Specific regulation of IL-8 and p53 promoters by NF-κB in HepG2 cells

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ABSTRACT

The purpose of the present study was to elucidate the role of the Nuclear Factor kappa B (NF-κB) subunits p50 and p65 in regulating on one hand the pro-inflammatory genes represented by interleukin-8 (IL-8), and on the other hand the anti-inflammatory and proapoptotic genes represented by p53.

The present disertation showed that IL-1β and tumor necrosis factor alpha (TNF-α) in both normal cultured human keratinocytes and HepG2 cells induced an nuclear expression of NF-κB dimers, which also by others have been shown to by dysregulated in involved psoriatic skin. It was demonstrated that the HepG2 cell line, due to its intact NF-κB system and its ability to synthesize pro-inflammatory cytokines like IL-8, is a good model for studying NF-κB activation in the inflammatory response.

By relating our results with the results from other groups, we were able to propose a model for the regulation of NF-κB during an inflammatory response in HepG2 cells. Our results confirmed that IL-8 expression is closely related to the nuclear expression of the NF-κB p65:p65 and p65:p50 dimers.

During the IL-1β induced inflammatory response the induced NF-κB binding to the p53 κB motif did not affect the expression of total p53, suggesting that p53 is not regulated by the canonical NF-κB pathway. Interestingly, a knock down of cellular p65, but not of p50, induced an increase of total p53, demonstrating that p53 was dependent on the levels of the NF-κB p65 subunit in HepG2 cells, even though that total p53 was independent of the IL-1β induced NF-κB activation.

Experiments showed an IL-10 induced inhibition of NF-κB after two or more repeated stimulations of HepG2 cells. Despite of this, the results only documented a weak inhibition of the IL-8 expression, and an unaffected total p53 protein expression.

Reporter plasmid experiments on keratinocytes and HepG2 cells indicated that another transcription factor, NFAT-1 was implicated in the transcription of the IL-8 gene in keratinocytes, but not in HepG2 cells. Leading to the hypothesis that NFAT-1 might be a key factor in chronic inflammatory skin deseases.

This thesis was written while a lot of questions were still unanswered. Especially we would prefer to conduct more siRNA experiments to confirm presented effects of reducing the p50 snf p65 subunits on the level of nuclear import of NF-κB and binding of dimers to the κB motifs. Monitoring the p53 transcriptional activity, by using a reporter gene assay with a construct containing the strictly p53 binding response element from the MDM2 promotor instead of measuring total p53, might be a better way to evaluate the p53 expression and activity.

The present study might in the future prove useful to explain the role of NF-κB in primary epidermal keratinocytes during the inflammatory response, and in chronic inflammatory skin diseases.