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Mesenchymal Stem Cells: The Past, the Present, the Future

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In the summer of 1964, I took a graduate pathology course for non-MDs given by Professor Guido Mino at Harvard Medical School. Dr Jerry Gross gave a 2-hour lecture in that course which went from the chemistry and structure of collagen up through the collagenase-mediated destruction of collagen during tadpole tail resorption and metamorphosis. This lecture changed my life forever and set the tone of my thinking and research by demonstrating the continuum of molecules through the control of biological phenomena as seen through the eyes of a biochemist and developmental biologist. In 1967, I started a postdoctoral fellowship with Nathan O. Kaplan, PhD, at Brandeis University where I met and joined the lab of Edgar Zwilling, PhD. Professor Zwilling was a renowned embryologist and developmental biologist[1,2] who taught me about these subjects as I started studying the differentiation capacity of undifferentiated embryonic chick limb mesodermal cells in culture.[3] These cells were mesenchymal stem cells (MSCs) that have the capabilities to differentiate *in vitro* into a number of mesodermal phenotypes by controlling the initial seeding density in culture and the medium additives and conditions.[4-6] Thus, I was able to study the molecular control of embryonic chick limb cell differentiation and limb development[7] in ways complementary to those described by Dr Gross 5 years earlier. This was the start of my MSC journey and experimental exploits.

The Past

In the late 1960s, I showed that the initial plating density of chick limb bud mesodermal cells into culture controlled whether they would or would not form cartilage in culture.[4] For example, at 5×10^6 cells per 35-mm dish, massive chondrogenesis was observed and, indeed, the time-dependent changing pattern of synthesis of the cartilage proteoglycan (PG) aggrecan observed in these cultures mimics the changing PG pattern which is observed in the developing and aging human joint cartilage.[8] If 2×10^6 cells/35-mm dish were seeded, no cartilage could be observed to form; this was the optimal seeding density for bone formation.[9]

In the late 1970s and early 1980s, we used the chick limb bud cell culture system as an assay to purify molecules extracted from demineralized bone[10] following the protocol of Marshall Urist, MD, and others.[11] By testing extract fractions in cultures seeded at 2×10^6 cells/35-mm dish, where control cultures never exhibited chondrogenesis, we obtained fractions that caused a dose-dependent differentiation of the cells into chondrocytes. We called these stimulatory molecules chondrogenic stimulating activity (CSA), now known as BMPs. In the late 1980s, John Wozny, PhD, and colleagues cloned the genes for BMPs,[12] and it was obvious that they had beaten us to this goal. In agonizing over this "failure," it occurred to me that there must be cells very similar to the undifferentiated limb bud cells, but in adults. This stemmed from the fact that demineralized bone matrix, when implanted either subcutaneously or intramuscularly into adult animals, caused the accumulation of multipotent progenitors that formed cartilage and/or bone.[13,14] I called these cells adult MSCs and proposed the simplistic scheme seen in Figure 1. It must be remembered that in the 1980s and early 1990s the dogma of the day was that there were no adult stem cells except for the hematopoietic stem cells (HSCs) and cells that gave rise to sperm or eggs. We now know that there are progenitors called neural stem cells (NSCs),[15] cardiac stem cells,[16] liver stem cells,[17] etc. in adults, and these cells function as sources for cellular replacement of differentiated cells that naturally expire or are injured.

Based on the logic of Figure 1 and *in vitro* and *in vivo* assays using MSCs, tissue engineering studies have been reported by us and others in which adult MSCs with appropriate scaffolds are used to fabricate tissues *ex vivo* that cannot regenerate by themselves in adults.[18-21] For example, cartilage[18] or tendon/ligament tissue[21] is initiated in culture using MSCs and then implanted in *in vivo* defects in animal models and in some cases into humans. This area of experimentation is quite active with MSCs from marrow, muscle, and adipose tissues from both animals and humans.

