

# Transcriptional Regulation of the Platelet-Derived Growth Factor $\beta$ -receptor by p53 Family Members

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## ABSTRACT

**Aims:** The platelet-derived growth factor  $\beta$ -receptor (PDGFRB) is critically involved in embryonic development and has a role in many diseases. In cells, signaling through PDGFRB affects growth, migration and death. The role of PDGFRB in these crucial processes necessitates strict regulation and therefore it is regulated in many ways, including transcription, dephosphorylation, internalization, and degradation. Of these, transcriptional regulation is the least studied. Previously, PDGFRB transcription has been shown to be under the control of the transcription factors nuclear factor  $\gamma$  (NF- $\gamma$ ), specificity protein 1 (Sp1) and the p53 family member p73. In the present thesis we investigated the role of p53 family members and their mechanisms for transcriptional regulation of PDGFRB.

**Results:** In search for the mechanism behind p73 $\alpha$ -mediated repression of the PDGFRB, we found that p73 $\alpha$  competed with histone acetyltransferases for binding to NF- $\gamma$ . The recruitment of p73 $\alpha$  and  $\Delta$ Np73 to the PDGFRB promoter corresponded with PDGFRB expression. In repression of the PDGFRB promoter, p73 was recruited with the co-repressor HDAC1. Binding of  $\Delta$ Np73 and the co-activator p300, on the other hand, corresponded to PDGFRB promoter induction.

Overexpression of the p53 interacting viral large T antigen (LT) in NIH3T3 fibroblasts resulted in repressed PDGFRB promoter activity and decreased expression of PDGFRB protein and mRNA. The same type of overexpression in c-Myc<sup>-/-</sup> HO15.19 fibroblasts, Rb<sup>-/-</sup> NIH3T3, and pRb- and p53-lacking osteosarcoma, Saos2, did not repress PDGFRB promoter activity, showing the importance of these molecules for LT-mediated repression of PDGFRB.

In order to identify the role of p53, we overexpressed p53 in mouse embryonic fibroblasts (MEF), p53<sup>-/-</sup> MEF, and Saos2, which induced repression of PDGFRB promoter activity and decreased mRNA and protein expression. Endogenous p53 activated by mitomycin treatment also downregulated PDGFRB expression. Experiments showed that p53 could bind the PDGFRB promoter region surrounding the CCAAT-motif. Upon p53 induction, when PDGFRB expression was repressed, p53 and HDAC1 bound the PDGFRB promoter and the co-activator p300 was dismissed.

The role of  $\Delta$ Np73 in PDGFRB expression was investigated using the neuroblastoma cell line IMR-32 which had dysregulated PDGFRB expression and SH-SY5Y which had regulated expression. Silencing of  $\Delta$ Np73 repressed PDGFRB promoter activity and protein expression in IMR-32 but not in SH-SY5Y and  $\Delta$ Np73 was constitutively bound to the PDGFRB promoter only in IMR-32. Treatment with the anticancer drug cisplatin decreased PDGFRB protein, mRNA and promoter activity in both cell lines. In IMR-32, cisplatin was found to dismiss  $\Delta$ Np73 and p300 from the PDGFRB promoter and recruit HDAC4.

**Conclusions:** Results presented in this thesis suggest a role for p53 family members in downregulation of PDGFRB expression upon growth stimulation or in response to DNA damage. In addition, we demonstrated that  $\Delta$ Np73 have a role in dysregulated PDGFRB expression. Also, we propose a potential for tyrosine kinase inhibitors in the treatment of neuroblastoma with high  $\Delta$ Np73 and PDGFR expression.

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- I. Uramoto, H., **Wetterskog, D.**, Hackzell, A., Matsumoto, Y., Funa, K. p73 competes with co-activators and recruits histone deacetylase to NF-Y in the repression of PDGF  $\beta$ -receptor.  
*Journal of Cell Science* 117 (2004) 5323-5331.
- II. Uramoto, H., Hackzell, A\*, **Wetterskog, D\***, Ballagi, A., Izumi, H., Funa, K. pRb, Myc and p53 are critically involved in SV40 large T antigen repression of PDGF  $\beta$ -receptor transcription.  
*Journal of Cell Science* 117 (2004) 3855-3865. **\*joint second authors**
- III. Yang, W\*, **Wetterskog, D\***, Matsumoto, Y., Funa, K. Kinetics of repression by modified p53 on the PDGF  $\beta$ -receptor promoter.  
*International Journal of Cancer* 123 (2008) 2020-2030. **\*joint first authors**
- IV. **Wetterskog, D.**, Ozaki, T., Nakagawara, A., Funa, K. Dysregulation of PDGF  $\beta$ -receptor expression by  $\Delta$ Np73 in neuroblastoma.  
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