Review of Caplan on cell-based therapeutic technology using Mesenchymal Stem Cells

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ABSTRACT

This classic discusses the original 1991 publication 'Mesenchymal Stem Cells (MSCs)' by Dr. Caplan on the emergence of a new therapeutic technology of self-cell repair using MSCs. After the original classic publication, a large number of methods to regenerate injured tissue have been reported. Currently, MSCs are used clinically to repair articular cartilage defects, liver cirrhosis, cerebral infarction, spinal cord injury, graft-versus-host disease (GVHD) and others. As a result, MSCs are considered one of the most important cell sources for Regenerative Medicine. An MSC has been demonstrated to be a multipotent stem cell in cell culture and was thought to contribute to the regeneration of injured tissue at transplant sites, but recently the concept of MSCs has changed such that they are now referred to as Medicinal Signaling Cells, owing to their often indirect effects on tissue repair and regeneration. Regardless of the name, either Mesenchymal Stem Cells or Medicinal Signaling Cells, MSCs will be used to regenerate injured tissue more widely in near future.

INTRODUCTION

Regenerative Medicine has gained worldwide acceptance for the repair of many kinds of tissues. Cell source choices for tissue repair remain very important. Since 2006, induced pluripotent stem (iPS) cells
have been thought to be the possible cell source, but clinical use is still limited due to cost effectiveness, and adverse events such as tumorigeneses. Currently, MSCs are considered an acceptable cell source for clinical practice. Dr. Caplan's 1991 publication "Mesenchymal Stem Cells (MSCs)" introduced the concept of MSCs being used for cell-based therapies at a time when regenerative medicine was in its infancy. As such, this publication has been selected as The Classic topic.

MSCs are multipotent stem cells whose culture-expanded progeny can give rise to mesodermal tissues, such as cartilage, bone, muscle, fat, tendon, ligament etc. In that way, MSCs are similar to the stem cells that exist in an embryo that give rise to mesenchymal tissues. In 1991, Dr. Caplan assumed that MSCs existed not only in embryo but also in adults, and advocated the basis for the emergence of a new therapeutic technology of self-cell repair using autologous MSCs (Figure 1). Initially, MSCs were used to repair bone and cartilage probably because bone marrow contains a subset of committed osteochondral progenitor cells that gave rise to these tissues. In 1990s, MSCs from multiple sources have been used to repair many kinds of tissues in preclinical animal models, and as of May 2011 an NIH website (clinical trials.gov) listed 19364 cell-based therapies, and 206 of those are considered MSC-related. The list of MSC-related candidate applications include diverse clinical targets, such as bone marrow transplantation, graft-versus-host disease (GVHD), acute myocardial infarction, stroke, spinal cord, lung (asthma, chronic obstructive pulmonary disease), acute kidney failure, liver fibrosis, tendinitis, juvenile diabetes, radiation syndrome, burn and wound healing, osteoarthritis, rheumatoid arthritis, systemic lupus erythematosus, autism, inflammatory bowel disease, multiple sclerosis, amyotrophic lateral sclerosis, urinary incontinence and sepsis. However, the
results from these MSC-related translational research projects had made scientists reconsider the mechanism of MSC-mediated repair in vivo, and to propose new concepts of cell therapy using MSCs beyond the initial concept of self-cell repair. Although several tissue-derived MSCs are currently in clinical use, their functions and activities differ depending on the original source tissue. Moreover, their effects would be assumed to alter depending on the target organ, disease, and the application, as supported by published reports. This article focuses on the work of Dr. Caplan, who brought the concept of MSC treatment to bone and joint tissues in the field of orthopedics, and describes the evolution of MSC research and its future challenges and directions.