Two important groups of studies were published using MSCs and the tissue-engineered fabrication of cartilage. First, we developed an *in vitro* method for inducing both animal[22] and human[23] MSCs into the chondrogenic pathway. The development of the pellet or aggregate culture

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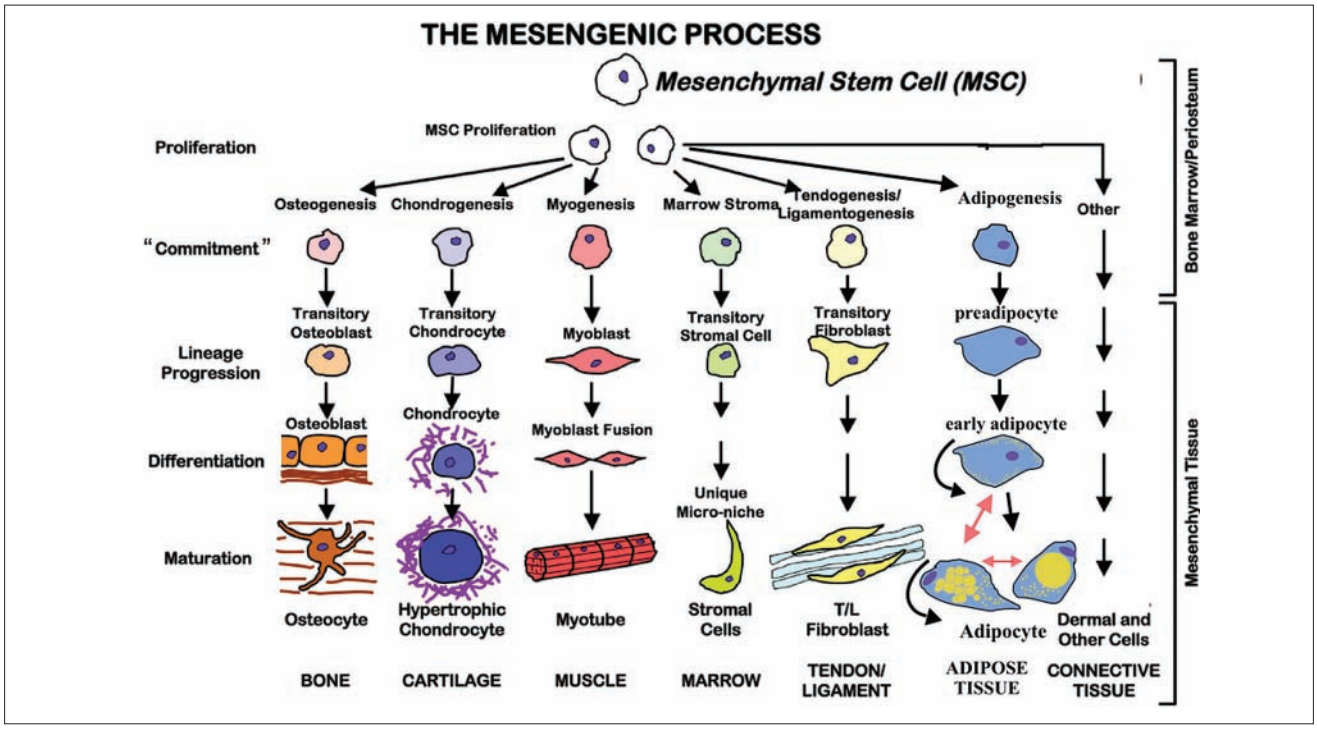

Figure 1. The mesengenic process. Hypothesized scheme of a multipotent MSC self-renewing and having the capacity to be induced into several mesenchymal lineage pathways resulting in the formation of definitive tissues such as bone, cartilage, muscle, etc.

system allows the assay of MSCs from any tissue source. For example, 2.5×10^5 cells are spun down to the bottom of a 15-ml conical tube or 96-conical well plate. The growth medium is replaced with a defined medium that contains TGF-β. Human marrow MSCs will form a ball of chondrocytes under these conditions, while human adipose-derived MSCs require both TGF-β and BMP-6.[24]

The second important study was performed by Shigeyuki Wakitani and collaborators in which rabbit MSCs were placed in a full-thickness osteochondral defect in the medial condyle of an adult rabbit.[18] Such successful animal models led Wakitani and his colleagues to explore the use of human autologous MSCs in human osteochondral defects.[25] The refinement of these early observations will have considerable clinical impact. As pictured in Figure 1, the use of MSCs for the tissue engineered repair/regeneration of skeletal tissues is an ongoing pursuit.

