and to repair its wounds in response to injury. Due to their

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Competing interests

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proliferative nature and ability to regenerate tissue, adult stem cells have the potential to treat a variety of degenerative diseases as well as aging. In addition, since stem cells are often thought to be the source of malignant tumors, understanding the mechanisms that keep their proliferative abilities in check can pave the way for new cancer therapies. While adult stem cells have had limited practical and clinical applications to date, several clinical trials of stem cell–based therapies are underway. This report details recent research presented at the New York Academy of Sciences on March 14, 2019 on understanding the factors that regulate stem cell activity and differentiation, with the hope of translating these findings into the clinic.

Keywords

adult stem cells; age-related degeneration; aging; differentiation; epidermal stem cells; hematopoietic stem cells; immune–stem cell interactions; inflammation; lung stem cells; mesenchymal cells; muscle stem cells; regenerative medicine; stem cell niche; stem cell signaling; tissue regeneration; tumor stem cells

Introduction

Adult stem cells have high proliferative potential and the capacity to differentiate into various cell types, depending on their tissue of origin. In the body, adult stem cells are responsible for generating new tissue in response to injury, disease, or regular maintenance. Adult stem cell–based therapies have therefore garnered much attention in treating a variety of degenerative diseases and in rejuvenating aging tissue. However, their proliferative nature also makes them dangerous. Dysregulation in the mechanisms that keep stem cells in a quiescent, nonpro-liferating state can lead to cancer. While this poses safety concerns for stem cell–based therapies, it also paves the way for new approaches to treat cancer. Understanding the molecular mechanisms that go awry in stem cells during cancerogenesis could lead to new cancer therapeutics.

While many clinical trials are investigating the use of adult stem cells to treat disease, few have resulted in approved therapies. Bone marrow transplant, which uses hematopoietic stem cells (HSCs) to regenerate blood cells, is used to treat several types of hematologic cancers and other conditions,[1] and skin stem cell therapy has been successful in regenerating skin in burn victims. In the United States, the only stem cell–based products approved by the U.S. Food and Drug Administration (FDA) are hematopoietic progenitor cells derived from cord blood.[2] In Europe, an eye stem cell–based therapy, Holoclar®, was approved in 2015 for cornea damage.[3] Other uses for stem cell–based therapies remain experimental.

On March 14, 2019, researchers from academia and industry convened at the New York Academy of Sciences to discuss new research in understanding the function and regulation of adult stem cells. Themes throughout the day included the importance of the stem cell niche in regulating stem cell function, the potential for stem cell therapy to address age-related degeneration and cancer, and the role that inflammation and stress play in activating and modulating stem cell activity. Much of the research presented focused on basic science; nonetheless, the presenters were clearly focused on the potential to translate their research into clinical applications.

Intrinsic and extrinsic cancerogenic factors in skin stem cells

Elaine Fuchs from the Rockefeller University gave the first keynote address, highlighting work in her laboratory on the basic science of how skin stem cells respond to stress and inflammation. She also spoke about the role of skin stem cells in cancerogenesis, particularly squamous cell carcinoma (SCC), a common and potentially life-threatening form of skin cancer that develops from hair follicle and epidermal stem cells. By selectively genetically modifying skin stem cells in mouse embryos, Fuchs' laboratory can interrogate which pathways are important for maintaining stem cell quiescence and activity in hair regeneration and wound repair, and also unravel what goes wrong in diseases like skin cancer.[4]

Using RNA-seq, Fuchs' laboratory has identified a number of transcription factors (TFs) expressed by hair follicle stem cells and has systematically knocked out each one in mice to determine their role in stem cell quiescence and repressing differentiation, and in mobilizing the stem cells to regenerate tissue. This group of TFs regulates approximately 350 genes, including the TF genes themselves as well as other genes involved in stem cell quiescence, such as genes expressing BMPs and Wnt signaling pro-teins. Some of the TFs can also repress genes in the Wnt signaling pathway that are required for tissue regeneration. Chromatin landscaping analysis revealed that the TF binding sites are found within large open chromatin domains, which are called *super-enhancers*. The opening and closing of these super-enhancers control stem cell identity, lineage commitment, and plasticity.[5,6]

