Functional role of ZBTB33 in clear renal carcinoma

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DNA methylation is crucial for proper vertebrate's development. Proteins interacting with methylated DNA can both interfere with the binding of other transcription factors and affect the formation of a new chromatin landscape. Often methyl-DNA binding proteins possess non-methyl-DNA binding activity. ZBTB33 can bind methylated CpGs and sequence containing CTGCNA. ZBTB33 is not necessary for mouse development, but it can influence the formation and progression of colon tumors due to DNA-methylation depending regulation of tumor suppressor genes. Deficiency of ZBTB33 in spontaneous colon cancer model and in breast cancer cells results in delay of tumor growth, influences the tumor size, lifespan. The aim of this work was to create and characterized the model system of renal carcinoma cells deficient for ZBTB33. We generated 3 clones of ZBTB33 knockout clear renal carcinoma cell line Caki1 by frame shift via CRISPR/CAS9 genome editing technology. Frame shift was generated in the middle of N-terminal BTB/POZ domain. Clones were analyzed for tumorigenic potential in Nude mice. Deficiency of ZBTB33 led to misregulation of 1587 genes (padj< 0,01). We performed ChIP-seq analyses for ZBTB33 binding sites. For negative control we used ZBTB33 deficient cells. We detected that ZBTB33 binds within regulatory elements and genic regions of 200 and 180 genes that was upregulated or downregulated after ZBTB33 depletion respectively. DNA methylation status of ZBTB33 binding sites was determined by whole genome bisulfite sequencing analyses. KEGG pathway analyses revealed downregulated genes from p53 pathway. Also, we detected that deficiency of ZBTB33 results in upregulation of several tumor suppressor genes. This work was supported by the Russian Science Foundation (19-74-30026) and the Russian Foundation for Basic Research (19-29-04139).