VI.I Abstract

Bone marrow stromal cells (BMSC) were first identified by their adherence to plastic tissue culture dishes and termed “colony forming-unit fibroblasts” (CFU-Fs). These had the ability to differentiate into different mesenchymal cells like osteoblasts, chondrocytes and adipocytes. For that reason, BMSC are also called mesenchymal stem cells (MSCs).
In recent years several publications showed the differentiation of BMSC *in vitro* or *in vivo* into myoblasts, epithelial cells, cardiac muscle cells and neural phenotypes, i.e. BMSC differentiate even into cells of different germ layers. The fascinating capacity of BMSC to develop a neuronal phenotype *in vitro* was for example shown by Woodbury and colleagues (2000). The differentiation of BMSC towards a neuronal phenotype raises the question of the mechanisms underlying these processes. Furthermore, the exact (*in vivo*) identity of BMSC had not been known in the beginning of this study.
Therefore, gene expression analyses of murine BMSC using cDNA-microarrays were performed to establish a molecular profile of these cells and their neuronal differentiation.
Undifferentiated BMSC express genes that code for proteins of different cells like neural cells, different muscle cells, mesenchymal cell types and vascular-associated cells. Literature-analyses of the expressed genes showed a high similarity to pericytes. Pericytes are localized in the basement membrane of small vessels like capillaries. Also the morphology of BMSC and pericytes *in vitro* is very similar. To confirm a vascular-associated phenotype of BMSC *in vivo*, slices of adult murine bone marrow were marked for proteins that are expressed by BMSC *in vitro*, e.g. Cadherin 13, Vimentin, Slug and VE-Cadherin. All antibodies stained blood vessel-associated cells and therefore support the proposed pericytic nature of BMSC *in vivo*.
The neuronal differentiation of murine BMSC was validated with several antibodies against neural proteins (e.g. neurofilament-M, neuron-specific enolase, nestin, RC2, GFAP, O4). About 85% of the cells were positive for the neuronal marker NF-M after 2 days of differentiation, none of the cells expressed markers for astrocytes (GFAP) or oligodendrocytes (O4, GalC).
The time-course analysis of the neuronal differentiation of murine BMSC with cDNA-microarrays revealed gene expression changes of genes that code for proteins of for example the cytoskeleton, the extracellular matrix or components of signal transduction pathways. Many of those were already correlated to the process of neuronal differentiation.
Because of the differentially expressed gene of the transcription factor Slug, that is responsible for epithelial-mesenchymal transition (EMTs) in the development of neural crest cells, the expression of other neural crest-specific genes was analysed (Twist, Snail and Noelin). These genes are also already expressed in undifferentiated BMSC. The differential expression of Sox-8, -9 and -10, Wnt-1 and -3a, as well as Pax-3 and -6 in course of the differentiation reveals similarities of the neuronal differentiation of BMSC with processes of early neural development, i.e. the specification and differentiation of neural crest cells.
One explanation for these similarities would be that BMSC activate a transcriptional program that recapitulates early steps of neuronal development. Another possibility, which had to be proven in future, would be that BMSC are descendants of neural crest cells themselves. A neuroectodermal origin of BMSC could also explain their differentiation capacity and would raise a completely new perspective to investigate their developmental biology.