Defective chondrogenic differentiation of murine embryonic stem cells treated with RGD-containing peptides

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The interplay between cells and their extracellular matrix (ECM) is of utmost importance in tissues like cartilage where cells are completely surrounded by ECM and cell-cell contacts thus take a minor role. A large number of these interactions are mediated by members of the integrin family. Chondrogenic development has been characterized in detail by using numerous model systems. Of these, murine embryonic stem cells (ESC) are of special importance because they are widely available without ethical concerns and because they render a large portion of animal experiments unnecessary. It has been proven that all physiologic stages of chondrogenic development are adequately mimicked by ESC in vitro. Since we found high expressions of the ECM molecule fibronectin (FN) and its major cellular receptor in cartilage, α5β1-integrin, during early chondrogenic development, namely during the formation of mesenchymal condensations that require active cell migration, we treated murine ESC either with RGD-containing blocking peptides mimicking the cell attachment domain of FN or RGE-containing control peptides to test the importance of FN-α5β1-integrin-interactions. RT-PCR analysis of α5 and FN-expression showed significant alterations demonstrating activity of the RGD-containing peptides. As shown by Alcian blue staining, the formation of chondrogenic nodules was significantly reduced although the number of PNA-positive cellular condensations and Collagen II-positive nodules remained unchanged. Confocal laser scanning microscopy did not show morphological differences between cells treated with blocking peptides and cells treated with control peptides. In addition we performed a phosphorylation-sensitive western blot to analyze Focal Adhesion Kinase (FAK)-activity as an integrin downstream signaling target. Taken together our results suggest that interactions between FN and α5β1-integrin play an important role during early chondrogenic differentiation stages of murine ESC that is independent from the formation of mesenchymal condensations.