Investigation of the Mechanism of the Gene Regulation of OPG (Osteoprotegerin) by Cx43

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The main objective is to determine whether the gene regulation of OPG, osteoprotegerin, by Cx43, is at the promoter level. In a recent project, it was found that deletion in Cx43 from osteocytes resulted in a decreased OPG expression. Furthermore, it was found that deletion of Cx43 from osteocytes resulted in enhanced osteoclast differentiation. Osteoprotegerin (OPG), an osteoblast-secreted decoy receptor that modulates osteoclast formation could be directly controlled by Cx43 at the promoter binding sites of p53 and Sp1. Cx43 and OPG in turn are widely up regulated by Wnt, lipid-modified signaling proteins that influence cell proliferation, differentiation, and survival. The activation of Wnt signaling results in the binding of the transcription factor Tcf in gene promoters, which leads to increased gene expression. The investigation was carried out using reporter constructs in which the activation of the promoter resulted in the transcription of the enzyme luciferase. Luciferase activity, in turn, can be measured using a commercially available substrate that emits luminescence when luciferase is present. OPG-Luc and Tcf-Luc were grown in *E. coli* and purified using a kit from Qiagen. Transfection of OPG 1 and Tcf-Luc reporter constructs on MLO-Y4 osteocyte cells deleted for Cx43 (Cx43shRNA) and Cx37 (Cx37shRNA) was conducted after seeding of the cells a day in advance. For each cell line, regular and Lithium Chloride (to mimic the effects of Wnt) induced medium was used, and cells were cultured for 24h. From the assay, it was deemed that luciferase activity was higher in Wnt induced cells. OPG is a target of Wnt signaling downstream of the transcription factor Tcf. We therefore also measure Tcf-mediated transcription using a Tcf-luciferase construct. Expression of OPG-Luc and Tcf-Luc was higher in cell lines that are not silenced for Cx43 and Cx37. According to ANOVA test, the results did reach statistical significance. However, future trials will be conducted to mimic the results.

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