MAYO CLINIC

PM&R Update

Physical Medicine and Rehabilitation News Vol. 2, No. 2, 2012

INSIDE THIS ISSUE

Stem Cell–Based Regeneration: The Next Frontier of Cardiac Tissue Repair

Coronary artery disease (CAD) is a major cause of morbidity and mortality. Potent pharmacotherapy, revascularization strategies, cardiac rehabilitation, and aggressive management of cardiovascular risk factors have reduced the recurrence of cardiac events.

"A major barrier in the treatment of cardiovascular disease is the inability of the myocardium for self-renewal," explains Carmen M. Terzic, MD, PhD, chair of Mayo Clinic's Department of Physical Medicine and Rehabilitation. Testing the hypothesis that replacing injured tissue with healthy tissue could rescue a failing phenotype, Dr Terzic and other Mayo Clinic researchers have focused on developing a stem cell–based strategy to repair diseased cardiac tissue.

Stem cell–based regeneration offers the next frontier of medical therapy through delivery of unlimited pools of progenitor cells to achieve structural and functional restoration.

Points to Remember

- Mayo Clinic researchers are seeking to promote myocardial self-renewal with stem cells by gaining a better understanding of the role of nuclear transport during stem cell differentiation and optimizing their properties for cardiac commitment.
- Preliminary data indicate significant variation in the cardiogenic potential of stem cells among patients.
- Correlating the response to stem cell treatment with presence of specific risk factors for cardiovascular disease may ultimately allow physicians to better predict who can best benefit from stem cell therapy.

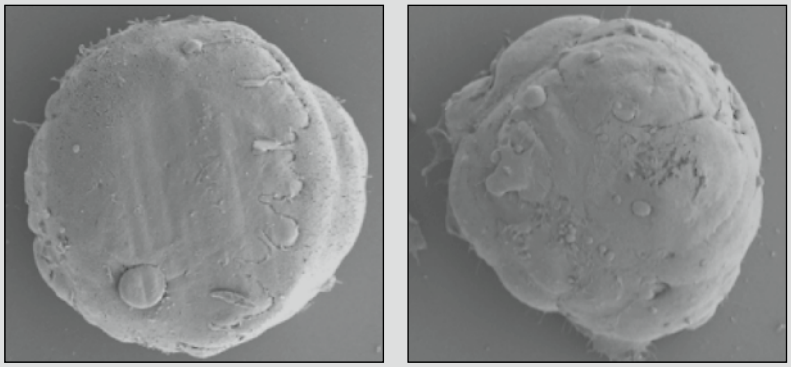

Figure 1. *Electron microscopy images of stem cells. Left image: Wild-type control (WT). Right image: Gene knockout, calreticulin gene (crt).*

Ultimately, augmentation of natural healing or rejuvenation exemplifies on-demand tissue self-renewal without the need for invasive intervention.

Internationally, the use of stem cell therapy in patients is currently limited, but preliminary data suggest that this approach may yield modest improvements in cardiac function and structure when treating acute myocardial infarction or chronic heart failure.

Dr Terzic has engaged in a variety of research efforts to direct stem cells toward cardiogenesis, to assess the role of nuclear transport during stem cell differentiation and optimize their properties for cardiac commitment. These efforts include developing techniques by which direct injection of stem cells in