SUMMARY OF THE CLASSIC

The original paper argues that stem cells exist in adult tissues and can give rise to mesenchymal tissues. Dr Caplan, called this multipotent stem cell "Mesenchymal Stem Cells (MSCs)" and proposed a new therapeutic technology of self-cell repair using MSCs for regenerative medicine. The original paper discussed the involvement of MSCs in bone and cartilage repair and turnover, but MSCs subsequently have been applied into the clinical translational research in other disease areas. More recently, the concept of self-cell repair of MSC has broadened into other areas.

HISTORIC PERSPECTIVE
It was reported in the 1950’s that bone was formed after transplantation of bone marrow. After transplantation in diffusion chambers, the development of a considerable mass of bone in the chambers was observed. Marrow cell suspensions or fibroblasts harvested from in vitro culture of marrow cells, gave rise to a mixture of bone, cartilage and fibrous tissue in diffusion chambers implanted into the peritoneal cavity.

In Dr. Caplan’s laboratory, Dr. Ohgushi reported heterotopic osteogenesis in porous ceramics induced by bone marrow cells and used these to repair bone defects in rat model. Later, Dr. Wakitani reported successful osteochondral defect repair using autologousMSCs from bone marrow or periosteum in the rabbit model. He reported that articular cartilage defects were repaired using autologous MSC in human. After that, others reported the use autologous MSC to repair articular cartilage defects.

In 1995, in Dr. Caplan’s laboratory, Drs. Wakitani and Saito reported that MSCs could be induced to differentiate into muscle cells and adipocytes. In 1998, Dr. Johnstone reported that chondrocytes could be formed in vitro fromMSCs, and in 1999 it was shown that MSC clones could be induced in vitro to form osteocytes, chondrocytes, and adipocytes. Moreover, it had already been reported the cells derived from tissues other than bone marrow, such as fat, had multipotency and phenotypic plasticity in vitro.

In 2006, minimal criteria for defining MSC was proposed by the International Society for Cellular Therapy (ISCT), these included 1. Adherence to plastic dishes, 2. Cell surface expression of CD105, CD73, and CD90, and 3. Differentiation into osteoblasts, chondroblasts and adipocytes in vitro. This was a result of the need to redefine MSCs in line with their functions as MSCs were being applied in a clinical setting.
In Dr. Caplan’s laboratory, in addition to demonstrating their multipotential capacity, showed that MSCs secrete bioactive factors that influence the local micro-environment, as demonstrated in a hematopoiesis study by Dr. Haynesworth in 1996. Drs. Caplan and Dennis termed this function of MSCs the Trophic Effect, and distinguished it from the immunomodulatory function which had already known in 2004 (Figure 2). Since 2004, some studies with the dramatic effects of MSCs for preclinical use had been published, which cannot be sufficiently interpreted simply by the original 1991 Diagram of Mesengenic Pathways, i.e., the multipotency of MSCs alone (Figure 1). And the nomenclature of MSCs expanded with the development of cell processing technology, and some discrepancies between the cell nomenclature and biological characteristics appeared, resulting in confusion and misunderstanding in the scientific community.

To reflect the function of MSCs more accurately, Dr. Caplan proposed that MSCs be amended to name "Medicinal Signaling Cells". It also led to the correction that MSCs did not meet the formal definition for “stem cells” as they did not show the property of self-renewal. Dr. Caplan also noted that most mesenchymal cells have phenotypic plasticity in vitro, and stated that "the stem cell moniker is inappropriate. Call them MSCs, but please, not stem cells". He has been a leader for a scientifically sound understanding and argued for ethical clinical use of MSCs in the scientific community. The ISCT recommended that MSCs isolated by plastic-adherent be called multipotent mesenchymal stromal cells in 2005, and continues to support the use of the acronym "MSCs" with their definitions, while referring to the works of Caplan et al.
SCIENTIFIC AND SOCIETAL IMPACT

This original 1991 publication discussed in this article was the catalyst for bringing MSCs into clinical use for tissue repair. Osiris Therapeutics, whose aim was to develop the MSC therapy was officially incorporated in 1992 with Dr. Caplan as one of the founders. Osiris later received regulatory clearance in 2010 for its first systemically administered stem cell drug.

