cells with extracellular matrix. Integrin α2β1 is one of four collagen-binding integrins and an important receptor for collagen I in bone tissue. Our group showed that integrin α2β1 deficiency in mice enhances the expression of collagen I and accelerates fracture healing. We will identify the basic mechanisms by which integrin α2β1 influences the bone metabolism.

Based on the well characterized roles of members of the TGFb family in collagen deposition and tissue repair, we investigated TGF-β1 and BMP-2 and their SMAD signaling cascades as these are prominent factors in the regulation of collagen gene expression and mineralization of bone tissue. By immunofluorescence staining and western blot analysis we identified the localization and level of phospoSMAD2,3 *in-vitro* in wild type (WT) and integrin α2-deficient ($ITGA2^{(-/-)}$) cells.

The western blot analysis showed an elevated basal level of phosphoSMAD2,3 in $ITGA2^{(-/-)}$ cells. Additionally, we detected different localization of phosphoSMAD2,3 in $ITGA2^{(-/-)}$ and WT cells. In WT cells signals for phosphoSMAD2,3 were detected in the cytosol, at the nucleus and ER as well as at the cell membrane. In the $ITGA2^{(-/-)}$ cells most of the signal was located at the ER and nucleus where phoshoSMAD2,3 binds to the promotor region of collagen type I and activates gene expression.

We could show that the loss of integrin α2β1 regulates collagen gene expression by increasing phosphorylation of SMAD2,3. Therefore, we hypothesize that integrin α2β1 influences collagen synthesis and the mineralization process by modulating the activity of members of the TGF-β superfamily and can be considered as therapeutic application for dysfunctions in the bone metabolism like non-union fractures.

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P097

Osteogenic effect of the association of VEGF-A and BMP-9 on mesenchymal stem cells

Helena Lopes, Maria Paula Gomes, Julia Lima, Georgia Quiles, Gabriela Totoli, Gileade Freitas, Marcio Beloti, Adalberto Rosa
Bone Research Lab, University of São Paulo - School of Dentistry of Ribeirão Preto, Ribeirão Preto, Brazil

Mesenchymal stem cells (MSCs) can differentiate into several cell types, including osteoblasts. The bone morphogenetic protein 9 (BMP-9) and vascular endothelial growth factor A (VEGF-A) play a role in osteogenesis, both increasing osteoblast differentiation of MSCs. However, the effect of combining BMP-9 and VEGF-A on osteogenesis is unknown. Thus, the aim of this study was to evaluate the *in vitro* effect of the association of VEGF-A and BMP-9 on the osteogenic potential of MSCs derived from bone marrow (BM-MSCs) and adipose tissue (AT-MSCs). All animal procedures were approved by the Local Committee of Ethics in Animal Research. Firstly, we determined that 100 ng/mL of VEGF-A and BMP-9 is the most effective concentration to induce osteoblast differentiation of MSCs. Then, BM-MSCs and AT-MSCs from male Wistar rats were grown in culture medium supplemented with VEGF-A (100 ng/mL), BMP-9 (100 ng/mL) and VEGF-A + BMP-9 (100 ng/mL of both) for 7 days. Cells grown in non-supplemented medium were used as controls. The gene expression of osteoblastic markers (n=3) and alkaline phosphatase (ALP) activity (n=5) were evaluated and the data were compared by One-Way ANOVA and Tukey's test ($p< 0.05$). In general, VEGF-A did not induce osteoblast differentiation while BMP-9 alone and BMP-9+VEGF-A enhanced this differentiation with the later exhibiting the most pronounced effect (Fig. 1). The association of BMP-9 and VEGF-A increased the osteoblast differentiation of MSCs from both sources; however, this effect was more intense on AT-MSCs suggesting that these cells are more responsive to such combination of growth factors.

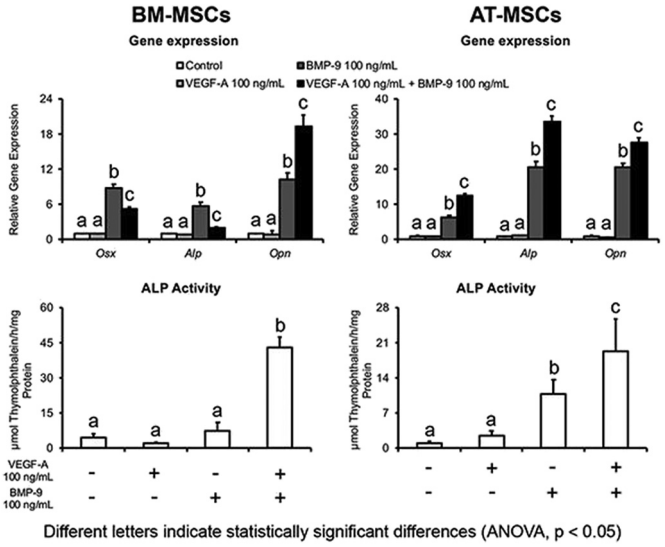

Fig. 1. Osteoblast differentiation of BM-MSCs and AT-MSCs induced by VEGF-A and BMP-9.

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P098

Serotonin promotes osteogenic differentiation of MC3T3-E1 cells

YuRi Song[a], Si Yeong Kim[a], Hyun Ah Lee[a], Jeong-Hwa Baek[b], Hyun Jeong Kim[c], Hee Sam Na[a], Jin Chung[a]
[a]*Department of Oral Microbiology, Pusan National University School of Dentistry, Yangsan-si, Republic of Korea*
[b]*Department of Molecular Genetics, School of Dentistry, Seoul National University, Seoul, Republic of Korea*
[c]*Department of Dental Anesthesiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea*

Background: Periodontitis is characterized with structural bone destruction of periodontium. Bone resorption was caused by the imbalance of bone homeostatis by osteoclast and osteoblast. Serotonin (SER; 5-hydroxytrypamine [5-HT]), a neurotransmitter involved in mood control, is involved in bone metabolism. However, the effect of serotonin on bone metabolism is still controveral and remains to be elucidated. Thus, the aim of this study was to investigate the effects of serotonin on osteoblastogenesis.

Method: To examine the effect of serotonin on cellular proliferation, MC3T3-E1 cells were treated with serotonin and MTT assay was performed. To evaluate whether serotonin promotes the osteogenic differentiation, the expression of RUNX2, osteocalcin (OCN), RANKL and osteoprotegerin (OPG) was observed by real-time PCR. The effect on mineralization was assessed by the alkaline phosphatase (ALP) acitivity assay.

Results: Serotonin treatment increased MC3T3-E1 cell proliferation until 3 days to 5 days. The mRNA expression of RUNX2 was increased after 72 hour culture with serotonin. In addition, serotonin enhanced the mRNA expression of OPG and RANKL. Serotonin increased OPG mRNA expression more than that of RANKL and then, it upregulated the OPG/RANKL ratio. Furthermore, serotonin enhanced osteogenic mineralization with ALP mRNA expression of and ALP activitiy.