CAIX and MCT4 Suppression Down-Regulate the Cell Viability in Clear Cell Renal Cell Carcinoma

Introduction and Objective: To identify potential targets in clear cell renal cell carcinoma (ccRCC), we performed a transcriptome analysis of ccRCC and normal kidney specimens. Based on the results, we analyzed the functional significance of carbonicanhydrase IX (CAIX) and monocarboxylate transporter 4 (MCT4) in ccRCC.
Materials and Methods: We extracted the total RNA from 60 ccRCC and 20 normal kidney specimens and performed a gene expression analysis on the human exon 1.0 ST array (Affimetrix). The expression profiles were analysed by unsupervised hierarchical average linkage clustering algorithm. The five best candidates showing the highest differences between ccRCC and normal kidney were further studied by immunohistochemical analysis. The mRNA and protein expression levels were evaluated in 7 ccRCC cell lines. To study the effects of CAIX and MCT4 cell lines were transfected with CAIX- or/and MCT4-siRNA. Furthermore, we measured the lactate concentration in the culture medium of the transfected cell lines.
Results: The transcriptome analysis revealed numerous highly overexpressed genes in ccRCC. The overexpression of the five best candidate markers (PHD3, FABP7, CAIX, NADH-1α; >10-fold, MCT4; 9.6-fold differential expression) was confirmed by immunohistochemical analysis. Staining patterns were strong and homogeneous for all the candidate genes except for FABP7. The down-regulation of CAIX and MCT4 at the mRNA and protein level persisted for 7 days after siRNA transfection. Based on MTT assays, functional suppression of CAIX or/and MCT4 resulted in significant growth inhibition, but no additive effect of MCT4 suppression on CAIX knockdown was observed. The functional suppression did not affect cell-migration. MCT4-suppression decreased the lactate level in the culture medium of the transfected cell lines.
Conclusions: We have identified several potential biomarkers for ccRCC related to hypoxia, metabolism and pH regulation. Functional suppression for CAIX and MCT4 down-regulates cell viability in ccRCC cell lines. MCT4 suppression might reduce the extracellular lactate level in the ccRCC, which might suppress angiogenesis. Further studies are required to evaluate their genuine value as therapeutic targets.