Intronic Noncoding RNA expression in Renal Cell Carcinoma by Quantitative RT-PCR

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A novel set of tumor suppressor gene candidates in Clear Cell Renal Cell Carcinoma (ccRCC) including intronic noncoding RNAs (ncRNAs) was identified previously in our group (Brito et al, Mol Carcinog.2008;47(10):757-67). To investigate a possible correlation of intronic ncRNAs in changes involving renal cell carcinoma (RCC) transformation we have extended the study using a 44K combined intron-exon oligoarray platform (Nakaya et al., Genome Biol.2007;8(3):R43). We analyzed 17 paired clear cell RCC patient samples divided into four pools of adjacent non-tumor kidney and four pools of tumor kidney tissues. We identified a subset of 13 pairs of antisense Totally Intronic Noncoding (TIN) RNAs and corresponding protein-coding gene from the same genomic locus as having correlated patterns of expression (FDR = 10%; p = 0.05). We selected two pairs of TIN RNAs and corresponding overlapping exons to validate their differential expression individually in each of the 17 tumour and non-tumour samples that comprise the pools analyzed with microarrays. Total RNA extraction was followed by DNAse treatment to eliminate genomic DNA contamination, by reverse transcription with oligo-dT(30bp) primer and by quantitative real-time PCR. Until now we have analyzed samples from three patients. Microarray expression patterns have been confirmed for SEMA3B and ICAM1 genes ($p<0.05$); the intronic expression pattern has not been statistically confirmed so far. Next step is to extend the quantitative analyzes to the remaining 15 patient samples. This result illustrates the relevance of validation patient-by-patient of data obtained with pooled samples, in order to assess individual variation. These noncoding RNAs may be new biomarker candidates of ccRCC.

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