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Unlocking the Mysteries of Adult Stem Cells and Regenerative Medicine

Since its inception in 1991, the Geriatric Research, Education and Clinical Center (GRECC) at the Miami VA Medical Center, Miami, FL has focused much of its research on the aging skeleton. In working toward the overriding goal of improving the range of therapies available to veterans with age-related bone and joint damage, the staff of the Miami GRECC has undertaken a journey with unexpected but very rewarding twists and turns.

This journey began with examinations into the complex cells responsible for the skeletal system's development, maintenance, and repair. Through these explorations, our research team has made fascinating discoveries involving bone marrow mesenchymal stem cells (BM-MSCs), unspecified progenitor cells located in the bone marrow that hold the potential to differentiate into various mature cell types.

As a result, Miami GRECC researchers are setting the stage not just for repairing bone and cartilage but also for regenerating a wide range of tissues (including vascular, glandular, and neural) that have been damaged by aging or by a variety of other causes, such as traumatic war injuries and malignancies. This work has vast implications for improving the lives of veterans—and nonveterans—of all ages.

INSIDE SKELETAL TISSUE

Although the skeleton is viewed mainly as providing support and protection for the body and its organs, it is actually a complex and dynamic metabolic tissue. As we age, biological, behavioral, genetic, and environmental factors influence the growth, cell differentiation, and repair of bone and its various components, which include osteoblasts (bone forming cells), osteoclasts (bone resorbing cells), adipocytes (fat cells), and vascular spaces and their cellular components.

In addition, bone marrow contains a number of cell types—including BM-MSCs, hematopoietic precursor cells, and endothelial precursor cells (EPCs)—whose proliferation and release are essential to successful repair and regeneration of damaged tissues throughout the body. Given their restorative potential, these various cells became a focus of our research into potential biotherapies for degenerative bone and joint diseases, such as osteoporosis and osteoarthritis.

MARROW STEM CELLS: A SEA OF POSSIBILITIES

In particular, BM-MSCs are of great interest. In our work, we have demonstrated that these cells can differentiate into osteoblasts and chondrocytes (cartilage forming cells), which together create a bone and cartilage matrix; neuronal-like cells; and pancreatic islet-like cells that express messenger RNA for insulin. BM-MSCs embody a heterogeneous population of uncommitted, pluripotent stem cells as well as lineage-committed progenitor cells. After isolation from the marrow, BM-MSCs attach to the surface of culture dishes and form colonies. The number of undifferentiated BM-MSCs can be

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The VHA's Geriatric Research, Education and Clinical Centers (GRECCs) are designed for the advancement and integration of research, education, and clinical achievements in geriatrics and gerontology throughout the VA health care system. Each GRECC focuses on particular aspects of the care of aging veterans and is at the forefront of geriatric research and clinical care. For more information on the GRECC program, visit the web site (http://www1.va.gov/grecc/). This column, which is contributed monthly by GRECC staff members, is coordinated and edited by Kenneth Shay, DDS, MS, director of geriatric programs for the VA Office of Geriatrics and Extended Care, VA Central Office, Washington, DC.

expanded in culture, while keeping them in an undifferentiated state using the appropriate expansion culture conditions. Thereafter, using other defined culture conditions (such as the introduction of certain hormones, growth factors, or cytokines) according to the final cell type desired, the expanded BM-MSCs can be directed to differentiate into specific phenotypic cell lineages. The exact process or mechanism by which BM-MSCs differentiate or mature into a given phenotype is currently under investigation at our GRECC and many other laboratories around the world.

A recent, exciting discovery has been the identification and development of a unique subpopulation of BM-MSCs, which we have named marrow-isolated adult multilineage inducible (MIAMI) cells.[1] Analysis of very early stem cell markers indicates that the MIAMI cells are developmentally immature and resemble primitive stem cells in their capability to differentiate, in culture, into functionally mature cells of various types (Figure). MIAMI cells are relatively small and are characterized by a unique molecular profile that distinguishes them from other marrow stromal cell populations.

Although the study of stem cells for potential therapeutic use has been the subject of much ethical debate in the United States and elsewhere, this debate has centered on the use of stem cells obtained from embryos.[2] Our research, by contrast, has focused exclusively on stem cells obtained (with informed consent from the next of kin) from the whole bone marrow of male and female donors who died of fatal traumatic injury. Using this type of stem cells—commonly called "adult" stem cells, even though the donors are not always adults—has allowed us to avoid this controversy.

