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Introduction: Bone Marrow derived mesenchymal stem cells (bMSCs) can be differentiated into chondrocyte-like cells, but the true cell function and biochemistry of the resulting cell population is poorly understood. We hypothesize that, due to higher phenotypic flexibility, this cell population will readily respond to hormonal stimuli triggering terminal differentiation.

Testing dexamethasone (dex) and triiodthyronine (T3) as in-vitro inductors of endochondral ossification, we show the potential of terminal differentiation in chondrogenically differentiated bMSCs, as well as the significant difference to the response of true primary chondrocytes submitted to the same protocol.

Materials and Methods: bMSCs were isolated from the bone marrow of two calves , expanded in monolayer, then encapsulated in alginate beads and submitted to a standard chondrogenic medium containing 10 ng/ml TGFß1 and 100 nM dex for three weeks. Treatment groups then received either serum free medium containing 1 µM dex or 100nM T3, or 10% FCS as control, for a culture period of 5 weeks. Histological sections as well as biochemical assays for DNA and glycosaminoglycan (GAG) content and activity of alkaline phosphatise (ALP) and were completed and mRNA expression of collagen types I, II and X was monitored using real time RT-PCR. Data was compared between groups, over time and to results of primary chondrocytes submitted to the same protocol.

Results: During chondrogenic differentiation, levels of collagen type II mRNA increased almost five orders of magnitude, while collagen type I mRNA remained unchanged. There was an increase in both DNA and GAG content. Collagen type X mRNA already showed some increase of one to two orders of magnitude, consistent with raising activity of ALP.

During treatment, dex group showed superior cell survival and total GAG production compared to control and T3 group. PCR revealed decreasing expression of collagen type I mRNA in the dex group, in contrast to increasing levels in control and T3-treated group, as well as high collagen type II expression, while in T3 and control group it declined. Dex also induced a high expression of collagen type X mRNA, increased by three orders of magnitude and accompanied by the highest activity of ALP.

Discussion: After three weeks of differentiation, collagen type II mRNA levels and total GAG reached amounts comparable to those found in primary chondrocytes in alginate culture. However, collagen type I mRNA remained high, most likely related to the monolayer expansion period of the stem cells before encapsulation. This, together with the rising levels of collagen type X mRNA suggests a less stable chondrocyte-like phenotype.

In the next step, we could demonstrate that dexamethasone enhances the chondrogenic phenotype by increasing GAG production and further increasing the collagen type II/I ratio in favour of collagen II. In means of terminal differentiation, only dex showed a positive effect on the cells, marked by an increase of collagen type X mRNA and ALP activity. Primary chondrocytes in the same setting, as shown in a study recently submitted for publication by the authors, would respond to T3 instead, being more delayed and less pronounced in their response.

In summary, we could demonstrate a significant influence of dex on chondrocyte like cells in means of chondrogenic as well as terminal differentiation, while serum medium or T3 failed to show such effects. Also we could prove a quicker and more distinct answer of the differentiated stem cells to the hormonal stimuli than found in primary chondrocytes, indicating their readiness to change their phenotype even after differentiation, an ability that can be of use for future bone tissue engineering applications or models of endochondral ossification.