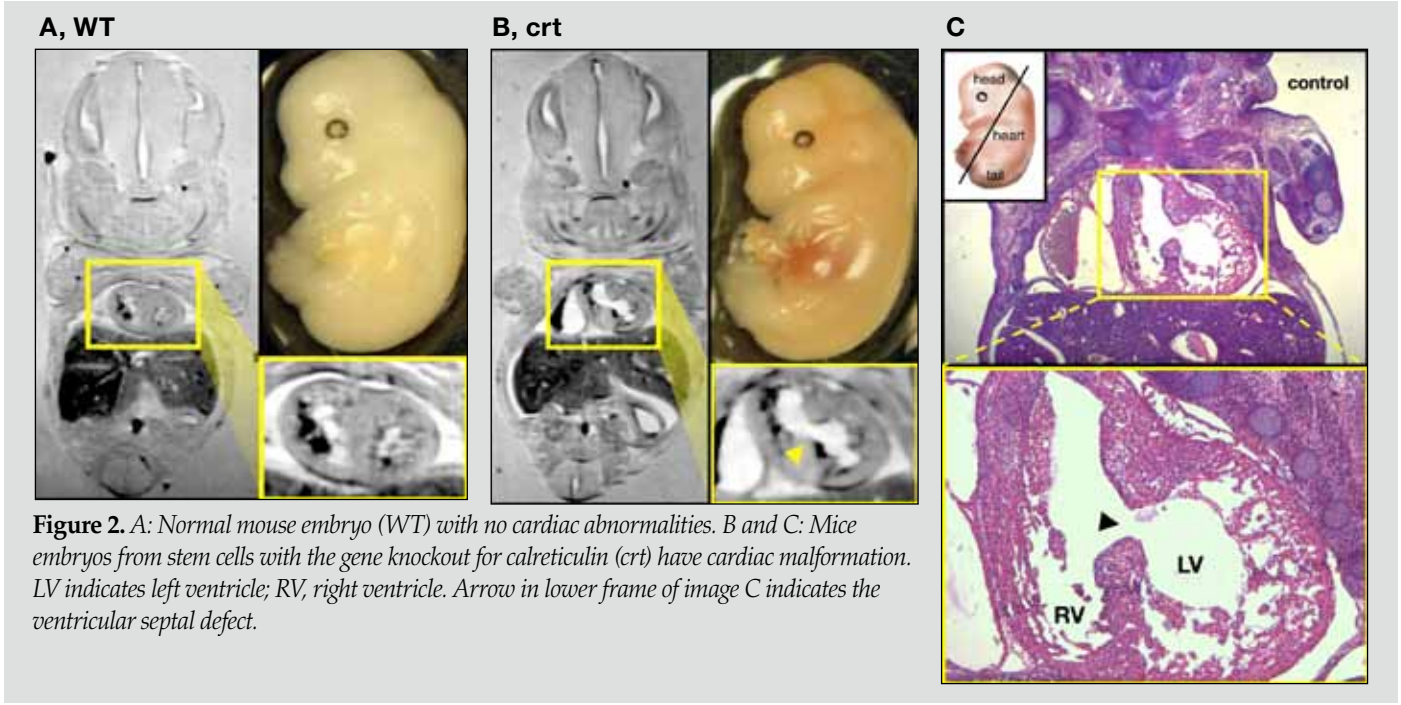

Figure 2. *A: Normal mouse embryo (WT) with no cardiac abnormalities. B and C: Mice embryos from stem cells with the gene knockout for calreticulin (crt) have cardiac malformation. LV indicates left ventricle; RV, right ventricle. Arrow in lower frame of image C indicates the ventricular septal defect.*

Carmen M. Terzic, MD, PhD

a murine model of cardiac infarction engrafts and repopulates the diseased heart with cardiac cells derived from the stem cells (Figures 1 and 2). The ultimate goal is to establish cardiovascular regenerative medicine as a new therapeutic modality for heart disease.

Dr Terzic's team has also gathered preliminary data indicating significant variation in the cardiogenic potential of stem cells among patients. Based on these findings, Mayo Clinic's Cardiovascular Health Clinic, Department of Physical Medicine and Rehabilitation, and Cardiovascular Research Laboratory are joining forces to introduce stem cell science into the innovative practice of prophylactic medicine to advance personalized wellness.

This project involves exploring the individual variation in the number of circulating progenitor cells and their potential to differentiate into cardiac tissue and examining the individual correlation between the cardiogenic potential of circulating progenitor cells and cardiovascular disease risk factors, risk indicators, and clinical outcome. These findings have the potential to help researchers identify novel biomarkers of cardiovascular disease risk, severity, and prognosis; an individual's self-repair capacity; and the restorative/regenerative effectiveness of secondary prevention therapies.

