Determination of hTERT promoter methylation status using methylation specific PCR

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Introduction: DNK methylation is significant for process that participate in embryonic development,cell differentiation,maintenance of homeostasis in the cells. The regulation of gene expression and maintenance of genome stability are mechanisms that ensure that these processes are carried out.Renal Cell Carcinoma (RCC) is the most common renal cancer in adults and includes several subtypes that may be distinguished by their histology, genetic background, clinical course and treatment. Human telomerase reverse transcriptase (hTERT) is a crucial enzyme for telomere maintenance, has been linked to RCC development.

Aim: The aims of this study were to search for genetic and epigenetic alterations in hTERT methylation and to establish a possible association between molecular and clinico-pathological characteristics of RCC.

Methodology: DNA was extracted from 31 formalin-fixed, paraffin-embedded tumor samples and 23 blood samples from 54 patients with RCC. Polymerase chain reaction (PCR) products were sequenced and analyzed using the Sequencher software. The hTERT promoter methylation status was determined by methylation specific PCR (MSP). In order to detect DNA sequence alterations after bisulphite treatment, MSP was used to distinguish methylated from unmethylated alleles. Genomic DNA from lymphocytes of healthy donors was used as control for unmethylated genes and the same DNA, treated in vitro with SssI methyltransferase, was used as control for methylated genes.

Results: HTERT promoter was methylated in 17 of the 31 tumor samples (54.8%). Interestingly, in 71% of papillary carcinomas the promoter was unmethylated, wereas in 100% of chromophobe carcinomas it was methylated. An association was established between methylation and histological type of RCC (p=0.047).

Conclusions: HTERT, via its promoter methylation seems to play a role in renal cell carcinoma biology. hTERT promoter methylation status is related to RCC histology.