The pattern of active super-enhancers changes based on the stem cell's environment, such as when a cell is exposed to cell culture or during wound repair. Fuchs showed that the super-enhancers turned on during the early stages of cancerogenesis are similar to those that are activated during wound repair.[7] The active TFs in this state represent a mix of hair follicle stem cell–related TFs and epidermal stem cell–related TFs. However, in cancer, this "lineage infidelity" becomes permanent, likely due to the activity of ETS2, a target of RAS/MAPK signaling that is transiently activated in wound healing but constitutively activated in some forms of SCC.[8]

In addition to describing the intrinsic pathways involved in cancerogenesis of skin stem cells, Fuchs also showed that interactions between stem cells and their environment affect tumor stem cell behavior. Fuchs' laboratory has identified a subset of tumor stem cells in SCC tumors in mice that reside next to blood vessels and are affected by TGF-β signaling. While these cells divide more slowly than stem cells further away from the vessel, they respond to the signal and become invasive.[9] Fuchs showed that these TGF-β–sensing cells are equipped with an arsenal of weapons that enable them to resist chemotherapy and immunotherapy and can cause cancer relapse after the vast majority of the tumor has been eliminated. Therapeutic strategies that target the arsenal harbored by these cells may be key to preventing drug resistance and tumor regrowth.[10]

Finally, Fuchs' team showed that the skin stem cells exhibit an inflammation-mediated memory that allows them to respond to injury more quickly. Using a mouse model, they discovered that when the skin is re-wounded in a similar location as before, it heals more

quickly after the second wound. The skin appears to “remember” that it had encountered a wound or inflammatory experience and is more efficient at dealing with it the second time. Her group showed that epigenetic changes in the chromatin of epidermal stem cells can persist for months after the initial wound, and allow faster activation of early responders to subsequent inflammatory stimuli.[11]

Using and targeting HSCs in cancer

In the second keynote address, **Irving Weissman** of Stanford University discussed his work on translating basic science discoveries in stem cell research to clinical applications. Weissman focused on HSCs—the cells responsible for developing and maintaining the various cells of the blood, including red blood cells, white blood cells, and platelets. In the 1990s, Weissman showed in a small pilot study that high-dose chemotherapy followed by autologous transplant of HSCs purified from mobilized peripheral blood had higher survival rates among patients with metastatic breast cancer than autologous transplant of unmanipulated mobilized peripheral blood (median overall survival = 60 versus 28 months). Weissman believes that this improvement is due to the removal of tumor cells in the mobilized peripheral blood, which in direct tests was a 250,000-fold depletion.[12,13] Autologous mobilized blood transplant is no longer used to treat metastatic breast cancer but is an option for some patients with hematopoietic malignancies, such as multiple myeloma and lymphomas.

Weissman also discussed his work in understanding the stem cell niche of HSCs. Work in Weissman’s laboratory has identified a marker, *Hoxb5,* that is expressed only in pluripotent HSCs. Transplanting *Hoxb5*$^{+/+}$ cells into irradiated mice could reconsti-tute the entire hematopoietic lineage, while transplanting *Hoxb5*$^{-/-}$ cells could not. Using *Hoxb5*-driven expression of a red fluorescent protein as a marker, Weissman showed that in the bone marrow, HSCs attach as single cells to the abluminal surface of the endothelium lining venous sinusoidal vessels.[14] Studies of other types of stem cells have also shown that they reside near blood vessels, suggesting that this may be a common theme.

Weissman next discussed his laboratory’s work on understanding HSCs and acute myeloid leukemia (AML). Using bone marrow samples with a specific translocation (AML1/ETO) from leukemia patients, Weissman showed that only AML1/ETO^{+} cells from the multipotent progenitor pool, not the stem cell pool, were able to transfer leukemia to immunodeficient mice. Although the AML1/ETO translocation was present in approximately 5–40% of HSCs, transplanting these cells into immune-deficient mice resulted in normal hematopoiesis. These results suggest that mutations involved in leukemia do not provide the ability for self-renewal; rather, leukemia develops in cells that already have the capacity for self-renewal.[15–17] Exome sequencing of HSCs from AML patients has also shed light on the order in which mutations occur during leukemogenesis. Weissman showed that the first mutations to occur in a set of AML patients were involved in regulating epigenetics and DNA accessibility. Interestingly, a mutation in the gene *Flt3* often most closely associated with AML was the last to develop.[18] Weissman hopes that these types of results can guide preclinical and clinical research.