Furthermore, adult stem cells are more appropriate than embryonic stem cells for our research, which is aimed at developing autologous stem cell therapies for bone and joint diseases. In this type of therapy, the patient's own stem cells are isolated, developed into a particular cell type, and then injected back into the patient in order to regenerate and repair a specific tissue or organ. BM-MSCs currently are being used in the treatment of nonunion fractures (by placing BM-MSCs directly in the fracture site, facilitating formation of new bone and subsequent fracture repair) and of myocardial infarction (through injection of BM-MSCs into damaged heart muscle to improve ejection volume and decrease scar formation). Examples of other uses under investigation are the injection of BM-MCSs that have been differentiated down the neuronal pathway into the site of a spinal cord injury to facilitate repair and the reintroduction of stem cells that have been differentiated down the pancreatic islet-like cell pathway into a diabetic donor as a means of producing insulin after the pancreas has failed.

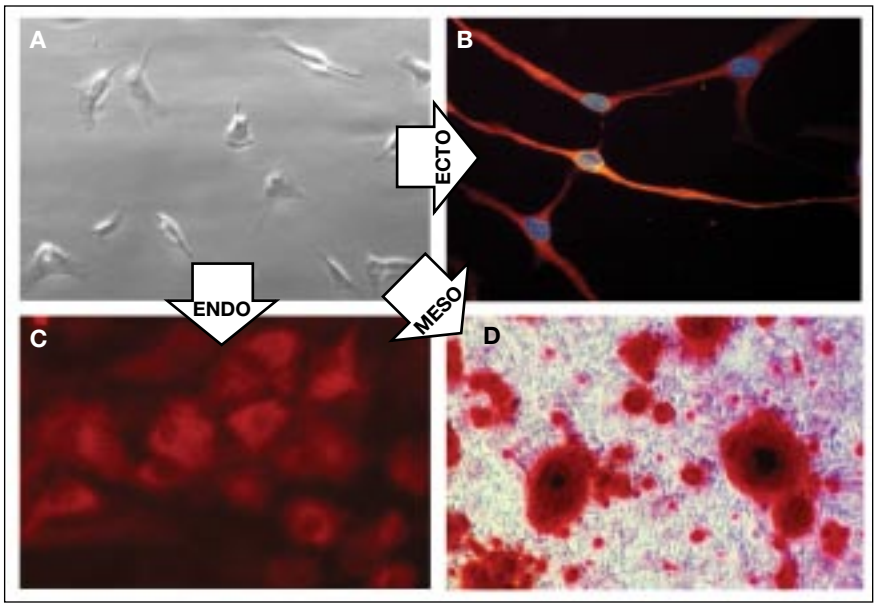

Figure. The differentiation potential of marrow-isolated adult multilineage inducibile (MIAMI) cells (A). These cells can be differentiated into ectodermal lineages, such as neuronal cells (B) (image shown with class III β-tubulin immunostaining); endodermal lineages, such as hepatocyte-like cells (C) (image shown with albumin immunostaining); or mesodermal lineages, such as osteoblastic cells (D) (shown here with extracellular matrix mineralization).

FACTORS THAT INFLUENCE DIFFERENTIATION

In order to develop adult stem cell therapies, and to bolster the stem cell populations available to researchers, Miami GRECC investigators recently have been studying the influences of various factors on BM-MSCs. The idea is that, if we can increase our understanding of the conditions under which these cells differentiate, we can learn to harness this differentiation for therapeutic purposes.

Age

Although evidence is growing that primitive, pluripotent stem cells are

present in bone marrow, very little is known about the effect aging has on regulating their "stemness" (the capacity of primitive stem cells to preserve their full differentiation potential), self-renewal, or maturation. Our investigations have demonstrated that, in the vertebrae, the number of BM-MSCs with the potential to mature into osteoblasts decreases nearly 40% by age 45—which may contribute to age-related bone loss.[3] Information about osteoblast maturation in the spine is particularly important given that the vertebrae are a site of high-turnover osteoporosis and may be the earliest site of bone loss in age-related osteoporosis.[3]

One of the characteristics of MIAMI cells that is particularly promising is that their stemness does not seem to be diminished by aging. Although the prevalence of these cells among all marrow nucleated cells decreases from about 0.01% at age 3 to 0.002% by age 45, the population remains essentially unchanged thereafter, and levels of expression of markers characteristic of MIAMI cells remain constant independent of age and gender. In long-term cell culture expansion experiments, aging increased cell population doubling time by about 30%. But specific differentiation of MIAMI cells toward functional osteoblasts capable of synthesizing and mineralizing extracellular matrix in response to defined molecular cues was unaffected by age, gender, or expansion of the cells at low oxygen concentrations.

Although these results were only for the differentiation of MIAMI cells into the osteoblastic lineage, they suggest it may be possible to define generalized cell-differentiation protocols that could be used for repair or regeneration of damaged tissue in patients regardless of age or gender. That is, it may not be necessary to define a specific protocol for each individual who might need stem cell therapy for a given problem, but rather only a specific protocol for the desired tissue to be regenerated or repaired.

