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The Stem Cell: Life's Goblet or Poisoned Chalice?

Do Stem Cells Hold the Key to Understanding Aging and Curing Disease?

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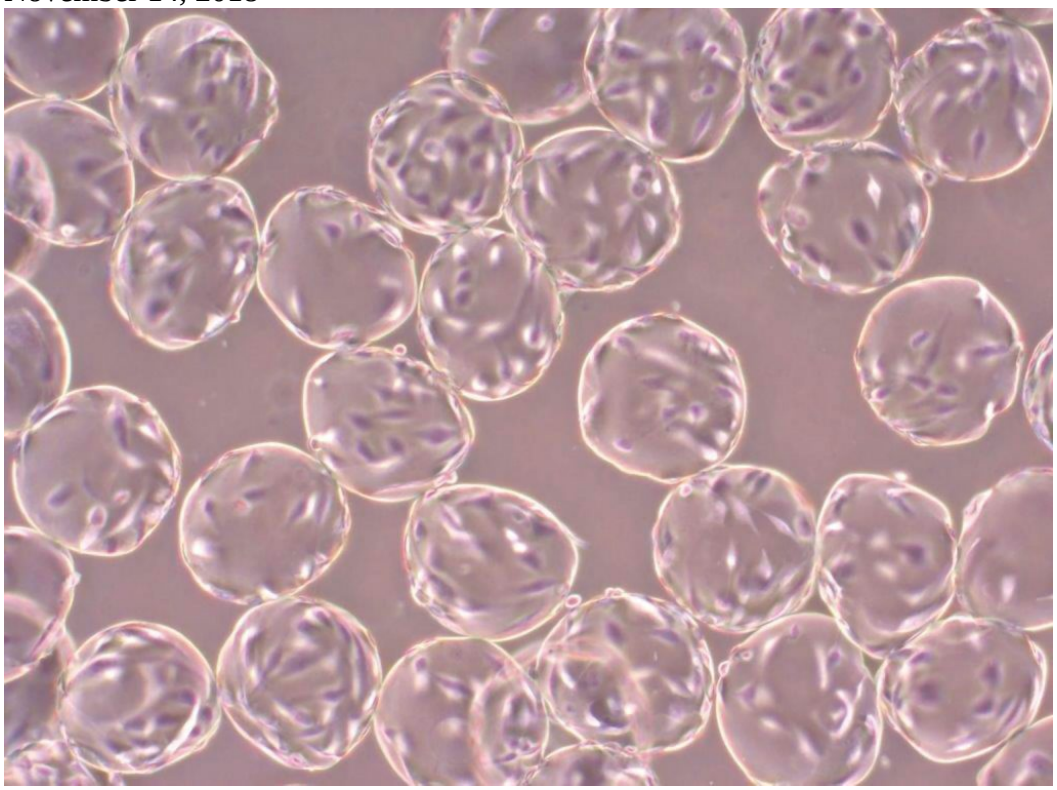

In bioprocess applications requiring the large-scale expansion of human mesenchymal stem cells, Corning Life Sciences' dissolvable microcarriers can simplify downstream purification and eliminate the need to physically separate the cells and microcarriers. Avoiding harsh cell-dissociation strategies can be especially advantageous when cell-based therapies or regenerative materials are being prepared. This image depicts cell expansion on Synthemax II-coated dissolvable microcarriers.

How stem cells follow their programming and how they remain open to reprogramming are topics of abiding interest to researchers. If all goes well, stem cells differentiate and sustain specialized cell populations, tissues, and organs. If not, stem cells may seed tumors or contribute to age-related degenerative disorders. They may even accelerate aging itself.

Because they hold so much potential for good or ill, stem cells are being investigated for myriad applications, including cancer treatments, "pain in a dish" models, and cardiac disease interventions. Such applications were discussed recently at the New York Stem Cell Foundation (NYSCF) conference, a leading scientific meeting on translational stem cell research. Here, researchers and tool providers convened to discuss their latest findings.

Examining Cancer Stem Cells

In myeloid cancers, most of the cancer cells are not driving the tumor; a relatively small subset of leukemic stem cells (LSCs) is responsible. LSCs are the product of a multistep transformation process that originates in normal hematopoietic stem cells. Transformation plays out over decades as stem cells' life spans allow the accumulation of aberrations. Most other blood cells have a half-life of only a few hours, days, or weeks—not enough time to accrue all the mutations required for tumor stem cell transformation.

Patients with the preleukemic condition myelodysplastic syndrome (MDS) often progress to acute myeloid leukemia (AML) within a few years after the initial diagnosis. Using longitudinal samples collected from patients from MDS diagnosis through AML transformation, researchers from two academic laboratories in the New York area found that the MDS-stage stem cells already carry the majority of the mutations that are exhibited after progression to AML.