Weissman also showed that CD47 is upregulated on leukemia stem cells. CD47 acts as an antiphagocytotic signal and prevents macrophages from destroying the cell. While CD47 is an antiphagocytotic signal, leukemia and all tested cancer cells also contain prophagocytotic signals. Weissman showed that calreticulin is secreted by activated macrophages and binds to unwanted aging cells, such as aging neutrophils and cancer cells via an asialoglycan moiety, enabling their clearance by macrophages. The balance of CD47 and calreticulin signaling largely determines whether a cell is phagocytosed by macrophages.[19,20]

CD47 may be an actionable target for several types of cancer. Anti-CD47 enabled phagocytosis of AML cells, depleted AML in the bone marrow of mice, and cleared xenografted breast tumors and glioblastoma in mice.[21] In a phase 1 study published in 2018, 50% of 22 patients with advanced non-Hodgkin's lymphoma treatment experienced an objective response after treatment with the combination of anti-CD47 and rituximab. Larger studies with longer follow up are necessary to confirm these results.[22]

Panel discussion: bridging the gap between laboratory and clinic

The morning session featured a panel discussion with **Fuchs, Weissman, Heinrich Jasper** from Genentech, and **David Glass** from Novartis. The panelists discussed what they viewed as some of the more exciting recent developments in stem cell research, such as the increasing appreciation for the role of cross talk between stem cells and the immune system in both homeostasis and disease and the potential for dedifferentiation of progenitor cells and reverting partially differentiated cells to a nearly stem-like state.

The panelists recognized that the field still faces major hurdles in translating basic research into clinical and practical applications. In particular, it is difficult to find funding for the large, expensive clinical trials necessary for stem cell–based therapies. Academic research often does not have the resources for such trials, and pharma can be unwilling to take a risk on cell-based therapies, preferring the more established small molecules and antibodies. The panel agreed that new approaches and systems are necessary to bridge this gap. Weissman pointed to recent success in California with the California Institute for Regenerative Medicine (CIRM). CIRM was established in 2004 after California voters approved Proposition 71, which amended the state constitution to make stem cell research a constitutional right and provides funding to help realize the potential of stem cell research in the clinic.[23] Weissman hopes that CIRM can serve as a model for other states and organizations interested in advancing stem cell research.

Another significant hurdle to stem cell–based therapies is safety. Jasper remarked that there are many unknowns on the potential risks of stem cell manipulation. One of the primary concerns with stem cell activation is the promotion of cancerogenesis. The bar for safety will depend a lot on the disease being treated—for serious diseases with few options, the potential benefits of stem cell therapy may outweigh the potential risks. However, there are still many unanswered questions that must be resolved before broadening research into less serious diseases.

Despite the intrinsic difficulties for stem cell–based therapies, there are reasons for optimism. Glass noted that, while pharma does prefer to work with small molecule therapies, there is precedence for FDA-approved cell-based therapies. In 2017, Novartis received FDA approval for the first CAR-T cell therapy for patients with relapsed/refractory B cell ALL.[24] In addition, deeper understanding of basic stem cell biology may allow therapeutic approaches that target endogenous stem cells with small molecules or biologics, which would likely be safer than delivery of exogenous stem cells. It is likely that there are applications for which perturbation of endogenous stem cells will be more appropriate than stem cell transplant and vice versa.

Regardless of the approach, stem cell niche will be an important factor to consider when delivering stem cell–based therapies. Culturing stem cells *ex vivo* and transplanting them into an environment that does not support their growth and function will likely be futile, likewise for perturbing endogenous stem cells without considering the effects of the niche. Glass foresees a potential role for dual therapies—codelivery of stem cell therapy with small molecules or antibodies that also affect the stem cell niche—that work together to maximize the effectiveness of stem cell–based therapies.

Dynamics of quiescence in muscle stem cells

Thomas Rando from Stanford University presented work from his laboratory on understanding the biology of quiescence in muscle stem cells (MuSCs), also known as satellite cells. MuSCs are generally highly quiescent but can break quiescence and regenerate new muscle tissue in response to injury. Rando showed that, in contrast to being a state of passive dormancy, quiescence is actually an actively maintained and dynamic state, with cells exhibiting varying degrees of quiescence.

