ABSTRACT

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Title of Doctoral Thesis **Regulation of Human Carbonyl Reductase 3 (CBR3) Expression**

The regulation of human carbonyl reductase 3 (CBR3) expression has been complete mystery until recently and is still not well understood. Because the transcriptional regulation of a gene is closely related to the function of encoded protein, the elucidation of the regulation of CBR3 might help to understand its physiological role which has not been elucidated up to the present. The promoter of CBR3 has been described in 2009. The CBR3 promoter contains several putative binding sites for various transcription factors. In 2010, we have shown that CBR3 is regulated via the Nrf2/ARE signaling pathway. This was the first study about the transcriptional regulation of CBR3. The involvement of Nrf2 in the regulation of CBR3 has been recently confirmed by another research group.

The functional antioxidant response element (ARE) is located at 2698 bp upstream of the translation initiation codon of CBR3 (−2698ARE). However, the analysis of CBR3 promoter encompassing 2500 bp indicated the presence of cis regulatory upstream element in sequence between 2500 bp and 500 bp upstream the initiation codon. Thus, another response element than −2698ARE appears to contribute to CBR3 regulation. The existence of putative NFκB binding sites in the promoter region of the CBR3 gene indicates that CBR3 may be a target gene of the NFκB signaling pathway. This hypothesis is further supported by data obtained from microarray analyses that show the up regulation of CBR3 mRNA in pro-inflammatory environments.

The theoretical part of this thesis summarizes current state of understanding of human CBRs. The aim of the experimental part was to investigate the possible involvement of the NFκB pathway in the transcriptional regulation of CBR3. We have studied the effect of NFκB activation and NFκB inhibition on the mRNA level by means of RT-PCR. After that, the effect of NFκB activation has been evaluated on the protein level using western blot analysis. The last aim was to examine the functional impact of putative NFκB binding sites in mediating the transcriptional regulation of CBR3.

We showed that CBR3 mRNA expression is inducible in the human cancer cell lines HT 29 and HepG2 by NFκB dependent mechanism. Treatment with the NFκB activators TNF α in HT 29 cells and with IL 1β in HepG2 cells regulated the expression of CBR3 mRNA in a time- and concentration-dependent manner. The HT 29 and HepG2 cell lines responded differently with respect to both the degree of CBR3 mRNA inducibility and the kinetics of CBR3 mRNA expression pointing a cell-specific regulation of the CBR3 transcription. In addition, we proved by means of vector-based overexpression of the NFκB subunits p65 and p50 that the inducing effect on CBR3 mRNA level is mediated via NFκB pathway.

An interesting finding of this work was that the activation of the NFκB pathway clearly enhanced the CBR3 mRNA level, but this effect did not correlate with protein expression. In HT-29 cells, only marginal changes in the CBR3 protein expression were detected after treatment with TNF α at various concentrations and for different times. Furthermore, the overexpression of NFκB subunits p65 and p50 enhanced the expression of CBR3 protein only slightly. However, it has frequently been observed that mRNA and protein levels of a certain gene do not correlate. It can be hypothesized that miRNA-based regulation of CBR3 is responsible for lack of CBR3 up-regulation on protein level.

In the last part of the experimental work, we pinpointed areas on the CBR3 promoter that may regulate the transcription of CBR3. It was demonstrated that the −1160NFκB binding site may be predominantly responsible for the constitutive activity of the CBR3 promoter construct in HepG2 cells. Furthermore, the −1160NFκB and −593NFκB binding sites may act as bona fide functional elements to activate NFκB mediated gene transcription in the presence of NFκB activators. In conclusion, we provide for the first time

clear evidence that the NFκB signaling pathway is involved in the regulation of the CBR3 gene.