"We are hopeful that a personalized algorithm based on progenitor cell potential would help us identify a high-risk target population with ischemic heart disease that could benefit from cell-based therapy designed to replace progenitor cell deficiencies," says Dr Terzic.

"Because we've found that stem cell therapy is not beneficial for all patients, we're examining whether risk factors such as smoking and high glucose may mediate the effectiveness of stem cell therapy for a given individual or a group of individuals with similar risk profiles," explains Dr Terzic. Correlating the response to stem cell treatment with presence of specific risk factors for cardiovascular disease may ultimately allow physicians to better predict who can best benefit from stem cell therapy.

Inducible progenitor cells are another type of recently described stem cells that have been used by Dr Terzic. What makes these cells "incredible" is the fact that inducible progenitor stem cells are obtained by expressing a set of 4 genes into a mature cell, such as a fibroblast. The fibroblasts expressing these genes "regress" to a state of undifferentiated stem cells that are similar to the embryonic stem cells.

"This approach is of importance because it could allow us to obtain pluripotent embryonic cells without using embryos, eliminating ethical issues," says Dr Terzic. "It also prevents the rejection of these cells as we can obtain the pluripotent cells from fibroblasts derived from the skin of the same individual who is in need of the stem cells."

Translating cardiac cell repair therapy into clinical practice is an intriguing and challenging objective. Dr Terzic and her colleagues are hopeful that this new knowledge will eventually guide clinicians in choosing the most effective reparative phenotype for each patient and help them optimize the delivery, dosing, and timing of these new forms of intervention. This fascinating field is making rapid progress that certainly merits close attention.

Mayo Clinic Researchers Study Role of Enzyme Drivers of Inflammation and Neurodegeneration in Multiple Sclerosis

Multiple sclerosis (MS) is the leading cause of nontraumatic neurologic disability in young adults in North America, yet there are no uniformly effective therapies and none yet proven effective at blocking long-term progression. The difficulty in predicting disease course and in developing effective treatments is complicated by the heterogeneity of the disease, which can manifest with a benign, relapsing/remitting, or progressive disease course. Since effective therapeutic options are very limited for MS patients, particularly when the disease is in its more progressive stages, it becomes essential to define both pathophysiologic mediators of each stage and markers that would permit individualized therapeutic intervention. The clinical manifestations of MS, whether relapsing or progressive, are believed to relate in part to the degree of inflammation relative to neurodegeneration, which characterizes the individual disease course.

Advances in genetics, protein chemistry, and bioinformatics and, most importantly, a skilled community of research and clinical experts create a fertile environment for Mayo Clinic's MS research team. Recently, Mayo researchers have discovered that select members of a family of secreted serine proteases known as tissue kallikreins play key roles in both the inflammatory and neurodegenerative processes that drive MS and may serve as important biomarkers of disease stage and future disability.

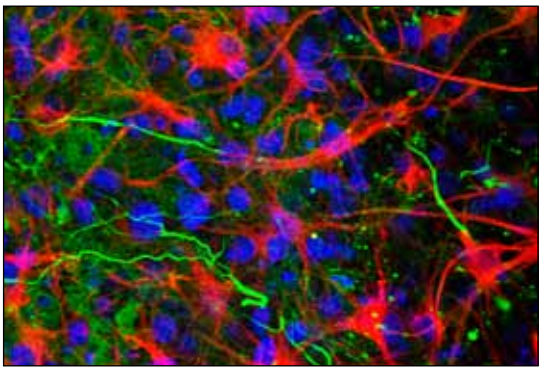

Figure 1. *Whole sections of mouse spinal cord were grown in an organotypic slice culture system to study the interactions between nerve impulse–conducting axons (stained for neurofilament protein [green]) and glial cells referred to as astrocytes (stained for glial fibrillary acidic protein [red]). Cells were visualized using confocal fluorescence microscopy. Cell nuclei were stained blue with 4′,6-diamidino-2-phenylindole. (Picture by Hye-Sook Yoon, PhD, Research Fellow, Mayo Clinic Physical Medicine and Rehabilitation Research)*