Initially, the main scientific tasks were to prove the origin of MSCs in the tissues and to elucidate their mechanism of action in vivo, and to isolate and clone MSCs in vitro. The mechanism of action of MSCs in vivo has been attributed to their heterogeneous cell population characteristics and not simply the multipotency of MSCs. This is the second paradigm shift for MSCs beyond the concept of self-cell repair and is thought to be the reason why cell therapy has been applied to new disease areas.

CURRENT EVIDENCE AND RELATED TO THE ORIGINAL ARTICLE

The question of the origin of MSCs in vivo, especially in bone marrow tissue, remained unclear. It had been demonstrated that microvascular pericytes could differentiate into chondrocytes and adipocytes as well as osteoblasts, and are multipotent cells in cell culture, and it had been suggested that a subset of endothelial cells have a generalized progenitor potential, according to the study of skeletal and cardiac muscle, and bone marrow MSCs had been found to have specific markers for pericytes and smooth muscle cells. A cell culture study by Drs. Crisan, Peault, et al. showed that perivascular cells have osteogenic,
chondrogenic, and adipogenic, and myogenic potential,\textsuperscript{35} which led Dr. Caplan to propose that “MSCs are members of the pericyte family of cells”.\textsuperscript{36} Then, he concluded that MSCs arise from pericytes, and dismissed the original hypothesis that MSCs are stem cells.\textsuperscript{37} An interesting recent report indicates that even within the bone marrow, MSCs differ between metaphysis and diaphysis, and that they are regulated at the level of gene transcription, related to their regional specialization and fate specification.\textsuperscript{38}

In Dr. Caplan’s laboratory, the cloning of stem cells from murine bone marrow derived progenitor cells (mesenchymal progenitor cells; MPC) had been accomplished by Dr. Dennis.\textsuperscript{39, 40} They had identified the immortalized MPC clone, BMC9, with four mesenchymal phenotypes: chondrocyte, adipocyte, stromal (support osteoclast formation), and osteoblast, and used BMC9 cells in preclinical animal studies.\textsuperscript{39, 41} However, the clone BMP9 has not been concluded to be an MSC as BMC9 cells do not show the potential to differentiate into multinucleated myotubes,\textsuperscript{39} which contrasts with the results of Wakitani and Saito who showed myogenic potential in a mixed MSC population.\textsuperscript{12, 13} Dr. Dennis discussed in his article “The existence of a mesenchymal stem cell is still a possibility, but on a functional level, a mixed population of multipotential MPCs with a range of response characteristics may be more physiologically appropriate than would a homogeneous population of MSCs that would all react identically to physiologic signals.”\textsuperscript{39} Dr. Caplan also argued that the in vivo trophic effect and the mechanism of the action, along with the heterogeneity of MSCs, could explain the phenomenon of those in vivo niches and provides the basis for the MSCs (cell-based) therapy.\textsuperscript{20, 21}
After the report of the repair osteochondral defects using autologous MSCs from bone marrow or periosteum embedded in collagen gels in rabbit model, Dr. Wakitani reported that articular cartilage defect repair using autologous culture expanded MSCs from bone marrow in two human cases. After that, they reported that MSC transplantation for repair of articular cartilage defects in human osteoarthritic knees. Twelve knees were transplanted with MSCs, and the other 12 subjects served as cell-free controls. Although the clinical improvement, measured using the Hospital for Special Surgery knee-rating scale, was not significantly different, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group 42 weeks after transplantation. They concluded that this procedure highlights the utility of autologous culture expanded bone marrow mesenchymal cell transplantation for the repair of articular cartilage defects in humans’ osteoarthritis. Subsequently, they performed this procedure in 41 patients between January 1998 and November 2008. Neither tumors nor infections were observed between 5 and 137 (mean 75) months of follow-up. Autologous MSC transplantation is a safe procedure and will be widely used around the world. Some other groups reported the utility of MSC transplantation for articular cartilage defects. The Saris procedure with freshly isolated chondrons (autologous) plus ten-fold more-allogeneic fat-derived MSCs showed NO engraftment of MSCs. Thus, their effect is totally paracrine from MSCs and positive in support of the autologous chondrocytes.
Dr. Gobbi reported a one-stage cartilage repair method using MSCs concentrated from bone marrow during surgery. Intraoperatively, bone marrow aspirate concentrate (BMAC) including MSCs was concentrated to create a malleable clot. It was then combined with a hyaluronic acid-based material (Hyalofast; Anika Therapeutics) secure with using a polydioxanone suture (PDS II 6-0; Ethicon) and/or fibrin glue (Tissucol; Baxter Spa). This treatment can reduce the cost of the procedure, because of the complete in an one-step surgery without cell cultivation, and shows good clinical outcomes evaluated with some clinical outcome measures in long term studies until ten years. The active cells in concentrated BMAC could be MSCs but also the hematopoietic progenitors which are, themselves, professional secretory cells.

