

3. AIMS

The WT1 gene is commonly expressed in human neoplasia (see *Introduction*). However, the cause of pathological upregulation remains unknown. The key objective of this work is an exploration of the mechanisms underlying abnormal WT1 gene expression in human neoplasia.

The first part focuses on correlation between expression levels of WT1 and its physiologic regulators in human carcinomas. The aims of this part are:

1) measurement in carcinoma cell lines and tumor tissue samples of the expression abundance and expression levels of WT1 and the three regulator genes, which expression has been shown to activate expression of WT1 in vitro: PAX2, PAX8 and GATA1, 2) investigation of possible correlations between expression levels of the studied genes.

The second part aims at further clarification of a potential relationship between WT1 expression and expression of its regulators by RNA interference. The aims of this part are: 1) establishment of efficient RNA interference system, 2) synthesis of siRNAs against WT1, GATA1, PAX2 and PAX8, 3) RNAi studies of the influence of downregulation of regulators expression on WT1 expression.

The third part is investigation of a possible role that mutation and methylation of the promoters of WT1, GATA1, PAX2 and PAX8 play in the pathogenesis of WT1 upregulation in leukemia. The aims of this part are: 1) establishment of PCR protocols that could be used for sequencing of the promoters, 2) sequencing of the promoters, 3) identification of possible variations and mutations, 4) establishment of a DNA methylation assay that could take advantage of Light Cycler quantitative PCR, 5) study of promoter methylation in relation to WT1 expression.