Points to Remember

- Select tissue kallikreins play key roles in the inflammatory and neurodegenerative processes driving multiple sclerosis (MS).
- Kallikrein 1 and kallikrein 6 are elevated in the blood and brain lesions of MS patients and appear to contribute to pathology.
- Kallikrein 1 and 6 each correlate with disability scores.
- These enzymes may be important therapeutic targets in treating both the neurodegenerative and inflammatory lesions in progressive MS.

Tissue kallikreins comprise a family of 15 mainly newly identified serine proteases. These proteases comprise the largest continuous cluster of serine proteases in the human genome, although they have as yet poorly defined roles in disease.

Mayo Clinic's MS researchers are focusing on two members of the kallikrein protease family that are elevated in the blood and brain lesions of MS patients and which show strong evidence of contributing to pathology. In a study published in 2008, Mayo researchers showed that kallikrein 1 and kallikrein 6 are selectively elevated in the serum of MS patients experiencing a progressive disease course (when compared with patients with relapsing/remitting disease or to non-MS controls). Importantly, in cell culture studies each of these was shown to exert neurotoxic effects toward central nervous system (CNS) neurons. Kallikrein 6 was also particularly toxic to the myelin-producing oligodendrocytes.

Interestingly, kallikrein 1 levels were found to correlate with Expanded Disability Status Scale (EDSS) scores at the time of serum draw, while kallikrein 6 serum levels were predictive of future EDSS worsening.

Additional research published in 2011 has shown that each kallikrein drives unique aspects of the inflammatory processes that underpin MS pathogenesis, with kallikrein 1 promoting lymphocyte proliferation while kallikrein 6 increased the resistance of lymphocytes to apoptosis. These findings predict a clinical model in which

Isobel A. Scarisbrick, PhD

elevated levels of kallikrein 1 and kallikrein 6 at sites of CNS inflammation likely contribute directly to sustained chronic inflammation and neurodegeneration. These enzymes thus become important therapeutic targets in treating both the neurodegenerative and inflammatory events that so often characterize lesions in patients experiencing a progressive disease course.

A major advance in understanding 1) the mechanism of action of kallikreins in MS and other neurologic disorders and 2) how these enzymes might be targeted therapeutically came with the discovery that kallikreins can activate protease-activated receptors to trigger discrete intracellular signaling cascades involved in inflammation and CNS injury.

Current research is therefore focused on determining the effects of genetic and pharmacologic modulation of these receptors in the CNS or in the immune compartments in animal models of MS. Mayo researchers are hopeful that the insights provided will point directly to new therapies for MS patients, particularly those at the more progressive stages of disease.

In a study published this year in *Brain Pathology*, Mayo researchers found that an antibody that neutralizes kallikrein 6 is capable of staving off MS in mice. "We were able to slow the course of disease through early chronic stages, both in the brain and spinal cord," says lead author Isobel A. Scarisbrick, PhD, of Mayo Clinic's Department of Physical Medicine and Rehabilitation.

Dr Scarisbrick and colleagues studied mice infected with Theiler's murine encephalomyelitis virus (TMEV), a viral model of MS, and assessed the effects of kallikrein 6–neutralizing antibodies on disease progression. RNA expression of kallikrein 6 was elevated in the brain and spinal cord by 7 days postinfection (dpi), and expression persisted primarily in the spinal cord, reaching a peak of five-fold over controls at mid-chronic stages (60 dpi–120 dpi). Significant elevations in kallikrein 6 RNA were also induced in lymphocytes stimulated with viral capsid proteins in vitro and in activated human acute monocytic leukemia cells.

