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**Dissertations-Kurzfassung**

# Promoter characterization of human receptor tyrosine kinase Axl, and its regulation in cancer


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*Axl*-receptor-tyrosine-kinase promotes antiapoptosis, mitogenesis, invasion, angiogenesis, and metastasis, and is highly expressed in cancers. However, the transcriptional regulation of this important gene has never been characterized. The present study was initiated to characterize the promoter, cis-acting elements, and promoter methylation driving expression of *Axl*. The 2.4kb sequence upstream of the translational start site, and sequential 5'-deletions, were cloned and revealed a minimal GC-rich region (-556/+7) to be sufficient for basal *Axl*-promoter activity in Rko, HCT116, HeLa, and K562 cells. Within this minimal region, five Sp-binding sites were identified. Two sites (Sp-a, Sp-b) most proximal to the translation start site were indispensable for constitutive *Axl*-promoter activity, whereas mutation of three additional upstream (Sp-c, Sp-d, Sp-e) motifs was of additional relevance. Gelshift and chromatin-immunoprecipitation identified especially Sp1 and Sp3 bound to all 5 motifs, mutations of all motifs abolishing binding. Mithramycin, inhibiting binding of Sp-factors to GC-rich sites, dramatically reduced Axl-promoter activity, Axl-, Sp1-, and Sp3-expression. In Drosophila Schneider SL2-cells, exogenous expression of Sp1/Sp3 increased Axl-promoter activity. Sp1/Sp3-siRNAs significantly reduced Axl-promoter activity and protein in Rko and HeLa cells. Methylation-bisulfite sequencing detected methylated CpG-sites within three Sp-motifs (Sp-a, -b, -c) and GC-rich flanking sequences, demethylation by 5-aza-2'-deoxycytidine upregulating Axl- and Sp3-expression in low Axl-expressing Colo206f/WiDr, but not high Axl-expressing Rko-cells. In addition, we studied TPA-inducible Axl-gene expression in K562 cells. Sequential 5'-deletion constructs, mutational, EMSA and supershift analysis revealed that the -660/-580 region (this region containing AP-1/CREB motifs, 5 partially overlapping TGCGTG repeats, and a GT rich region) of the Axl promoter is indispensable for TPA-induced promoter activity. Three AP-1 binding motifs, differentially bound with AP-1 family transcription factors but not CREB, were mainly required for TPA stimulated Axl gene expression in the K562 cell line. JNK (SP600125), ERK1/2 (U0216) and p38 (SB202190) inhibitors suppressed TPA-induced Axl gene expression and c-Jun activation. Taken together, data suggest that Axl gene expression in cancer cells 1) is constitutively driven by Sp1/Sp3 bound to 5 core-promoter motifs, 2) TPA-induced Axl gene expression is activated by AP-1 binding motifs at -660/-580 bp region through the JNK, ERK1/2, and p38 signaling cascade, 3) basic expression of Axl is restricted by methylation within/around the Sp-binding sites. This information enhances the understanding of essential mechanisms associated with Axl gene expression, and might foster ideas how to interfere with the expression of this important gene in cancer.