Tissue-specific differences in the regulation of *KIBRA* gene expression in kidney and neuroblastoma cells

Regulation der *KIBRA*-Expression in proximal tubulären Nieren- und Neuroblastomazellen

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The cytoplasmatic scaffold protein KIBRA is mainly expressed in kidney and brain tissue and is involved in human memory performance. In podocytes, KIBRA is proposed to serve as a linker molecule between the cytoskeleton and polarity protein complexes (Duning et al, JASN 2008). In the current study, we focused on the regulation of *KIBRA* gene expression and the functional characterization of its promoter region.

We screened 2705 bp of the 5'-flanking region of *KIBRA* in 56 patients with cardiovascular disease to characterize its variant structure. Patients’ DNA was subcloned to generate promoter deletion constructs. Identified SNPs were introduced by site-directed mutagenesis. Deletion constructs were transiently transfected into SH-SY5Y neuroblastoma cells and immortalized human kidney epithelial (IHKE) cells. Transcription start sites (TSS) were determined by rapid amplification of 5’cDNA ends (5’RACE). Endogenous expression was assessed by semiquantitative PCR.

We identified two distinct regions that direct significant transcriptional activity (TA): a proximal region between positions -730 and -1443 and a distal region between -2508 and -3005. 3' deletion constructs of the distal region were transcriptionally active only in IHKE cells. 5' RACE revealed two alternative TSS in both cell lines upstream of the listed TSS (NM_015238). Exclusively in IHKE cells, two additional TSS were detected in the first intron, resulting in two alternative exons, termed 2a and 2b. Deletion constructs of the upstream regions of both exons were transcriptionally active, consistently in IHKE cells only.

TA of the *KIBRA* promoter is separated by ~1060 bp into two distinct regions, suggesting differentially active promoter portions. *KIBRA* TSS utilization is cell type-specific, since two intronic TSS were detected in IHKE cells only. The upstream regions of both identified exons were transcriptionally active exclusively in IHKE cells, supposing two alternative *KIBRA* promoters leading to transcripts lacking the first exon. To identify the role of alternative *KIBRA* promoters in different cell types and their individual regulation by transcription factors will be the scope of future studies.