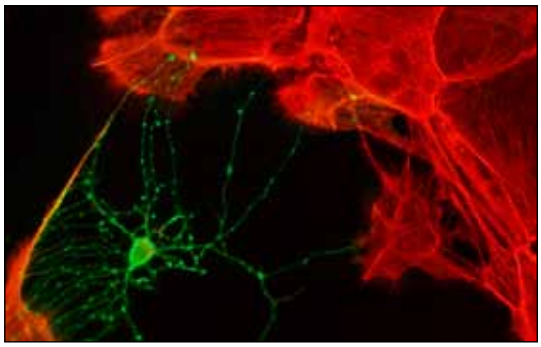

Figure 2. *Fluorescence photomicrograph shows dissociated cultures of myelin producing oligodendroglia (stained for sulfatide [green]) and astrocytes (stained for glial fibrillary acidic protein [red]). The culture system allows determination of factors such as kallikrein 6 which affect myelination. (Picture by Maja Radulovic, PhD candidate, Mayo Clinic Neurobiology of Disease Program)*

Kallikrein 6–neutralizing antibodies reduced TMEV-driven brain and spinal cord pathology and delayed-type hypersensitivity responses when examined at early chronic time points (40 dpi).

The effects of kallikrein 6 on spinal cord pathology included a decrease in activated monocytes/microglia (inflammatory white blood cells) and a reduction in the loss of myelin basic protein, a key component of the myelin sheath. By 180 dpi, pathology scores no longer differed between groups. The kallikrein 6–neutralizing antibody had reduced inflammation and demyelination through early chronic time points, and the investigative team hopes to develop more powerful enzyme-inhibiting techniques to determine if more long-term effects can be achieved.

"These findings suggest kallikrein 6 plays a role in the inflammatory and demyelinating processes that can accompany many types of neurologic conditions," says Dr Scarisbrick. "In the early chronic stages of some neurologic diseases, therefore, kallikrein 6 may represent an excellent drug target to coordinately reduce inflammation and myelin loss. "

Treating Recalcitrant Tendinopathy: Combination of Tenotomy and PRP Injections Produces Significant Improvement

Recently recognized as primarily a degenerative condition, tendinopathy (previously called tendinitis) is characterized by hypercellularity, vascular hyperplasia, and collagen disorganization. Nonoperative treatment of tendinopathy includes correction of biomechanical factors, kinetic chain deficits, and training errors; activity modifications, stretching, strengthening, endurance, and proprioceptive exercises; and bracing, physical modalities, and medications.

When nonoperative interventions fail to relieve symptoms, additional treatment options that attempt to stimulate tissue regeneration can be considered. Emerging therapies include tendon fenestration (percutaneous tenotomy), and platelet-rich plasma (PRP) injections.

During tendon fenestration, the practitioner uses ultrasound to make multiple needle passes through the injured area to stimulate tissue healing. This process disrupts tendinopathic tissue and induces bleeding. The bleeding leads to clot formation and release of growth factors.

"The goal of this treatment is to convert a chronic, nonhealing injury into an acute injury with increased healing potential, " explains Physical Medicine and Rehabilitation physician Jay Smith, MD, who specializes in sports medicine and musculoskeletal ultrasound at Mayo Clinic in Rochester.

Another promising new treatment for refractory tendinopathy is PRP injection. Used for more than a decade to facilitate the healing of difficult wounds, PRP is rich in growth factors linked to healing. Using ultrasound guidance, practitioners inject the PRP into the affected tendon, usually following a tendon fenestration procedure. It is theorized that when PRP is injected into an area of tendinopathy, the platelets release growth factors and stimulate a healing response. "The fenestration breaks up the abnormal tissue, and then we inject the platelets into the prepared area to promote healing," explains Dr Smith.

PRP is created from an autologous whole-blood sample through a platelet separation process, which results in an increased platelet concentration compared with the original whole blood sample.

In a recent study published in the journal *PM&R*, Mayo Clinic researchers reported that the combination of tenotomy and PRP injections produced significant improvement in many patients with long-standing tendon injuries.

