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Molecular characterization and differentiation of chromophobe renal cell carcinoma and renal oncocytoma

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Renal cell tumours display a morphological and biological diversity leading to difficulties in routine diagnosis and estimation of tumour prognosis. Histological discrimination of malignant chromophobe renal cell carcinoma (chRCC) and benign oncocytoma (RO) is important because each tumour posses different biologic potential. The data presented in the modern literature with regards to pathological features, gene and protein expression, cytogenetic characteristics, and prognosis of chRCC and RO were critically analyzed in the present study, and pinpointed the difficulties in evaluation of data without confirming the diagnosis of these tumours by genetic means.

Several genetic studies have analyzed chromosomal aberrations in chRCC and RO using approaches with a low resolution. In the first part of the project, a high density 250K SNP oligoarrays (Affymetrix, Inc.), with the capacity to characterize both copy number and allelic imbalance aberrations in a single assay, have been applied to detect loss of small DNA segments at specific chromosomal regions in chRCCs and ROs, which may mark the loci of genes involved in the tumourigenesis. As the results, the occurrence of small specific alterations was excluded with all certainty. These types of renal cell tumours are characterized by the monosomies of specific chromosomes. Comparing the genetic changes in 30 chRCCs, loss of chromosome 2, 10, 13, 17 and 21 occurred in 93%, 93%, 87%, 90% and 70% of chRCCs, respectively. None of the 42 ROs displayed loss of these chromosomes. Based on the results obtained, any microsatellites or BAC clones localised at these chromosomes can be used to establish the diagnosis of chRCCs in cases with uncertain histology. This can be achieved in most histopathological laboratories by applying microsatellite analysis or FISH to detect the specific genetic alterations.

The second part was aimed to find a gene expression profile for chromophobe RCC and RO by applying HG-U133 and HG-U133Plus2.0 microarrays (Affymetrix, Inc.) and to identify novel markers that discriminate between the two types of tumours. Unsupervised hierarchical clustering based on the raw data separated chRCCs and ROs into one distinct group among other types of renal tumours. Further stringent evaluation revealed a consistent relationship between under-expression of genes mapping chromosomes displaying monosomies in chRCC. As gene selection strategies are typically used to identify the relevant gene set from all genes on a microarray, 100 genes that were significantly differentially expressed between chRCC and RO were revealed using pooled and individual specimens. Microarray results for 12 most promising candidates were confirmed by real-time RT-PCR, and the difference in mRNA expression was larger in the latter assay. Using normal RT-PCR performed on a panel of larger number of samples, TMC5 and RBM35A were

confirmed as potential expression markers for chRCC, whereas AQP6 resembled a good potential marker for RO. Although the DNA analysis can determine chRCC unequivocally, pathologists prefer to use morphological methods in the differential diagnosis. To date, no valuable immunohistochemical markers are available to differentiate chRCC from RO. In the present study, CD82/KAI1 was found as an excellent marker for distinguishing chRCC from other types of renal cell tumours with overlapping phenotype. The significance of differential expression of other genes at the protein level might be improved after arising specific antibodies.

In order to identify proteins associated with characteristic proliferation and alterations of mitochondria seen in cells of RO and chRCC, respectively, two-dimensional gel electrophoresis followed by mass-spectrometry was applied in the third part of the study. Possible roles of revealed proteins in tumourigenesis was discussed, however further studies are needed to clarify these suppositions.