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Editorial

TMU: Stem Cells

1. Introduction

The subject of stem cells seems to have reached a captivating level such that everyone regardless of station in life seems to have an opinion about stem cells: what they are? What can they do? Are our hopes realistic? What are the problems inherent and unanticipated? Surely some of us realize that as we offer opinions and solutions to problems we often raise others, which were predicted and ignored or the benefits of a particular solution outweighed the predicted outcomes—often with a shrug of shoulders: oh well, we will cross that bridge when it appears; oh well, we can fix that later. It is almost as if in our infinite wisdom, we seem to ignore our own predictions in favor of an almost immediate solution to a particular problem. Problems then are like basic research: we answer a question but raise others, but in the case of stem cells, we should not be so cavalier (see also: offhand, inconsiderate, high handed; arrogant; haughty; casual; careless; in contrast to the antonym: considerate) about our technical prowess and ability to solve a problem. Considerate would encompass all sorts of outcomes and possibilities that in the case of stem cells impinge on human suffering and its alleviation.

What then are some of the immediate targets of stem cells? Cancer; Parkinson disease; Alzheimer disease, and diabetes—all of which in one way or the other could benefit by the "simple" insertion of stem cells into a denuded site with the promise of their acceptance, recolonization, and restoration of function. One crucial question? Is the target area a site of a "toxic" environment however small that caused the original demise still an area that will be hostile to new recruits, that is, stem cell transplants? Is the new area so independent that it will not invoke a long-held concept in transplantation immunology that *self* is acceptable but *non-self* is not?[1,2] Even if the inserted stem cells are *self*, there is no guarantee of success. If the area is not vulnerable to the laws of transplantation, then will that original area send out other possibly toxic signals that may now be classified as "danger"[3]?

Sally Lehrman is a science journalist and a fellow at the Markkula Center for Applied Ethics at Santa Clara University. There such questions as those that I raise are analyzed under the rubric: Bioethics. There are written: "Articles, cases, and links on medical ethics, biotechnology and ethics, clinical ethics, end-of-life decision making, culturally competent health care, and public health policy from the Markkula Center for Applied Ethics at Santa Clara University." Center staff and scholars work with hospitals, public health departments, and other agencies to analyze real-world ethical issues in medicine and biotechnology and to develop innovative tools and programs to address them.

In a recent article, Sally Lehrman[4] has addressed the question of stem cells. In reference to funds, she opens with a cautionary note proposing an analysis of the situation for funding stem cell research in California: "no breakthroughs? Blame the scientists; blame misplaced optimism by proponents and the media."

This article seems an appropriate background for one of my primary initiatives in these editorials, that is, raising awareness of what is being done in current research at Taipei Medical University (TMU). In this instance, there are substantial approaches to the future of stem cells with it is safe to say, TMU appears to be out front. This pertains to analytic approaches and certain seemingly less familiar sources such as teeth! Teeth are in marked contrast to the well-known sources, for example, embryos, umbilical cord, bone marrow, and so on.

To write this piece, I searched stem cells and TMU and found more than 50 articles published of course in reputable peer-review journals. To give credence to the discussion, I arbitrarily chose two studies each from 2010 and 2009 from those that seemed the most unique at least from my viewpoint.

One of the newest sources of stem cells at least in the minds of most citizens are those derived from teeth! From the School of Dentistry, TMU, we are reminded that human dental pulp stem cells (DPSCs) are a useful material for future analyses devoted to regenerative medicine. Lee and colleagues[5] have demonstrated that cryopreservation of intact teeth can successfully preserve the periodontal ligament for future autotransplantation (*self*), but with a cautionary note: they suggest that the effects of cryopreservation on the properties of DPSCs require clarification. For analyses, they sought to test whether DPSCs isolated from cryopreserved teeth could express stem cell-specific markers. Their approach is interesting because they use a novel programmable freezer coupled to a magnetic field to perform relevant experiments. First, the tested DPSCs were isolated from magnetically cryopreserved and noncryopreserved fresh teeth using an enzyme digestion procedure. For analysis, they measured a success rate following isolation, using growth curves, morphology, stem cell-specific markers, and differentiation capacity of isolated cells. Results revealed that the isolation rate of dental pulp cells from magnetically cryopreserved teeth was relatively high, that is, 73%. Even after culture for five generations, there was no significant difference in cell viability between cells isolated from

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magnetically cryopreserved teeth and those isolated from fresh teeth. In addition, there were no differences between the two groups of dental pulp cells with respect to morphology, expression of stem cell markers, or osteogenic and adipogenic differentiations. Encouraging results suggested that cryopreserved whole teeth are useful for autotransplantation and can provide a viable source of DPSCs.

