1. SUMMARY

The Wilms Tumor gene 1 codes a powerful transcription factor that appears to play a pivotal role in the regulation of cell proliferation and differentiation. The physiological expression of WT1 is restricted to a few types of developing tissues: blood precursor cells, kidney precursors and cells in the mammary ducts. The abnormal expression of WT1 had been found in various human neoplasias. WT1 expressed in neoplasias lacks mutations and reduction of its expression blocks proliferation and leads to apoptosis or cell differentiation. There are few possible causes that could explain the abnormal expression of WT1: the gene could be mutated, which could lead to constitutive expression, an abnormal expression of physiological activators could activate WT1 expression, and an epigenetic modification of DNA could activate WT1. All three possible causes for pathological upregulation of WT1 gene were investigated in this work.

The genes PAX2, PAX8 and GATA1 are physiological activators and regulators of WT1 expression. The expression patterns of these genes were analyzed in carcinoma cell lines and human carcinoma tissue samples. A close correlation was observed between expression of WT1 and PAX2 and PAX8. However, the correlation was not linear but rather of a threshold type. WT1 expression appeared when activators where expressed at certain levels. A model of an “on – off” regulation is proposed in which the expression of an activator had to reach certain level (termed activation threshold) to activate expression of WT1.

The method of RNA interference was used to further analyze the interactions between WT1 and activator genes. The genes WT1, GATA1, PAX2 and PAX8 were silenced in a series of experiments. It was shown that RNAi reduce the growth in WT1 expressing cells by 50%. However, the reduction of WT1 expression achieved with RNAi did not lead to an increase in apoptosis. Targeting of each of the regulator genes caused similar effects – downregulation of the activator was followed first by downregulation of WT1 and later by downregulation of housekeeping genes. When the WT1 regulators were targeted the relative downregulation of WT1 was lower than in the case of direct targeting. When regulators were downregulated also the growth reduction was less pronounced than in cases with direct targeting of WT1. Taken together, it can be implied that WT1 is regulated by PAX2, PAX8 and GATA1.

The promoter sequences of the studied genes were analyzed in the next step. They did not contain any mutations. There were few variations that were also present

in healthy controls. The lack of mutations in promoter regions enables the activation of WT1 by physiological activators in AML. Lack of promoter mutation allows PAX2, PAX8 and GATA1 to exert their influence upon WT1 also in the milieu of neoplastic cell.

Methylation changes of promoter sequences at CpG residues are the most common epigenetic change of the human genome. A novel method of gene methylation analysis was developed here to analyze, whether such changes could influence WT1 expression in human neoplasia. The method is based on digestion of a chosen CpG residue with methylation sensitive enzymes and subsequent quantification with quantitative PCR (Light Cycler). The methylation level of the GATA1 dependent enhancer of the WT1 gene was analyzed in AML patients. The GATA1 enhancer was chosen as the most probable site of epigenetic variation. GATA1 is expressed ubiquitously in AML, but the expression level varies to a large extent between samples and does not correlate with WT1 expression. Since GATA1 exerts its activity on WT1 in hematopoietic cells mainly through the enhancer it was reasonable to start looking for possible changes at that segment. There were expression changes identified that were related to the methylation status of the enhancer region – however, hypermethylation was connected with higher expression of WT1 and hypomethylation was connected with lower expression. GATA1 expression levels were similar in both instances. Since hypermethylation is thought to prevent transcription factor binding to target sequences, possible explanation of the observed inverse relation could be the presence of an unidentified negative regulator.