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Microenvironmental guidance of stem cell self renewal, differentiation and localization

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Stem cell fate decisions are influenced by the microenvironment in which the stem cell resides. One key component of such microenvironments is the mesenchymal stroma. Our studies focus on the interactions between hemopoietic stem cells and the mesenchymal stroma of the bone marrow and other tissue. Our past studies have highlighted the plasticity of the mesenchymal cell phenotype. These cells not only form the regulatory microenvironment of the bone marrow, they also constitute a remarkable pool of stem cells by themselves, in the embryo and in the adult, and can give rise to a vast spectrum of mature cell types (Figure 1). Our studies are therefore directed towards the analysis of the molecular constitution of mesenchymal stem cells (MSC) and the mechanistic basis of their plasticity. Furthermore, we examine the mode of action by which MSC affect the fate of other cells and influence the development of cancer.

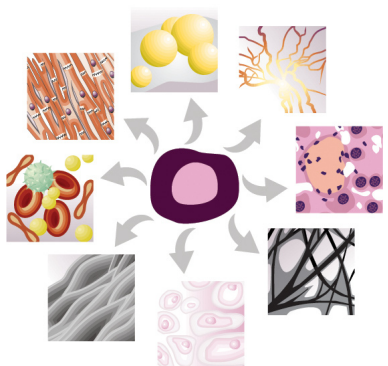

Fig. 1 *Adult stem cells are found to be highly plastic and can differentiate into unexpected lineages*

A single MSC may produce liver cells, bone, muscle, or nerve cells, to name just a few of the options. This capacity of the mesenchyme can be harnessed to create new tissues and organs. In the past 12 months we have developed culture conditions that allow us to direct the differentiation of MSC into muscle, bone, cartilage, fat and nerve cells (Figure 2). We have established a frozen cell "bank" of such highly potent cells, which are now being transplanted into animals in order to evaluate their capacity to integrate into diseased tissues. One model that we are studying concerns osteoarthritis (OA). We use MSC to re-establish a normal joint by differentiation of the MSC into cartilage and bone.

As indicated above, one major function of MSC is to produce the stromal microenvironment that supports hemopoiesis. The mechanism of this stromal cell function is not yet fully understood. We are studying the interactions of stromal cells with stem cells and the subsequent differentiation of these stem cells into maturing B lymphocytes. Based on *in vitro* studies, evidence was generated in our laboratory implicating stromal activin A, a transforming growth factor (TGF)β family member, in negative regulation of B lymphopoiesis. Activin A reduced the differentiation of bone marrow derived cells towards to B lineage. Accumulation of pre-proB and proB cells occurred and the generation of preB cells was reduced. We are now examining (a) whether activin A operates at the HSC level or downstream in the cascade and (b) how would modification of the microenvironment *in vivo*, by use of genetically manipulated mesenchymal stem cells, overexpressing different members of the TGFβ superfamily, affect B cell generation.

One immediate consequence of this study is the implication that activin A may affect not only normal B cells but also tumor B lineage cells. Multipe myeloma is a human disease of this cell lineage which is incurable to date. The pathogenesis of multiple myeloma in men shows that the tumor cells are dependent on the bone marrow mesenchymal microenvironment. This is due, in part, to interleukin (IL)-6, a survival factor for myeloma cells, produced

by the mesenchymal stroma. We previously showed that activin A is highly potent in blocking cell cycle progression and in inducing apoptotic death of myeloma cells. We further showed that activin A is an antagonist of IL-6 and thereby kills myeloma cells by depriving them of their survival signals. We propose that overexpression of activin A in mesenchymal cells, within the microenvironment of the hemopoietic organs, may lead to regression of myeloma. We noted that mouse myeloma (MM) tumors formed by RPC-5 cells in BALB/c mice, regressed spontaneously. We used bicistronic retroviral vectors for introduction of activin A and green fluorescence protein (GFP) cDNAs into mouse mesenchymal cells. We are now using a model of MM which resembles human MM in tropism to the bone marrow and in inducing bone damage, as a preclinical animal model to determine whether cell therapy using activin A modified mesenchyme may be used in humans.

