

The role of the Pax8 gene for the Wilm's tumor gene 1 (WT1) regulation in cancer

Daniel Koychev

Study objective

To understand the WT1 - Pax8 interactions in order to develop targeted approaches against this tumor growth promoting pathway. Furthermore, Pax2, Pax8 and WT1 relations would be investigated in the sense of possible regulation of apoptosis and cell growth in thyroid, but also renal tumors and in lymphoproliferative diseases.

Project report

PCR conditions for Pax8, Pax2 , WT1 and bcl-2 group – bcl-2, bax, bcl-XI

In the first stage of the study real-time PCRs for all existing Pax8 and Pax2 splice variants as well for total Pax8, Pax2 and WT1 genes were established. Furthermore, for Bcl-2, Bax and Bcl-XI genes real-time PCR conditions were determined. The primer pairs were constructed over two neighboring exons, thus eliminating a possible interference from DNA contamination. A novel model was set up to examine the level of inducible apoptosis – Bax/Bcl-XI protein ratio.

Pax8 A-E cloning

Full-length Pax8A, B, C, D and E splice variants were isolated from renal cancer cell lines and cloned into a pBudCE4.1 vector. The expression levels of WT1, Pax2 and Pax8 were investigated in about 70 tumor cell lines with real-time PCR and possible candidates for Pax8 transfection experiments were sorted out for further study.

Pax8 downregulation and induction of apoptosis

At a next step, different techniques for downregulating the Pax8 expression in follicular thyroid cancer (FTC) using inhibitory RNA – miRNA, shRNA, siRNA were explored. Possible presence of Pax8/PPAR γ 1 fusion was first excluded in the used in the study five FTC cell lines (8505c, BCPAP, CGTHW1, TT2609co2) were first investigated. Optimized were the conditions for, miRNA, shRNA and siRNA transfection establishing novel protocols for FTC, renal cancer and lymphoma cell lines. Using transient Pax8 siRNA and stable Pax8 miRNA transfection a considerable level of Pax8 down regulation was observed on gene level and to our knowledge this is the first report of successful Pax8 knock down in solid tumors. It is important to determine whether or not Pax8 protein acts at the mitochondria to understand the mechanism by which they block the apoptotic mitochondrial permeabilization and the extent of activation of the intrinsic and extrinsic apoptotic pathways. For this purpose the activation of the following apoptotic

markers were measured by means of flow cytometry and/or Western Blot: membrane phospholipids, mitochondrial membrane potential, Caspase3, Caspase8, Caspase9, Bcl-2, Bcl-xl, and Bax. In some renal (Caki1 and Caki2) and FTC (BCPAP) cell lines the downregulation of Pax8 induced significant induction of intrinsic apoptosis, which was proved by activation of Bax (transcriptional and post-transcriptional) and reduced levels of Bcl-2 and/or Bcl-xl. Activities of Caspase3 and Caspase9 were significantly increased in Pax8 downregulated cell lines compared to the mock transfected. However, the activity of Caspase8 was not significantly increased. In these cases significant WT1 downregulation (transcriptional and post-transcriptional) was observed. Furthermore, an inhibition of the cell growth was observed using the cFDASE assay. However, another group of cell lines (A498, TT2609co2) although activating the intrinsic pathway (Bax, Bcl-2, Bclxl, Caspase9) significantly suppressed the activation of the extrinsic pathway (Caspase8) - compared to the control - and this event was accompanied by low-scale WT1 downregulation. In the present study we also report that downregulation of Pax8 induced G2/M or G1 block in some cell lines, which also downregulated WT1. These results indicated a possible involvement of the Pax8 and WT1 genes in cell-cycle progression in cancer cells.

Conclusion

The present study has shown that downregulation of Pax8 isoforms induces WT1 downregulation, followed by growth inhibition and apoptosis, which includes predominantly the intrinsic pathway and in some cases, to a large extent inhibit the extrinsic pathway. As for downstream targets of Pax8 isoforms, proapoptotic Bax and antiapoptotic Bcl-2, Bcl-xl might be direct or indirect targets. However, all splice variants of WT1 seem to be subdued to transcriptional regulation by Pax8. Comprehensive studies, including microarray analysis are being planned to identify the targets of Pax8. Taken together, these results may indicate that Pax8 isoforms recruit some molecule(s), bind to their different target sequences and regulate the transcription of target genes to play antiapoptotic roles.