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RAGE dependent regulation of PPARα in bone metabolism

Promotionsfach: Innere Medizin
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The physiological homeostasis of the bone including the regulation of bone remodeling depends on the proper crosstalk between the immune system and bone cells. Prolonged inflammation derived from chronic diseases such as diabetes is known to inhibit bone forming activity of osteoblasts. RAGE, the receptor for advanced glycation end products (AGEs), has been shown to play an important role in chronic inflammatory processes. RAGE sustains activation of the proinflammatory transcription factor NFκB and thereby converts transient proinflammatory responses into perpetuated cellular dysfunction. Since RAGE expression itself is also up-regulated by NFκB, RAGE has been implicated in sustained inflammation-promoted bone destruction in diabetes, osteoporosis or peridontitis. The physiological role of RAGE in bone tissue, however, has not yet been deciphered. Unexpectedly, pilot experiments in the laboratory of the scientific supervisor Prof. Dr. Bierhaus have demonstrated that RAGE deficiency induces a proinflammatory phenotype in bones and osteoblasts of $RAGE^{-/-}$-mice. This proinflammatory phenotype is associated with reduced protein and gene expression and intranuclear translocation of the anti-inflammatory nuclear receptor PPARα indicating that RAGE might directly regulate PPARα expression and/or nuclear translocation and thereby controls inflammation. Therefore, the aim of this thesis was to study and characterize the molecular mechanisms linking RAGE and PPARα and to provide an explanation for the proinflammatory phenotype observed in RAGE deficient osteoblasts. Primary osteoblasts were isolated from wildtype and $RAGE^{-/-}$-mice and transiently transfected with PPARα promoter plasmids (generated and characterized in the laboratory of the supervisor) to analyze the regulation the PPARα promoter in the presence and absence of RAGE. The PPARα promoter-driven-reporter gene expression in $RAGE^{-/-}$ osteoblasts was compared with the expression in wildtype osteoblasts showing impaired PPARα gene expression at mRNA level, down regulation of PPARα expression at protein level and sparsely immunostaining of PPARα in bone sections in $RAGE^{-/-}$ mice as compared to the wildtype. Additionally Electrophoretic Mobility Shift Assay (EMSA) data showed reduced nuclear translocation of PPARα in the bone and osteoblasts derived from $RAGE^{-/-}$ mice. These data adumbrate a strong correlation between the absence of RAGE and the impaired PPARα gene expression and subsequent decreased PPARα protein biosynthesis. Moreover our PPARα-promoter activity experiments in $RAGE^{-/-}$ osteoblasts showed marginal differences among the different human PPARα-promoter plasmids but a reduced activity compared to the full-length murine PPARα promoter plasmid. This implies that RAGE regulates PPARα promoter activity through certain transcription factor binding sites located on the PPARα promoter. Furthermore, we performed sequential promoter deletions that identified transcription factor binding sites that are involved in the modulation mechanism of transcription factor binding to the PPARα

promoter in the presence of RAGE deficiency. Finally, PPARα transcription and expression was monitored in response to over-expression of the identified transcription factor Sp1 to define qualitative and/or functional defects of transcription factor recruitment to the PPARα promoter upon RAGE deficiency. Our results show that Sp1 over-expression significantly enhances, whereas co-transfection with siRNA to Sp1 significantly down-regulates mPPARα promoter activity in wildtype osteoblasts compared to the endogenous Sp1 level in untreated wildtype osteoblasts. This confirmed that the Sp1 site is functional. Based on these data we surmised that the regulation of PPARα promoter activity and the consequential PPARα gene expression are Sp1 dependant in wildtype osteoblasts. Additional studies were performed showing that fenofibrate, a synthetic PPARα agonist, can restore PPARα expression and reduce inflammation in $RAGE^{-/-}$osteoblasts. We demonstrated that PPARα induction with WY14643 in $RAGE^{-/-}$ osteoblasts up-regulates PPARα promoter activity. In addition we were able to show that WY14643 increases PPARα protein expression by two-folds and also improves PPARα DNA binding activity. This thesis outlines the important role of RAGE dependent regulation of PPARα in bone metabolism and concludes that PPARα promoter regulation is Sp1-dependant. According to this and in terms of a therapeutically approach our data indicates that the systemical inhibition of RAGE by sRAGE or other small RAGE-molecules, rather leads to sustained inflammation in bone than arresting it.

In this context our results imply that the transcription factor Sp1 and PPARα might represent novel target structures for therapeutic intervention in bone disease and chronic inflammation. Nevertheless, the functional role of PPARα, and thus its therapeutic potential as a drug target in the treatment of impaired bone due to chronic inflammation, needs to be further evaluated in vitro and subsequently in clinical trails.