Several lines of evidence show that quiescence is an actively regulated state. Rando showed that the Notch signaling pathway maintains quiescence and suppresses proliferation in MuSCs. Disrupting Notch signaling enables MuSCs to break quiescence and express markers of proliferation and differentiation in the absence of injury or stimulus.[25] In addition, knocking down the quiescent-specific microRNA mi-489, which is highly expressed in quiescent MuSCs and downregulated in activated cells, causes cells to spontaneously proliferate.[26]

Rando also showed that quiescence is not a single, defined state. When MuSCs break quiescence and begin to divide, the first cell division takes much longer, an average of 2 days, compared to the second, which takes an average of 12 hours. In addition, the range of time to the first cell division among a population of MuSCs is much broader than for the second division. This led Rando to propose that MuSCs are in varying states of quiescence —cells in a deeper quiescent state take longer to activate. Rando showed that MuSCs can be poised to activate more readily. In mice, an injury in one part of the body caused all MuSCs to enter a state of alert quiescence. While these cells are still quiescent, they are primed to respond more rapidly and regenerate tissue more quickly after a subsequent injury than in uninjured animals. This alert state is regulated by hepatocyte growth factor–mediated activation of mTORC1 signaling.[27,28]

Rando also proposed that aging can cause MuSCs to adopt a deeper state of quiescence. MuSCs from older animals take longer to break quiescence than those from younger animals. Rando's laboratory is investigating some of the molecular pathways that modulate quiescence with age. One characteristic of aged MuSCs is that they are more likely to die during regeneration than young MuSCs. The cells die due to mitotic catastrophe, a type of cell death associated with cell division and mitotic failure. Stabilization of p53, a well-known tumor suppressor, rescues aged MuSCs from mitotic catastrophe both *in vitro* and *in vivo*, presumably due to its ability to lengthen the cell cycle and allow for more time to repair DNA damage.[29]

Rando finished his talk by showing some more recent work from his laboratory in analyzing RNA transcription during MuSC activation. Most transcriptional analyses of SCs are performed after isolating and purifying cells from their niche. Comparing the *ex vivo* transcriptome of isolated and purified quiescent MuSCs with the *in vivo* transcriptome using an RNA-labeling technique showed that while there is broad overlap, there is also a significant amount of RNA degradation and transcription.[30] Rando is now using new techniques to investigate the dynamics of RNA transcription during MuSC activation *in vivo.*

Rejuvenating MuSCs in age and disease

Helen Blau from Stanford University shared her work on the possibility to rejuvenate MuSCs to improve muscle strength. Blau is especially interested in improving decreased muscle function associated with aging or localized muscle loss, for example, due to inactivity after a surgery. Currently, the only successful approach to regain muscle strength is exercise, which can be difficult for elderly populations. Pharmaceutical interventions are being developed that hypertrophy the muscle fiber; however, little clinical research is being conducted on interventions using or targeting MuSCs.

MuSCs are activated by tissue injury following a transient wave of inflammatory cytokines and growth factor signaling.[31] Using an *in silico* screen of input from three databases, Blau's laboratory has generated a gene signature of MuSC activation. During her talk, Blau focused on EP4, a key inflammatory mediator that is upregulated in activated MuSCs. EP4 is a receptor for prostaglandin E2 (PGE2), a proinflammatory lipid with a wide range of biological effects. Animal studies show that PGE2 is released by myofibers following muscle injury—a transient spike in PGE2 occurs approximately 3 days post injury.[32]

EP4/PGE2 signaling is required for MuSC function and regeneration. Impairing PGE2 signaling by conditional knockout of the EP4 gene (*Prger4*) in MuSCs impaired MuSC function, regeneration, and engraftment in mice. This translated to a decline in muscle strength following injury. In addition, blocking PGE2 production with nonsteroidal anti-inflammatory drugs—common, over-the-counter painkillers—reduced MuSC activity and impaired muscle strength and regeneration following injury in mice.[32]

While blocking EP4/PGE signaling impairs the ability of MuSCs to regenerate and restore muscle strength following injury, activating this signaling pathway increases MuSC activity.