The Present

The story today is quite different from what we imagined when Dr Wakitani and his colleagues did the first implantation of autologous MSCs into a cartilage defect in a patient.[25] Indeed, we did the correct first clinical trial[26] using MSCs for (retrospectively) the wrong reasons. In the 1990s, we assumed that MSCs could differentiate into bone marrow stromal cells and would, thus, add value to bone marrow transplantation (BMT) protocols. In this context, it was separately shown that human MSCs could support the expansion and differentiation of HSCs and their progeny.[27] The first safety study[26] and first use of human MSCs were to augment BMTs for cancer patients following chemotherapy and radiation ablation of their marrow.[28] The results of these clinical trials were that MSCs aided the kinetics of engraftment and hematopoietic recovery in MSC-supplemented protocols.

We now know that this added value of MSCs upon infusion into such patients is due to the massive amounts of bioactive agents secreted by MSCs[29] and because of their powerful immunoregulatory activities.[30] These newly recognized capacities allow clinical trials to be conducted using allogeneic MSCs to control and cure graft-versus-host disease and inflammatory bowel disease (Crohn's disease). Several publications now document that MSCs and their secretions can affect dendritic cells, T-cells, B-cells, T-regulator cells, and natural killer cells.[31]

Although the differentiation of MSCs into bone and cartilage is still an important and potentially useful capability for tissue engineering applications, the immunomodulation capacity may have a more profound and immediate effect on joint chemistry and biology by muting or eliminating the chronic inflammation observed in osteoarthritis, in rheumatoid arthritis, or with severe focal injuries to skeletal tissues.

The Future

This newly uncovered capacity of MSCs to secrete bioactive factors that are both immunomodulatory and regenerative opens up avenues for their clinical use not previously imagined. For example, asthma is a chronic inflammation of the lung. In a mouse lung inflammation model, human MSCs introduced intravenously can cure the degenerative effects of lung tissue.[32,33] Additionally, we have shown that the medium conditioned by MSCs can influence the differentiation pattern of NSCs to favor the differentiation of oligodendrocytes.[34] In a mouse model (EAE) of multiple sclerosis (MS), the intravenous infusion of human MSCs can cure the mouse by separately turning off the inflammatory processes that affect the myelin surrounding the axons of nerves and enhancing the host-mediated differentiation of host-intrinsic NSCs into oligodendrocytes.[35] Thus, MSCs home to sites of tissue damage, ischemia, or inflammation where they mute the inflammatory events and establish a regenerative microenvironment. Given this newly documented and complex activity, the future use of MSCs will focus on autoimmune diseases like diabetes, on scarless regeneration of skin following massive burns or injury, on stroke and spinal cord contusion or excision injuries, on acute and chronic cardiac events, and on acute renal or liver failure.

This new and evolving MSC science also involves the new realization that (maybe) all MSCs are perivascular cells or pericytes.[36] These cells reside on every blood vessel in the body, and some of these cells become MSCs upon focal injury. By secreting factors which mute the immune system, the MSC-pericytes inhibit T-cell surveillance of the damaged tissue and, thereby, prevent autoimmune reactions from starting. Moreover, bioactive agents are released by MSCs that establish a regenerative microenvironment by inhibiting ischemia-caused apoptosis, inhibiting the formation of scar tissue and stimulating angiogenesis by secreting VEGF and by becoming pericytes once again and stabilizing newly formed blood vessels.[37] Last, factors secreted by MSCs are mitotic to tissue-specific progenitors that add to the regeneration of adult tissues.

It may be that the future will hold a revolutionary new medicine in the form of MSC cell therapies that mimic what the tissues do themselves in a very limited sector of self-regeneration.[38,39]

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