Dr. Kamei reported a new cell delivery system using magnetic force, termed magnetic targeting. They have magnetized MSCs with ferucarbotran during cultivation, injecting them into the knee and then allowing the cells to accumulate within a focal articular cartilage defect using the 1.0-T compact magnet. Magnetic targeting of MSCs is thought to concentrate MSCs into mini-aggregated that become chondrogenic. The process was safely performed and showed complete coverage of the defects with cartilage-like tissues and significant improvement in clinical outcomes 48 weeks after treatment. The magnetic targeting of MSCs shows promise as useful as a minimally invasive treatment for cartilage repair.

MSC from periosteum
Shimomura et al. studied implantation of a scaffold-free tissue-engineered construct generated from autologous synovia-derived MSCs for repair knee chondral lesions. They reported this procedure was safe and effective to repair articular cartilage defect in knee joint.  

MSC from umbilical-cord-blood  

Human umbilical-cord-blood-derived mesenchymal stem cells (hUCB-MSCs) have recently been used in clinical cartilage regeneration procedures with the expectation of improved regeneration capacity. They demonstrated the safety, improvement of clinical symptoms following hUCB-MSC therapy, and that there was no clear difference in the comparison with bone marrow aspiration concentrate.  

MENISCUS REGENERATION  

MSC from synovium was studied to repair meniscus injury. Sekiya studied 5 patients with the use of synovial MSC transplantation following surgical repair of a complex degenerative tear of the medial meniscus of the knee. He reported this was effective to promote the repair of meniscus injury as evaluated with MRI images and some clinical outcome measures. This study showed 3 adverse events, but none necessitated treatment discontinuation, with the expectation for a future prospective study.  

OSTEOARTHRITIS
Knee osteoarthritis (OA) is one of the most prevalent diseases and, therefore, draws a great deal of attention as a target for cell-mediated repair.

Some procedures to repair articular cartilage defects by MSC transplantations (introduced in the former session) are used to repair articular cartilage defects in knee OA. To fix the MSCs in articular cartilage defect, a surgical procedure is necessary. However, injection of the MSCs in joint is a desirable approach as it is less invasive and preferrable for not only patients but also medical doctors. Adipose tissue derived MSC are injected to reduce pain in osteoarthritic knee. However, the efficacy of the treatment is still controversial.

FUTURE DIRECTIONS

As Dr. Caplan argued, MSCs likely exert the trophic effects to indirectly induce a favorable state in the local micro-environment at the site of tissue damage, not related to direct differentiation. Therefore, intra-articular administration of MSCs as a treatment for joint diseases, could be a promising option to affect tissue repair of via a trophic effect.