Treating MuSCs with PGE2 promoted MuSC expansion and engraftment in mice. These effects were seen both when cells were treated with PGE2 *ex vivo* and transplanted into injured mice, and when MuSCs and PGE2 were coinjected into injured mice. PGE2 even improved endogenous MuSC activity when administered via intramuscular injection after muscle injury, suggesting that MuSC activity can be enhanced endogenously, without the need for stem cell transplant.[32]

Blau also discussed whether modulating PGE2 activity could be a therapeutic strategy to improve muscle function during aging. The MuSC niche in aged muscle differs significantly from that of young muscle. Notably, in young muscle, injury induces a short, transient wave of inflammatory cells that help to coordinate the signals necessary for efficient, temporary activation of MuSCs. In the aged muscle, however, chronic inflammation can contribute to aberrant MuSC activation and eventual loss of quiescence.[31] Blau showed promising unpublished results demonstrating that PGE2 can augment MuSC function in aged mice. Blau hopes that these results will lead to strategies to stimulate endogenous MuSCs in humans to increase strength. She has founded a company, Myoforte Therapeutics, to help translate this research into clinical applications.

Data blitz presentations: a new stem cell, ongoing clinical trials, and delineating epidermal stem cell/immune cell cross talk

Shawon Debnath, from Weill Cornell Medical College, described work on identifying a new type of stem cell found on the periosteum—or outer surface—of the bone. Previous studies have identified skeletal stem cells in the endosteal—or inner compartment of the bone. However, a stem cell for the periosteum, the source of bone-forming osteoblasts, had not been identified. Upon noticing that a skeletal targeted cathepsin-K–Cre selectively labeled the periosteum mesenchyme, Debnath was able to isolate these putative periosteal stem cells (PSCs) and showed that they are distinct from endosteal stem cells. The putative PSCs fulfill the criteria for stemness; they can self-renew in serial transplantation and they sit at the apex of the differentiation hierarchy for periosteal cells. In addition, PSCs mediate bone formation when transplanted into a recipient. Debnath also showed that PSCs can differentiate into osteoblasts and they play a key role in both normal ossification and bone healing. Debnath's work shows that the bone marrow is composed of several distinct stem cell populations with different locations and functions.[33]

Anthony Oliva, from Longeveron LLC, provided a brief overview of the company's clinical trial programs. Longeveron is investigating the use of allogeneic mesenchymal stem cell (MSC) transplant to treat aging-related diseases. MSCs have several qualities that make them attractive candidates to alleviate aging-related disease: they are anti-inflammatory, antifibrotic, and neuroprotective, and can improve endothelial and mitochondria function. In addition, because MSCs are immuno-privileged, MSC transplants do not require a tight donor/recipient match.

Longeveron is currently evaluating its allogeneic MSC transplant approach in several clinical trials. A phase 2b study is underway to investigate whether the treatment improves aging frailty, which is evaluated by changes in a number of endpoint measures, including

proinflammatory biomarkers and performance on the six-minute walk test.[34] A second ongoing phase 1/2 study is investigating the ability of MSC therapy to improve vaccine response in patients with aging frailty.[35] Finally, a third phase 1 study is recruiting patients with mild Alzheimer's disease. While the primary objective is to assess the safety of MSC therapy, the trial is also designed to monitor changes in cognition, quality of life, and Alzheimer's-related biomarkers.[36]

Sangbum Park, from Yale University, presented unpublished work on understanding interactions between immune and epithelial stem cells in the epithelium. Using a technique that enables realtime cellular imaging in living animals, Park showed the distribution of immune cells within the mouse epithelium and how changes in epithelial stem cells affect that distribution. Future work will investigate the molecular mechanisms that govern these interactions.

Inflammatory signals in HSC activity

Emmanuelle Passegué, from Columbia University Irving Medical Center, presented work on the role of inflammation in regeneration of HSCs. Inflammation plays an important role in regenerating damaged tissue by helping to remove damaged cells while activating stem cells. HSCs can survive inflammatory stress that kills other blood cells, and many proinflammatory cytokines have been shown to play a role in hematopoiesis, affecting both the size and lineage distribution of the blood system by directly reprogramming HSCs.[37]

Passegué focused on the role of the proinflammatory cytokine TNF-α on HSC survival and lineage distribution. Upregulation of TNF-α is associated with many chronic inflammatory diseases as well as bone marrow failure, aging, and hematological malignancies. Data on the effects of TNF-α on HSCs have been conflicting. Some studies have shown that TNF-α supports survival and differentiation of HSCs,[38,39] while others suggest that TNF-α impairs survival and supports the elimination of HSCs.[40,41]