One of the laboratories is led by Ulrich G. Steidl, M.D., Ph.D., professor of cell biology and medicine (oncology), Albert Einstein College of Medicine–Montefiore Medical Center; the other is led by Amit Verma, M.D., professor of medicine, oncology, and developmental and molecular biology, Albert Einstein College of Medicine.

"We discovered a much more diverse pool of preleukemic stem cells (pre-MDS stem cells in this situation) than previously thought," stated Dr. Steidl. "This unexpected finding challenges the linear divergence tumor evolution model and is changing how we think about these myeloid cancers and strategies to target them therapeutically."

Earlier, the group had found consistently high upregulation of an endogenous inhibitor of p53, MDMX, in AML. This observation extented to leukemic stem cells.[1] ALRN-6294, a dual inhibitor of MDMX and MDM2 (not upregulated in leukemia stem cells) developed by Aileron Therapeutics, was tested and found to reactivate p53 function and inhibit leukemia cell growth in vitro as well as in mouse xenotransplantation models of human leukemia.

ALRN-6294 is a molecule of a new chemical substance class called stapled peptides. Modeled after a small peptide that is part of the p53 molecule, it acts as a decoy binding MDMX and MDM2. The encouraging study results have led to the development of a new clinical trial for individuals with advanced MDS and AML.

Probing Hematopoietic Stem Cells

Three transcription factors, GATA2, FOS, and GFI1-B, will reprogram human fibroblasts to human hematopoietic progenitor cells, said Kateri Moore, D.V.M., professor, cell, developmental, and regenerative biology, Icahn School of Medicine at Mount Sinai. The process recapitulates developmental hematopoiesis where the fibroblasts reprogram to endothelial-like cells that undergo an endothelial-to-hematopoietic transition.

The reprogrammed cells display a human hematopoietic stem cell (HSC) cell surface phenotype and will repopulate immunodeficient mice for three months. Mechanistically, the factors bind open chromatin in a cooperative manner with GATA2 displaying dominance. Together, GATA2 and GFI1-B recruit FOS to silence the fibroblast program and activate endothelial and hematopoietic genes.

Dr. Moore's laboratory has also investigated the divisional history of HSCs and the slow-cycling HSC population.[2] These cells enter a state of dormancy after four symmetric self-renewal divisions. Hypothetically, the dormant cells function as a reserve population in case the organism experiences times of extreme stress that might require the production of large volumes of mature and downstream progenitor cells.

Some preliminary data suggest a combination of epigenetic factors and protein stoichiometries play a role in HSC information storage about divisional history, but it remains unclear if this is the underlying mechanism of the four cell divisions. The cells are likely capable of storing information through multiple mechanisms.

"If the maintenance mechanism of self-renewal was understood, it would have widespread clinical applications, enabling us to expand stem cells for use in all types of therapeutic situations, which would obviously be a great gain for the medical community," added Dr. Moore. "From a scientific perspective, if we could determine how stem cells self-renew, then theoretically we would be able to understand what regulates their mortality and what prevents them from self-renewing, which may have widespread implications for understanding organismal aging."

Conquering Pain

Sodium channel Nav1.7, identified by studying cohorts with genetically inherited loss-of-function and gain-of-function diseases, is encoded by the gene SCN9A, and it acts to amplify small stimuli—thus setting the gain on pain-signaling peripheral neurons. This finding suggests that Nav1.7 blockers might alleviate pain without the central side effects of current pain pharmacotherapy.

Individuals with inherited erythromelalgia (IEM) have gain-of-function Nav1.7 mutations that make the pain-signaling neurons hyperexcitable. A double-blind, placebo-controlled study in IEM patients demonstrated gene variants that enhance responsiveness to existing medications. Molecular modeling and functional analysis confirmed carbamazepine engagement of Nav1.7 for two patients with one particular mutation (S241T).[3]

"Using molecular modeling of Nav1.7, we were able to predict response with one particular polymorphism. We can do it only for a tiny group of patients now, but we are establishing proof-of-principle that a precision medicine approach, using genomics and molecular modeling, for treatment of pain is not unrealistic and can be achievable," discussed Stephen G. Waxman, M.D., Ph.D., a professor of neurology, neuroscience, and pharmacology at Yale University, and a researcher at Veterans Affairs Connecticut.

Individuals afflicted with IEM appear to display a number of different mutations in SCN9A that lead to gain of function. In a functional sense, the mutations all do the same thing. Two broad drug classes may work: one by inhibiting overall activity regardless of defect, the other by focusing on a specific mechanism. Initial studies with Biogen's BIIB074, which blocks Nav1.7 along with many of the other sodium channel subtypes, are promising.