With respect to stem cells, specifically mesenchymal sources, a group in the Graduate Institute of Clinical Medicine has been most active; led by Ho et al,[6] analyzed cells during stages of differentiation. Focusing on changes of actin filament organization, they analyzed cell fate commitment of mesenchymal stem cells (MSCs). Their focus included the following approach. Thymosin beta-4 [Tbeta(4)], a major G-actin sequestering peptide, regulates the cytoskeleton. The analyses thus viewed pathways wherein Tbeta(4) regulates cell fate determination in MSCs during differentiation. Tbeta(4) decreased F-actin formation, reduced the F-actin/G-actin ratio, and inhibited osteogenic differentiation. This actin reorganization was not associated with changes of Runt-related transcription factor 2 gene expression during early osteogenic induction. In contrast, Tbeta(4) reciprocally facilitated adipogenic differentiation. Tbeta(4) treatment upregulated gene and promoted surface expression of adipocyte adhesion molecules during early adipogenic differentiation. This acceleration of adipocyte phenotypic maturation was not associated with differential expression of peroxisome proliferator-activated receptor gamma during the first week of adipogenic induction. Thus, Theta(4) initiated cell fate determination of MSCs by exerting biophysical effects through cytoskeleton reorganization and altered cell-cell adhesion, instead of direct regulation of lineage-determining transcriptional factors. These results suggest a rather obvious clinical application because Theta(4), a ubiquitous peptide, when its intracellular concentration is elevated, could be involved in affecting the dreaded condition of osteoporosis.

Intervertebral discs (or **intervertebral fibrocartilage**) lie between adjacent vertebrae in the spine. Each disc forms a cartilaginous joint that allows slight movement of the vertebrae and acts as a ligament to hold them together. As people age, the *nucleus pulposus* (NP) begins to dehydrate, which limits its ability to absorb shock. The *annulus fibrosus* becomes weaker and begins to tear. Although this may not cause pain in some people, in others one or both of these may cause chronic pain, associated with the back, sciatica, spinal disc herniation. Knowing this, Chen et al.[7] in the Stem Cell Research Center have developed an ex vivo degenerative intervertebral disc (IVD) organ culture system that can screen disc regeneration agents. Chymopapain was used to partially digest (NP) tissue that mimics human IVD degeneration. This system was then tested for evaluating different therapeutic regimens including MSC derived from enhanced green fluorescent protein (GFP)-transgenic porcine (MSC-GFP), platelet-rich plasma (PRP) and MSC-GFP/PRP combined treatment; this was confirmed in in vivo animal model.

Chondrogenic-specific gene products including Col II and aggrecan were upregulated, and chondrogenic matrix deposition was increased. This was evident by sustained fluorescent signals during 4 weeks, in the MSC-GFP implanted group. In earlier experiments, these investigators demonstrated in vitro stage-specific chondrogenesis of MSC by chondrocytic commitment. These same molecules upregulated for chondrogenesis were also observed in MSC-GFP group. PRP that promote NP regeneration also resulted in significant increased levels of mRNA involved in chondrogenesis and matrices accumulation. The ex vivo IVD regeneration results were repeated and supported by observations of the in vivo porcine degenerative system. The disc height index was significantly increased in both in vivo MSC-GFP and PRP regeneration groups. To the investigators' surprise, the MSC-GFP/PRP combined therapy revealed an inclination toward osteogenesis in the ex vivo system. The ex vivo degenerative IVD culture system could therefore serve as an alternative and more accessible model when compared with large animal model. Finally and of equal importance, this system also provides a high-throughput platform that can screen therapeutic agents for IVD regeneration, a point of great promise for this prevalent ailment.

Molecular imaging and immunohistochemistry are two other approaches used to analyze cell-based bone regeneration. A group led by Lo et al[8] (Graduate Institute of Clinical Medicine) used genetically modified NIH3T3 embryonic fibroblasts carrying enhanced GFP (NIH3T3-G). First, the fibroblasts were predifferentiated into osteoblast-like cells using PRP medium, followed by intraosseous transplantation into ovariectomized senescence-accelerated mouse prone substrain 8 (OVX-SAMP8 mice); the results are interesting. PRP-conditioned NIH3T3-G (PRP/NIH3T3-G) engraftment prevented the development of osteoporosis. Molecular imaging and immunohistochemistry demonstrated the migration of NIH3T3-G cells from the implantation site throughout the skeleton. In situ analyses revealed coexpression of osteopontin and GFP in the newly formed bone tissue. This result demonstrated that the transplant restored bone trabecular architecture and mineral density in treated OVX-SAMP8 mice. Another finding was promising: the life span of OVX-SAMP8 mice that received PRP/NIH3T3-G transplantation was significantly prolonged and similar to that of the congenic senescence-resistant mouse strain. The investigators concluded that this unique and yet simple approach has substantial potential. It could be used to treat senile postmenopausal osteoporosis or even inborn genetic syndromes associated with accelerated aging including Hutchinson-Gilford progeria syndrome; the prolongation of life expectancy.

2. Perspectives

I considered four promising approaches to understanding stem cells as may be applied to defined therapies. There are chances for breakthroughs with important implications. Like all basic science experiments, there is the need to do the translation, that is, to move the lab bench to the bedside! In other words, more and more results generated from animal models and or in vitro approaches are unquestionably relevant and valuable—but the leap must occur. What is the promise and is it realistic? Sally Lehrman ends the presentation with a rather promising but extremely cautious admonition about which I am in agreement with. "Progress may indeed be in the offing, but let's not predict wonders just yet." She quotes: As a Chicago Tribune business writer wrote about embryonic stem cells: "All the possibilities dreamed about long ago may yet come to pass—eventually."

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