Passegué's work shows that the effects of TNF-α on the blood system are highly cell-type dependent and that TNF-α is cytotoxic to all but the most immature HSCs. Administration of TNF-α in mice eliminated by apoptosis most of the bone marrow cells, including mature B cells, granulocytes, and many progenitors, but preserved HSCs via activation of the NF-κB pathway and prevention of necroptosis-mediated cell killing. In addition, TNF-α induced HSCs to exit quiescence, enter the cell cycle, and differentiate into myeloid cells via precocious activation of PU.1, a key transcriptional regulator of myeloid differentiation. These functions of TNF-α were found essential to keep HSCs alive and mediate the regeneration of the blood system in conditions of chronic inflammation associated with disease development and aging. Altogether, Passegué established the role of TNF-α as a key pro-survival and pro-regenerative factor for HSCs.[42]

Understanding lung stem cell/stroma cross talk

Lung diseases and cancer often involve injure or depleted epithelial cells; however, little is known about whether these defects are related to dysfunction in the lung epithelial stem cells

or whether stem cell therapy may represent an appropriate strategy for addressing these diseases.

Carla Kim, from Boston Children's Hospital/Harvard Medical School, presented work from her laboratory on understanding the role of lung epithelial stem cells in lung disease and cancer. Kim's laboratory has developed a coculture system that mimics the environment of the lung. By culturing lung epithelial stem cells with lung stromal cells, Kim's laboratory can create bronchiolar (tubular airways) or alveolar (air sac) organoids to model the two major cell types in the lung.[43]

Kim showed how her laboratory has used this model system to understand how the epithelial stem cells communicate with stromal cells. She showed that Lgr6, a marker of epithelial stem cells in intestine and skin,[44] actually labeled bronchiolar mesenchymal cells in the adult mouse lung, while another Lgr protein, Lgr5, marked alveolar mesenchymal cells. Coculturing epithelial lung stem cells with Lgr6-expressing mesenchymal cells produced large, bronchiolar organoids. Conversely, coculturing epithelial lung stem cells with Lgr5-expressing mesenchymal cells produced small, alveolar organoids. These data demonstrate that the mesenchymal cells influence the differentiation pathway that the stem cell chooses.[43] Kim's laboratory is working to understand the molecular mechanisms and secreted products that mediate this cross talk.

Kim also showed how her organoid model provides insight into lung disease and lung cancer. For example, her laboratory is using cells from a mouse model of bronchopulmonary dysplasia (BPD), a chronic lung disease that can affect premature infants. In premature infants, the alveoli are often not mature enough to function properly, necessitating respiratory support with oxygen and/or a ventilator. While these measures are often life-saving, they can also damage the airways and air sacs, resulting in BPD. Kim's laboratory is using organoid cultures to ask if defects in endothelial, stromal, or lung progenitor cells may drive disease in BPD. Understanding the mechanisms by which stromal cells influence epithelial cell development could lead to new therapeutic strategies for lung diseases. However, her data demonstrate that a more holistic approach is necessary and that understanding the mechanisms by which mesenchymal cells influence epithelial cell development could lead to new therapeutic strategies for lung diseases. Kim is also using the organoid coculture system to understand the early effects of *KRAS* activation on stem-like cells. *KRAS* is a well-known oncogene, and aberrant *KRAS* activation is a common occurrence in lung adenocarcinoma. Kim believes that the coculture system may allow them to model the early stages of lung cancer or later stages on a more rapid timescale.

Finally, Kim's laboratory is also working on using the organoid culture to grow transplantable cells, with encouraging results in mouse models.

Addressing age-related stem cell dysfunction

Stem cells are tightly regulated at various levels. All of these regulatory levels, whether they be intrinsic, niche-related, or systemic, are impacted with aging and contribute to stem cell decline. **Heinrich Jasper** (Genentech) proposed that despite some cell type–dependent

differences, common themes are emerging with regard to stem cells and aging that may pave the way to using stem cells to rejuvenate tissues and maintain homeostasis during aging.

Jasper discussed how age-related stem cell dysfunction can affect the barrier epithelium. Jasper's work uses the fly intestinal epithelium as a model; however, he hopes that the results will be translatable to other barrier epithelium systems, such as the airway epithelium.