Oxygen

All nucleated human cells respond to changes in oxygen bioavailability, a characteristic essential for both normal prenatal development and postnatal physiology. Physiologic oxygen concentrations inside the body are much lower than the concentration of oxygen in the atmosphere (about 21% at sea level) and vary by tissue, ranging from 1% in cartilage and bone marrow to 13% in the arteries, lungs, and liver. Low oxygen levels stimulate the creation of red blood cell precursors and promote the growth of BM-MSCs, which differentiate into various cell types when exposed to higher oxygen concentrations. These actions appear to occur through the same growth and differentiation activators (that is, hormones, cytokines, and oxygen levels).

As such, oxygen clearly plays a key physiologic role in the bone marrow microenvironment. It is directly involved in regulating the balance between stemness and differentiation. For instance, oxygen concentration influences the distribution of hematopoietic progenitor cells throughout the marrow space, with committed precursors localizing closer to the blood vessels (where oxygen concentrations are higher) and more primitive progenitors and those that are still proliferating localizing primarily to areas with lower oxygen concentrations.

Because the oxygen concentration in bone marrow ranges from 1% to 7%, Miami GRECC scientists have examined the role of oxygen levels in regulating the capacity of MIAMI cells to self-renew and remain pluripotent during long-term culture. Our results suggest that maintaining developmentally primitive human cells in culture at low oxygen levels mimics conditions found in the body and favors stemness, thereby preserving the multipotential tissue differentiation capability of the cells.[4]

Biomechanical stress

Although much of the Miami GRECC's research has focused on the influence of hormones, cytokines, and growth factors on BM-MSC and MIAMI cell differentiation, we have expanded our studies to evaluate the role of biomechanical stress. We have learned that dynamic compressive loading—without the addition of cytokines—stimulates the process by which BM-MSCs differentiate into cartilage precursors, including the expression of chondrogenic genes and the induction of corresponding proteins.[5]

Of tantamount importance from an extension of these studies using human cells is the finding that BM-MSCs and MIAMI cells derived from donors older than 50 years do not respond to dynamic compression-induced chondrogenesis as well as those derived from donors younger than 20 years. This suggests a need for caution in using autologous stem cells in regenerative medicine protocols for older patients (H.S.C., unpublished data, 2006). It is hoped that these studies will lead to methods for developing cartilage for repair of damaged or diseased joints.

BEYOND THE SKELETON

Since BM-MSCs and MIAMI cells have the potential to differentiate into a variety of other cell types besides bone and cartilage, Miami GRECC researchers are partnering with others to explore different avenues of inquiry and clinical application. For instance, a collaborative group—including investigators from the Miami GRECC; the University of Miami Miller School of Medicine (UMMSM) Miami Project to Cure Paralysis; and the University of

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Angers in Angers, France—is studying the differentiation of MIAMI cells into neuronal cells, with the goal of using the cells to regenerate and repair tissue damaged by spinal cord injury and to treat brain tumors.[6] Already, this research has identified various factors that direct MIAMI cells down the neuronal cell pathway.[6] Animal studies are under way, and human trials are being planned.

Another partnership between the Miami GRECC and UMMSM is looking into connections between BM-MSCs and EPCs, with the aim of improving treatment of cardiovascular disease. These studies are centered around the fact that, within the bone marrow, osteoblasts and osteoclasts interact to expand and mobilize EPCs into the circulation. Mobilization of EPCs is primarily the responsibility of osteoclasts, which are derived from a hematopoietic stem cell precursor and which share a common progenitor with macrophages and, possibly, EPCs.[7] It may be possible, therefore, to harness MIAMI cells with EPC potential to develop new cardiovascular therapies.

These and other collaborative studies (including those with members of the Diabetes Research Institute and the UMMSM Center on Aging) are facilitating the Miami GRECC's participation in the newly established University of Miami Interdisciplinary Stem Cell Institute. The overall plan is to leverage what has been discovered and developed already into stronger science, translational research, and regenerative medicine.

More than 30 years ago, the VA established its system of GRECCs to ensure that the health needs of elderly veterans would be addressed by the best research and clinical practice available. In combining sound principles of basic research and emerging technologies, with an eye toward applying the findings to clinical care, Miami GRECC investigators today are validating the wisdom of the VA's investment. Given the expansive potential of adult stem cell research, it is likely that the beneficiaries of these scientific and medical endeavors will include not only elderly veterans but also veterans of all ages whose service to their country came at a steep price: the loss of tissue and function. ●

Author disclosures

The authors report no actual or potential conflicts of interest with regard to this column.

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