**Ruprecht-Karls-Universität Heidelberg**

**Medizinische Fakultät Mannheim**

**Dissertations-Kurzfassung**

# Differential analysis of the differentiation potential of mesenchymal stromal cells

Autor: Irena Brinkmann

Institut / Klinik: Institut für Transfusionsmedizin und Immunologie

Doktormutter: Priv.-Doz. Dr. K. Bieback

Mesenchymal Stromal Cells (MSC) have a therapeutic effect in many applications in regenerative medicine due to their differentiation potential, immunomodulatory capacities and provision of a pro-regenerative milieu. The broad differentiation potential, especially into the mesodermal lineage, permits the use in tissue engineering. Moreover, MSC are immunomodulatory, anti-apoptotic, anti-fibrinolytic and pro-angiogenic enabling them for the application in several areas like fibrosis, organ transplantation or inflammatory diseases. Many tissues can be used to isolate MSC, including bone marrow (BM), adipose and perinatal tissues. To identify the best source for therapeutic application, characterization of these cells is required, including their migration, proliferation and differentiation potential and the disparities among MSC from different tissues. The main part of this study focuses on the analysis of the differentiation potential of cord blood (CB)-, BM- and adipose tissue (AT)-MSC including the development of a non-invasive method permitting online monitoring of differentiation. Another part of this study concentrates on the adhesion capacity to laminin of AT-MSC cultivated in cell culture medium supplemented with fetal calf serum (FCS) and with pooled blood group AB human serum (HS).

**Molecular analysis of the differentiation potential of CB-, BM- and AT-MSC**

In our laboratory, we isolate human MSC from BM, AT and CB exhibiting differences in their differentiation potential: CB derived MSC fail to differentiate into adipocytes in contrast to BM- and AT-MSC. This has been shown by immunohistchemical staining and gene expression analysis of adipogenic associated genes. Due to gene expression profile, Preadipocyte factor-1 (Pref-1, DLK-1, FA-1) has been identified as possible inhibitor responsible for the impaired adipogenic differentiation potential of CB-MSC. This presumption has been encouraged by the inhibitory effect of cord blood plasma, containing high concentrations of Pref-1, on the adipogenic differentiation of AT-MSC. Our findings suggest an early influence of Pref-1 on CB-MSC from their original environment with a long term effect on their adipogenic differentiation potential. Probably, after further examination of contents and action of CB plasma, this could be used for treatment of obesity. For cell therapeutic applications, CB-MSC are not recommend in adipose tissue regeneration, but are attractive candidates for treatment of bone fractures.

**Electrochemical impedance sensing for the analysis of differentiation potential**

As application of MSC in tissue regeneration requires standardized quality control assays, impedance sensing has been tested to discriminate adipogenic and osteogenic differentiation of MSC. So far, analysis of the differentiation potential is performed using histochemical methods, gene expression profiling or protein expression analysis. All these methods evaluate the differentiation potential at the endpoint but do not indicate the differentiation process. Within this study, we demonstrated impedance sensing as a method to non-invasively monitor adipogenic and osteogenic differentiation. This method enables real time analysis of differentiation that indicates differences already after a few hours.

**Adhesion capacity of HS and FCS cultivated AT-MSC**

For therapeutic application of MSC, alternatives to FCS as supplement for expansion medium are necessary, due it's critically discussed applicability in human therapy. Primary results of the group have detected differences in the expression of adhesion molecules in AT-MSC cultivated in FCS and the alternative supplement composed of pooled blood group AB human serum (HS). This part of the study concentrated on the adhesion capacity of MSC to laminin, as this is important for engraftment in

the body and thereby for the therapeutic efficacy. Integrin α6 (CD49f) is described to form complexes with integrin β1 (CD29) and β4 (CD104) mediating adhesion to laminin. We could show that functional blockade of CD49f and CD29 leads to a clearly reduced adhesion to laminin, whereas CD104 seems not to be involved in this process. CD49f and CD104 expression is reduced with higher confluence of the cells. Furthermore, our results reveal donor dependant variability in the expression of CD49f without correspondence to the supplement. These data evidence no differences between AT-MSC cultured in FCS or HS regarding their adhesion capacity to laminin, recommending HS as an appropriate alternative supplement for the expansion of MSC.