Intestinal stem cells (ISCs) within the fly intestine give rise to two differentiated cell types via an intermediate cell type, the enteroblast. The two differentiated cells are enterocytes, which represent the bulk of the intestinal epithelium and maintain its barrier function, and enteroendocrine cells. These cells are frequently damaged and replenished by ISCs. During aging, ISCs are more proliferative and differentiate abnormally, ultimately disrupting the epithelial barrier. In flies, this leads to death.[45]

Jasper presented a model for how epithelial homeostasis within the intestine declines with age. During aging, changes in gene expression disturb the compartmentalization of the intestinal tract. Gastric epithelial cells, which normally acidify the gastric region, transdifferentiate into enterocytes. This decreases the acidity of the gastric region and changes the composition of the microbiome in the gut, leading to an inflammatory response. Inflammation activates the ISCs, which become highly proliferative and accumulate on the basal side of the epithelium, disrupting its function.[45,46] In flies, this results in a leaky epithelium and ultimately to death. Several of the molecular players in this process have been identified, including JAK/STAT, FOXO/REL, and JNK. Perturbing these pathways in ISCs can help to restore homeostasis and extend lifespan in flies.[45,47,48]

Jasper went into more detail on how stem cell–intrinsic pathways contribute to the dysfunction of the intestinal epithelium during aging. He focused on mTORC1, which is normally suppressed in stem cells to prevent differentiation. He showed that repeated stem cell activation and regeneration via transient mTORC1 signaling cause stem cell number to decline both in fly intestine and mouse trachea. Inhibiting mTORC1 with rapamycin, which has been shown to increase lifespan in mice, abrogated this effect. Rapamycin also inhibited age-related basal cell decline in the mouse trachea and restored basal cell numbers when given to older mice.[49] While more research is required to extend these results to humans, these results suggest that it is possible to restore stem cell activity and rejuvenate cells in aged animals.

A new type of stem cell to regenerate the lung

Mark Krasnow from Stanford University talked about his work on understanding how lung stem cells regenerate the lung. Krasnow focused on the alveolar cells of the mouse lung. These cells are found at the end of the bronchial tree and are responsible for mediating gas exchange from the lungs into the bloodstream. There are two main types of alveolar cells: type I cells, which are very flat to enable oxygen diffusion, and type II cells, which secrete a surfactant that lines the lung lumen. During embryonic development, the two types of

alveolar cells differentiate from the same bipotentprogenitor. However, by birth, all the bipotent progenitors have been depleted.[50,51]

Krasnow was interested in understanding how the lung continues to regenerate the alveolar epithelium after birth. Lineage labeling of alveolar type II cells in mice showed that 1 month after birth, alveolar type I cells carried the type II lineage label. Krasnow proclaimed that, in addition to their secretory function, type II cells can also function as a stem cell. He coined these cells “differentiated stem cells,” that is, cells that have a function in addition to their role in regenerating damaged tissue.[50]

Krasnow’s laboratory has detailed the molecular signals that activate an alveolar type II cell’s stem cell activity. EGFR released by an injured or dying type I cell triggers the stem cell activity of the nearby type II cell via the KRAS pathway. Another signal from a neighboring fibroblast stromal cell, Wnt5a, determines which of the daughter cells differentiates into a type I cell and which remains a type II cell. The daughter cell closer to the fibroblast receives the Wnt5a signal and remains a type II cell with stem cell potential, while the other daughter cell differentiates into a type I cell. Inhibiting Wnt activity in type II cells caused them to differentiate into type I cells, while activating Wnt activity inhibited their ability to differentiate into type I cells.[52]

Only a small portion of the type II cells expresses Wnt pathway markers and has the potential for stem cell activity. Krasnow proposed that alveolar type II stem cells are the origin of lung adenocarcinoma and described how these results can have clinical implications for lung cancer. Based on his results, lesions that contain alveolar type II cells that do not express Wnt signaling markers are likely to be benign, while lesions with alveolar type II cells that test positive for Wnt signaling have likely arisen from an alveolar type II stem cell and will progress to a malignant tumor. With regard to therapies for lung cancer, many patients currently receive EGF antagonists for lung adenocarcinoma. However, the tumor inevitably develops resistance and progresses. Krasnow suggested that a dual therapeutic approach that blocks both EGF and Wnt signaling, that is, the two messengers that activate alveolar type II stem cells, may be able to stop tumor growth and reprogram the type II cells into benign type I cells.

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