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The mineralization of the extracellular matrix during the osteogenic differentiation of mesenchymal stem cells correlates with increased levels of human xylosyltransferase I

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Introduction:

Mesenchymal stem cells (MSCs) are multipotent adult stem cells capable to differentiate into osteoblasts. Therefore, they represent attractive cell sources for tissue engineering applications, especially for bone replacement. Proteoglycans (PGs) exhibit a crucial role for matrix assembly and remodeling. Nevertheless, since bone development is a highly dynamic and complex process, the regulation of the extracellular matrix (ECM) formation remains elusive. Consequently, the aim of this study was to investigate the mRNA expression levels of genes involved in PG assembly. Hence, we analyzed mRNA and protein expressions of crucial PG core proteins and key enzymes involved in glycosaminoglycan (GAG) biosynthesis in different stages of osteogenesis.

Materials and Methods:

Human MSCs were induced to differentiate into osteoblasts in appropriate medium. The

progress of osteogenesis was confirmed using histological stainings. The mRNA levels of osteogenic marker genes as well as the investigated target genes were analyzed by real-time RT-PCR. The xylosyltransferase activity was monitored in the culture supernatant.

Results:

For one of the rate-limiting enzymes in GAG biosynthesis xylosyltransferase I (XT-I), maximal mRNA expression levels (3.89 ± 0.83-fold increase) and elevated enzyme activities (285 ± 17 dpm/μg DNA) were observed 10 days after osteogenic induction, simultaneously to the beginning mineralization of the ECM, whereas the highly homologous protein XT-II showed no specific alterations. The differential expression of chondroitin sulfate, dermatan sulfate and heparan sulfate chains were determined by analyzing the mRNA expression of EXTL2 (α -1,4-*N*-acetylhexosaminyltransferase), GalNAcT (β -1,4-*N*-acetylgalactosaminyltransferase), and GlcAC5E (glucuronyl C5-epimerase) as they

represent crucial enzymes in GAG biosynthesis. Besides GlcAC5E, all key enzymes showed upregulated mRNA contents (up to 3.6-fold) around day 10. Except for decorin, which exhibited heightened mRNA levels even in the early stages of osteogenesis, we found similar upregulated mRNA contents (up to 14.6-fold) for all investigated PG core proteins.

Discussion and Conclusions:

The tight synchronized expression profiles demonstrate the coordinated biosynthesis of the PGs and underline their importance for the formation of bone and for the osteogenic stem cell differentiation. Furthermore our results indicate that prominent assembly and remodeling processes occur especially in later stages of osteogenesis and in parallel to the mineralization of the ECM.

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