The study included 34 patients with a wide range of tendon and soft tissue injuries, from tennis elbow (lateral epicondylitis) to plantar fasciitis, an inflammation on the bottom of the foot. "These disorders can be hard to treat, and patients tend to receive one therapy or the other (tenotomy vs PRP), depending on what a doctor happens to offer. Our study was the first clinical study to investigate the combination of both treatments in injured tendons," says Dr Smith.

Researchers found maximum benefits tended to occur within four months after the procedure. More than 70 percent of patients had better use of their tendons, and 76 percent reported improvement in pain. In addition, researchers found some indication of tendon healing, which was detected with sophisticated ultrasound imaging.

"Larger studies are still necessary to determine whether the combination is particularly helpful for certain injuries or types of tendons, but this investigation showed these therapies together are safe and effective for some people who have an ongoing tendinopathy," says Dr Smith.

Points to Remember

- During tendon fenestration (percutaneous tenotomy), the practitioner uses ultrasound to make multiple needle passes through the injured area to stimulate tissue healing.
- Used for more than a decade to facilitate the healing of difficult wounds, platelet-rich plasma (PRP) injections are another promising new treatment for refractory tendinopathy.
- Mayo Clinic researchers found that more than 70 percent of patients treated with a combination of tenotomy and PRP injections had better use of their tendons, and 76 percent reported improvement in pain.

Jay Smith, MD

Mayo Clinic
PM&R Update

Medical Editors:

Carmen M. Terzic, MD, PhD
Mary L. Jurisson, MD

Contact Us

Mayo Clinic welcomes inquiries and referrals, and a request to a specific physician is not required to refer a patient.

Arizona
866-629-6362

Florida
800-634-1417

Minnesota
800-533-1564

Resources

mayoclinic.org/medicalprofs

Clinical trials, CME, Grand Rounds, scientific videos, and online referrals

Education Opportunities

Mayo Clinic Symposium on Concussion in Sport

September 28-29, 2012
Scottsdale, Arizona

Offered both onsite and as a webcast, this state-of-the-art symposium highlights the epidemiology of concussion in sport; the pathophysiology of concussion; sideline and outpatient clinical evaluation; standard and novel diagnostic strategies; and implementation of return-to-activity guidelines. The format includes platform lectures, small group workshops, panel discussions, and live two-way remote audio/video concussion evaluations using robotic teleconcussion technology. Contact: 480-301-4580 or e-mail: mca.cme@mayo.edu

The Neurorehabilitation Summit

October 25-26, 2012
Rochester, Minnesota

This summit provides practitioners with updates that address three common areas of neurologic care: brain disorders, spinal cord dysfunction, and neurodegenerative diseases. Renowned speakers will discuss advancements in clinical practice, research, and innovation throughout the continuum of care. Contact: 800-323-2688 or e-mail: cme@mayo.edu

22nd Annual Mayo Clinic Symposium on Sports Medicine

November 9-10, 2012
Rochester, Minnesota

This case-oriented program provides an integrated approach to the injured athlete and includes case presentations, lectures, and video demonstrations. Health care professionals with an interest in sports medicine and athletic trainers will find this program appropriate. Contact: 800-323-2688 or e-mail: cme@mayo.edu

Update in EEG, EMG, and Clinical Neurophysiology

February 24 - March 2, 2013
Amelia Island, Florida

This course will review clinical neurophysiology techniques and topics, including basic physiology, pathophysiology, EEG, evoked potentials, EMG, movement disorders, and intraoperative monitoring. Focus will be on neurophysiologic tests for epilepsy, sleep disorders, movement disorders, peripheral nerve, and neuromuscular disorders. Contact: 800-323-2688 or e-mail: cme@mayo.edu

See all Continuous Professional Development courses at http://www.mayo.edu/cme

MAYO CLINIC | mayoclinic.org

4500 San Pablo Road
Jacksonville, FL 32224

200 First Street SW
Rochester, MN 55905

13400 East Shea Boulevard
Scottsdale, AZ 85259

MC1938-0712