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Thoughts Concerning the Origins and Functions of Human Mesenchymal Stem Cells

David W Scharp*

The Scharp-Lacy Research Institute, Scharp Technologies, Aliso Viejo, California, USA

***Corresponding Author:** David W Scharp, The Scharp-Lacy Research Institute, Scharp Technologies, Aliso Viejo, California, USA.

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This first, preliminary report of our adult human mesenchymal stem cells initiated from research that recently began in The Scharp-Lacy Research Institute and continued into the new company, Scharp Technologies, Inc. Adding this interest to our major research and clinical interest in human pancreatic islets and their global distribution through Prodo Laboratories, brought many new challenges and changes in our approaches into this new interest. We focused on establishing and standardizing tissue culture media and techniques that consistently permitted the long-term culture of adult human mesenchymal stem cells obtained from the bone marrow of organ donors. These efforts led to the ability to culture primary human bone marrow, mesenchymal stem cells (BM-MSC's) for many weeks with the evidence of several different types and differing concentrations of these cells on culture. The details of these long-term cultures of human BM-MSC techniques and results will be submitted for scientific publication in the near future. The purpose of this brief report is to simply identify our initial findings, open lines of potential communication, and continue forward with this project.

With the knowledge that human BM-MSC's normally differentiate to many different cell types, we established there are two major types of differentiation these cells can undergo that we have chosen to label as Hetero-Differentiation. This mean these stem cells can differentiate into many different cell types in the body, as well as within tissue culture conditions. Figure A in this report shows this Hetero-Differentiation capabilities that are well known in that BM-MSC's can differentiate into fibroblast cell types, as well as different types of muscle cells, cartilage, bone, fat as well as fibroblasts and fibrocytes. What we did not appreciate was that human BM-MSC's on appropriate tissue culture media will readily divide and differentiate into different morphologies of a few of these cells. As shown in Figure A, it is well known that the spindle shaped BM-MSC's will grow in tissue culture conditions and can differentiate in vitro to different cell morphologies. With additional study, we found we could force their culture forward into a series of increasingly flat cells. We also found that we could also return these flat cells back to the spindle cells in vitro by changing the culture conditions. These changes permitted multiple passages forward to flat cells and back to spindle cells. If we let these spindle cells continue to grow using the culture media we developed, these flat cells became increasingly flat and significantly enlarged. They went through morphologic changes as well as through 3 stages of flat cells as shown in the enclosed (Figure 1). The last cell morphology was a rather large flat cell that on further culture developed a number of cell surface invaginations in the cell membranes under ongoing tissue culture conditions. During these prolonged cultures, there were increasing concentrations of small, discreet particles that were determined to be mesenchymal exosomes. We also discovered a very small round cell that developed in culture over time, but has not been identified as yet. If we tried to culture these small cells in new culture media, they did poorly. But, culturing them in the conditioned media taken from the larger flat cells containing exosomes, these cells developed into newly formed spindle shaped cells that were similar to the original spindle shaped MSC's.

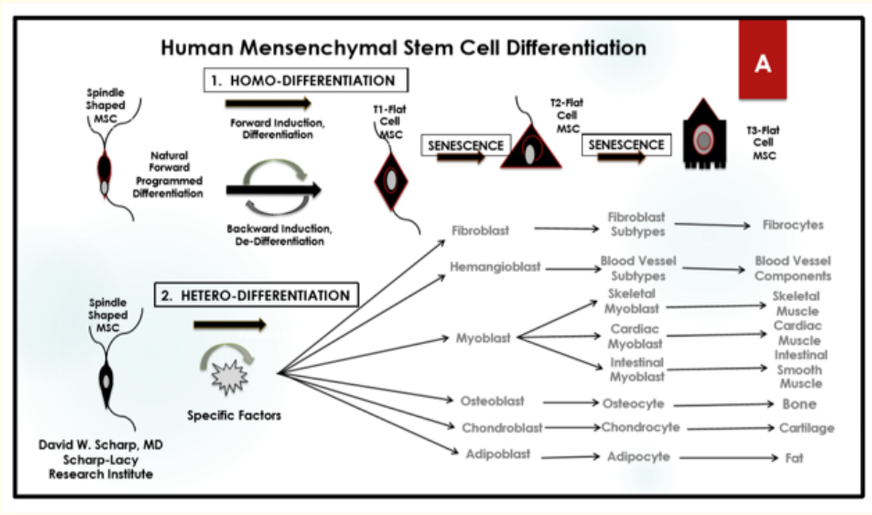

Figure 1: *Human mesenchymal stem cell differentiation potentials.*

Continuing these BM-MSC cultures permitted us to develop a potential pathway of understanding that we now term as BM-MSC Homo-Differentiation. This topic is presented in figure 2, entitled "Developing HuMSC Types". If one starts the review of this figure at the Spindle Shaped MSC's in the left/center under "Replication", one can culture these cells forward with one set of culture conditions resulting in their induction to flat cells morphologically. One can then reverse the culture conditions to drive the cells back to their original morphology. These back and forth culture conditions can be repeated several times. However, when one continues to culture in the forward direction, these flat cells increase their "flatness" and change their morphology to the large flat cells. It appears that these large flat cells are the producers of the exosomes that continue to increase in the culture media over time. The unidentified small cell types seem to also be developing into the spindle shaped MSC's when cultured in the conditioned media that originally was produced by the prolonged culture of the flat cells. Further studies are continuing to explore the potential mechanisms involved and the potential to more thoroughly study these cells and their growth responses under different culture conditions.

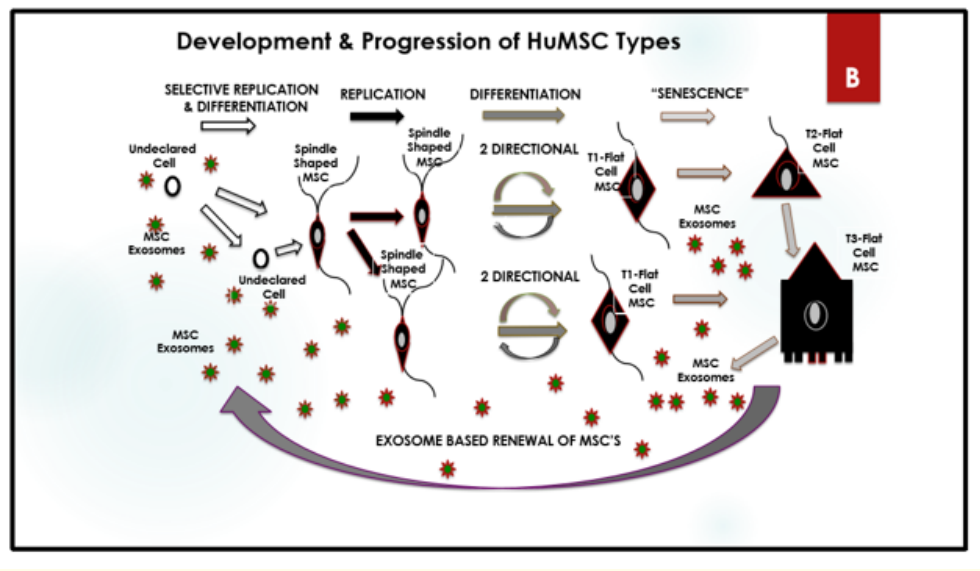

Figure 2: *The progression of human mesenchymal stem cell types and their relationships to exosome production and function.*

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