Activin A is thus one mesenchymal molecule that mediates several major functions relating to the regulation of blood cell proliferation and differentiation. Some of the molecules expressed by the mesenchyme are surprisingly shared by mature cells from unexpected lineages. In a recent study performed in our laboratory, we identified a novel T cell receptor (TCR)β transcript that lacks the variable region and possesses an extra 5' sequence derived from the presumptive intronic Jβ2.6 sequence (hence intronic J2β.6). This transcript is expressed by mesenchymal cells, specifically by mouse embryonic fibroblasts (MEFs) and by stromal cell lines. Additional studies have shown that a recombinant GFP-J^{int}-Jβ2.6-Cβ2-TCR fusion protein can be overexpressed in 293T cells and in MEFs. Such overexpression leads to apoptotic death of the cells. It is not yet known what might be the role of this protein in those cells. However, our recent studies indicate that the mesenchymal TCR may be involved with cell growth regulation and tumorigenesis.

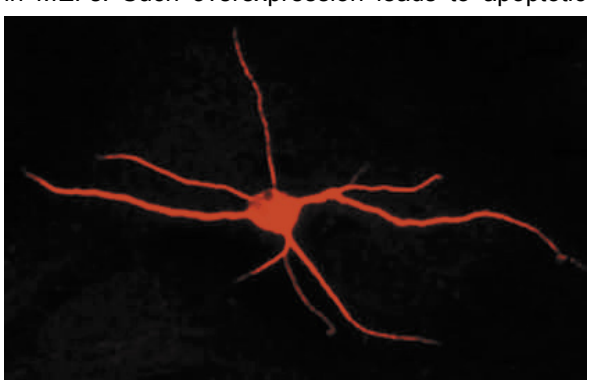

Fig. 2 *A neuronal cell derived from bone marrow mesenchyme*

Selected Publications

Zauberman, A., Lapter, S., and Zipori, D. (2001) Smad proteins suppress C/EBPβ and STAT3 mediated transcriptional activation of the haptoglobin promoter. J. Biol. Chem., 276,24719-24725.

Zipori. D., and Barda-Saad, M. (2001) Role of activin A in negative regulation of normal and tumor B lymphocytes. Leuk. Biol., 69, 867-873.

Shav-Tal, Y., Cohen, M., Lapter, S., Dye, B., Patton, JG., Vandekerckhove, J., and Zipori, D. (2001) Nuclear re-localization of the pre-mRNA splicing factor PSF during apoptosis involves hyperphosphorylation, masking of antigenic epitopes and changes in protein interactions. Mol. Biol. Cell, 12, 2328-2340.

Barda-Saad, M., Shav-Tal, Y., Rozenszajn, AL., Cohen, M., Zauberman, A., Karmazyn, A., Parameswaran, R., Schori, H., Ashush, H., Ben-Nun, A., and Zipori, D. (2002) The mesenchyme expresses T cell receptor mRNAs: relevance to cell growth control. Oncogone, 21, 2029-2036.

Shav-Tal, Y., and Zipori, D. (2002) PSF and p54(nrb)/NonO - multi-functional nuclear proteins. FEBS Lett., 531,109-114.

Shoham, T., Parameswaran, R., Shav-Tal, Y., Barda-Saad, M., and Zipori, D. (2003) Stromal activin A negatively regulats B cell lymphopoiesis. Ann. NY. Aca. Sci., 996, 245-260.

Prindull, G., and Zipori, D. (2004) Environmental guidance of normal and tumor cell plasticity: epithelial mesenchymal transitions as a paradigm. Blood, 103, in: 2892-2899.

Acknowledgements:

D.Z. is an incumbent of the Joe and Celia Weinstein professorial chair at the Weizmann Institute of Science; This work was supported by grants from the Israel Ministry of Health, the Ministry of Science Culture and Sport, and by the Minerva Stiftung; Supported by the Gabrielle Rich Center for Transplantation Biology Research, the Isabelle and Leonard Goldenson Association, Jerrald and Helene Wulff and by Daniel and Rhonda Shapiro.