MSCs have therapeutic potential including anti-inflammatory and immunomodulatory activities, as shown in clinical application and their effectiveness for GVHD and Crohn’s disease. One of the mechanisms of action on immune system is the formation of T-regulatory cells. Recently, in terms of joint disease, the immune system including T cells and macrophages in the synovium has been getting attention to regulate the inflammation in OA progression with joint destruction. The injection of the MSCs for OA described above have been studied on the basis of exactly this nature. And, it has also been reported that
chronic pain is associated with neuroinflammation and non-neuronal cells such as immune cells, moreover, it was recently reported that MSCs are also effective as a therapeutic treatment for neuropathic pain. Therefore, chronic pain associated with OA would also be a target for the intra-articular injection of MSC, and the effect for this factor would have been reflected in the results of OA study mentioned above. It is the still challenge to elucidate these complicated mechanisms of action in the future, and new therapeutic concepts may be created in the process.

THE LESSON(S) LEARNED.

The first lesson to be drawn from the classic is that it proposed that undifferentiated cells, MSCs, could be useful to transplant into various tissues, instead of transplanting already differentiated cells. The second lesson is the paradigm shift in the concept of MSCs have a trophic effect and that the main mechanism of action may not be the direct differentiation of the MSCs.

Finally, Dr Caplan's own lessons learned during his career were presented at the 50th anniversary celebration in Cleveland in autumn 2019, and these are shared with the reader at the end of this article. We might learn tips for serendipity from these lessons.

Caplan Lessons Learned, at the 50th anniversary in Cleveland in Autumn 2019

1) The single most important human characteristic that can bring success is perseverance.
2) Perseverance requires an open mind: repeating the same wrong approach will still be wrong. An open mind sees the many solutions to the problem.

3) Curiosity beats repetition but repetition brings reliability. If you cannot repeat it, it does not exist.

4) If it works the 1st time, you will never be able to repeat it. Then it is up to you to make it work.

5) Dogma is the “truth” of the moment. The more that you work on the problem, the more that the dogma is challenged.

6) Money is both the source of intellectual freedom and also the bondage that muffles creativity.
The important new conceptual piece is that every tissue in the body has its own unique committed progenitor cell. These cells give rise to the differentiated tissue in which they are found. Isolated and culture-expanded MSCs are found from all tissues, but they are distinctly different in each tissue because they come from pericytes which are in different tissue-specific microenvironments. Also, the MSCs from different tissues, or from different sites even in same bone marrow tissue, have very different populations of committed progenitors. Thus, the actual MSCs have core MSC transcripts but have tissue-unique (tissue of origin) transcripts in addition. Importantly, MSC populations from any tissue have core MSC-capabilities but in cell culture, these cells can be induced into pathways outside their capabilities and outside the pools of committed progenitors. Almost any mesenchymal cell in culture for a few passages can be forced to differentiate along an abnormal pathway of expression.

Regarding the nature of MSCs, I maintain, as I have for 30 years, that they are progenitor cells and might not be stem cells, which is why I referred to these cells as Mesenchymal Progenitor Cells in my publications. The “stemness” of a true MSC is the ability to self-renew as a progenitor cell. And while most MSC preparations are a mixture of progenitor cells, Bianco et al. showed the most convincing data that there are stem cells in this mixed population by showing that MSCs could form bone via serial transplantation in subcutaneous implants.
We thank Dr. Arnold I. Caplan and Dr. James E. Dennis for the English editing the manuscript and providing their own comments, and Dr. Thomas J. Kean for his co-operation.

This manuscript was supported by Japanese Society for the Promotion of Science; JSPS, Grant-in-Aid for Scientific Research, KAKENHI Grant Number JP21K09245.
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Fig. 1 The Schema shown in the original paper.
The original concept for MSC therapy was based on the multipotency of MSCs alone.
It is very simplistic and therefore easy to accept, but is now considered oversimplified.
Fig. 2 Schematic diagram, which was corrected, of MSCs including bioactive factors secretory function as dual role of MSCs.