

Sonntag, David and Coombs, Jaron

Chondrogenic Differentiation of Adult Hepatic Stem Cells: Investigating Candidates for Osteoarthritis Treatment

Faculty Mentor: Laura Bridgewater, Molecular and Microbiology

Osteoarthritis is a painful disease associated with the breakdown of articular cartilage, the protective protein coating of the major joints of the body. Over 20 million Americans are affected by this pervasive disease which is associated with the overuse of joints and aging. Osteoarthritis occurs when the proteins that compose articular cartilage are degraded by repetitive use and injury. These actions can lead to swelling, tearing, or flaking off of the cartilage at the surface of the bone. When cartilage loses its integrity in this manner the amount of friction in the joint greatly increases and the patient begins to experience pain, swelling, and increasingly limited function at the affected site.

By nature cartilage is an avascular tissue with little regeneration capability; this makes osteoarthritis an extremely difficult disease to treat. Currently most treatments focus on the symptoms of the disease and little can be done to reverse the disease's progression. With the advent of stem cell research and genetic engineering osteoarthritis sufferers have been given hope that new treatments can be developed to combat this degenerative condition.

Extensive research has been performed differentiating Mesenchymal Stem Cells into Chondrocytes, the cell type that produces cartilage. Mesenchymal stem cells are adult stem cells derived from the bone marrow of humans or other organisms. Research with lines of mesenchymal cells has shown that they can be differentiated into all the different cell types of the body. Adult stem cells are very attractive to researchers because they are easily isolated and can be worked on without dealing with the ethical problems inherent with embryonic stem cells. These advantages make it important to discover new sources of adult stem cells and to define their differentiation capabilities.

Hepatic Oval cells are a line of cells that are closely related to mesenchymal stem cells. Based on cell marker studies performed by Dr. Peterson of the University of Florida it was believed that they may have differentiation capabilities similar to those of mesenchymal stem cells. It was our proposal to study these capabilities to refine the contribution that oval cells can make to future treatment developments for osteoarthritis.

During embryonic development chondrocytes are differentiated from progenitor cells by the presence of two protein growth factors TGF- β 3 and BMP-6. In vitro studies have shown that pellets of mesenchymal stem cells can be differentiated into chondrocytes if cultured in a chondrogenic media supplemented with these same growth factors. We utilized this mesenchymal chondrogenic protocol with oval stem cell pellets to determine whether our oval cells could be induced to become chondrocytes in the same manner. Originally we did not see the collagen expression typical of chondrocytes that we expected, so we decided to examine more thoroughly the mechanism of chondrogenic activation.

We learned that controlled gene expression by BMP-6 and TGF- β 3 is dependent upon membrane receptor kinases and the phosphorylation of transcriptional cofactors called SMAD proteins. These proteins form the pathway through which BMP-6 and TGF- β 3 work to turn on genes such as collagen. We hypothesized that if the essential components of these activation pathways were present in both types of stem cells that both should be capable of chondrogenic differentiation. The absence of these genes in cells like oval stem cells could be the barrier that was preventing them from differentiating into chondrocytes. These experiments would help us to define more fully the potential of adult stem cells like oval cells. Through RT-PCR we verified the presence of these receptors and cofactors in both mesenchymal and oval cells. When we observed that the cellular machinery was present for chondrogenic activation in oval cells we decided to repeat our attempt at differentiating them into chondrocytes.

Differentiation was carried out by culturing the approximately 200,000 oval cells in chondrogenic media which consists of 500 ng/ml BMP-6, 10 ng/ml TGF- β 3, 10^{-7} M dexamethasone, 50 μ g/ml ascorbate 2-phosphate, 40 μ g/ml proline, 100 μ g/ml pyruvate, and 50 mg/ml ITS + Premix. This media was changed every 2 to 3 days throughout the course of the experiment. At 0, 7, 14, and 21 days we performed RT-PCR an assay which tests for the presence of collagen, the major product of chondrocytes. We were disappointed in our several attempts and were never able to conclusively find collagen in our pellets.

The discovery of the SMAD pathway in both mesenchymal and oval cells along with our failure to find collagen in any of our oval cell pellets led us to believe that there must be other factors involved in differentiation process. We were unable to continue our investigation into the mechanisms of differentiation beyond this point, but we believe that new lines of adult stem cells must be isolated and defined in order to overcome difficult diseases like Osteoarthritis.