

GENETIC CONTROL ON OSTEOBLAST DIFFERENTIATION: A GENE EXPRESSION ANALYSIS

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INTRODUCTION: Osteogenesis is an extremely complex process. At a molecular level, each factor can induce different responses activating multiple signalling and transcription factors with different biological effects.

We analyzed MSC (mesenchymal stromal cell) gene expression profile during osteogenic induction to investigate “critical” steps to osteoblast differentiation and mineralization.

METHODS: Bone marrow aspirates were collected from the femur during surgery in 4 male patients, without genetic or neoplastic diseases. A MSC culture schedule was defined to pinpoint relevant steps for genetic control of bone cell differentiation. mRNA was extracted before adding ascorbate and dexamethasone as differentiating agents (MD1), after 24h (MD2), at semiconfluence (MD3), at semiconfluent ‘Colony Forming Units’ (MD5), at full confluence before adding b-glycerophosphate mineralizing medium (MD4), 24 h after addition of mineralizing medium (MM1), 7 days later (MM2) and 14 days later (MM3), when mineral nodules were seen. Transcriptome analysis was performed on Human Genome U133 Plus 2.0 Array (Affymetrix GeneChip® Array). Differential expression of MD2/3/5 versus MD1, and MM1/2/3 versus MD4 were evaluated using bioinformatic analysis and 11,256 ‘probe sets’ with paired T test p value < 0.05 were selected.

RESULTS: Our analysis focused on upregulated genes with a biological function relevant to osteogenesis, such as cell communication, morphological and skeletal development, Wnt signalling, TGF-beta signalling, angiogenesis, cell cycle and apoptosis.

We selected 217 genes: 69 acting in well recognized pathways, 103 reported on literature as relevant to skeletal development, and 45 with functions not described in bone cells.

In early stage (MD2) of differentiation we observed genes mostly belonging to cell cycle pathway, while in further stages (MD3-MD5) the expression of growth factor-signalling pathway, bone related genes and adhesion

molecules, was gradually increased. Genes typical of angiogenesis and morphogenesis were upregulated in final steps of mineralization (MM2, MM3), suggesting a role of mature osteoblast in growth of other tissues involved in bone development.

DISCUSSION & CONCLUSIONS: Our results allow to design a gene expression profile of adult MSC during specific steps toward osteoblast differentiation, and to distinguish the genetic control of differentiation from mineralization. These informations can contribute to identify and clarify roles of new genes involved in osteogenesis regulation. Moreover, from a better understanding of molecular mechanisms of bone cells, molecular defects that hamper bone formation might be recognized.

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