Another study used selective targeting and showed that Nav1.7 blockade reduced firing in nociceptive neurons and provided pain relief.[4] In this study, induced pluripotent stem cells (iPSCs) were used as a patient-derived "pain in a dish" model to enable rapid screening of pain drugs. However, getting the iPSCs to differentiate into cells that resemble sensory neurons takes 2–3 months. In the future, whole exome screening may be a faster alternative approach.

Cardiac Applications

The use of iPSC-derived cardiomyocytes (iPSC-CMs) has become increasingly widespread in basic research, according to Joseph Wu, M.D., Ph.D., director, Stanford Cardiovascular Institute and professor, department of medicine and department of radiology, Stanford School of Medicine.

iPSC-CMs have been used for disease modeling to elucidate various cardiovascular diseases, for drug development (screening to assess cardiotoxicity),[5] and for precision medicine in the context of understanding variants of uncertain significance, and for understanding how various patients responded differently to drugs in a clinical trial.

Before iPSC- or ESC (embryonic stem cell)-based cardiac regenerative medicine can accelerate, more work is needed to overcome regulatory hurdles and pass the scrutiny involved with these cells. Therapies need to show no tumorigenicity and a need for only minimal immunosuppression usage.

Therapy must also show efficacy not just by improving patient symptoms but, ultimately, by improving patient morbidity/mortality. Dr. Wu indicated that this will be a very expensive clinical trial involving large numbers of patients. Advantages must be shown in comparison to ongoing adult stem cell therapies and traditional medical and surgical interventions.

Recently, in Japan, approval was given for an initial iPSC-CM test on three people.[6] Wafer-thin sheets of 100 million heart-muscle cells derived from iPSCs will be grafted onto diseased human hearts.

The lab of Dr. Yoshiki Sawa, M.D., a researcher in the department of surgical medicine at Osaka University, has demonstrated in porcine studies that grafting these tissue sheets can improve the heart's function. The cells do not seem to integrate into the heart tissue but instead appear to release growth factors that help regenerate the damaged muscle that

can be caused by a buildup of plaque or by a heart attack. After the initial year-long study, approval will be sought to conduct a clinical trial in 10 patients.

Reprogramming Tools

The stem cell market faces challenges establishing robust and reproducible processes and methods that easily transition from basic research to translational applications and also satisfy quality, regulatory, scalability, and cost requirements.

iPSCs intended for translational applications need to be of high quality, free of reprogramming transgenes, clear of adventitious agents, genetically stable, and functionally pluripotent. Generation of these cells requires consistent reprogramming, defined culture reagents, and comprehensive characterization.

Scientists at Thermo Fisher Scientific used the company's CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit, the first off-the-shelf reprogramming system designed for clinical and translational research, in combination with Gibco CTS Essential 8 Media, to generate and expand iPSCs under defined conditions for translational applications. The authenticated clones were free of mutations associated with cancer hotspots that serve as the minimal qualifying criteria for iPSC banks intended for translational applications.

"We integrate and validate our cell therapy systems, media, reagents, and services to work together to maintain consistency from research to cell therapy GMP-compliant environments. These products help address emerging challenges by providing regulatory documentation and extensive safety testing," explained Uma Lakshmipathy, Ph.D., director of R&D, cell biology, Thermo Fisher Scientific.

iPSCs are immensely valuable not just in stem cell–based therapies but as a renewable allogeneic source of therapeutic immune cells, such as T and NK cells. The enablement of efficient genetic modification of cell banks to produce even more potent differentiated cell products will become increasingly critical.

Optimized Systems

Human mesenchymal stem cells are currently the most common adult stem cell type used for cell therapy applications, due to their regenerative properties and ability to differentiate into multiple cell lineages. A key part of bioproduction is removal of the viable cells from the growth surface.

Corning Life Sciences' dissolvable microcarrier bioprocessing technology not only allows the growth of stem cells in a more physiologic environment, but also facilitates the removal and isolation of the cells with high viability and yields. To ensure the correct state of differentiation, a range of surface variations exists for different applications.

The 100–300 micron-sized microcarriers are composed of polygalacturonic acid (PGA) polymer chains crosslinked via calcium ions. Microcarrier dissolution is achieved with

the addition of EDTA (to chelate calcium ions and destabilize the polymer crosslinking), pectinase (to target degradation of the PGA polymer), and a standard protease (to break down cell and extracellular matrices).

“We used plant-derived polymers to avoid contamination with polymers of animal or human origin. After digestion, the resultant small-molecular-weight monomers become a very low sugar component of the media,” commented Richard M. Eglen, Ph.D., vice president and general manager, Corning.

Controlling production variation in terms of biomaterial yields and the correct genotype and phenotype of stem cells requires careful control of the culture conditions. The major goal is to eliminate cell-to-cell variation through the use of closed, sterile environments. This includes the use of vessels in different configurations and the adoption of closed-system, single-use disposable technology, such as sterile bags with customized inlet/outlet ports, together with a wide range of specialized surface treatments and optimized media.

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