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BOOK OF ABSTRACTS

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P2: Analysis of Alternative LDLRAD4 Gene Promoters and Transcripts in Colorectal Cancer

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Gene LDLRAD4 plays a role in cell proliferation, apoptosis, immunosuppression and cancer progression. Transcription of LDLRAD4 is regulated by several alternative promoters, two of which were indicated by *in silico* analyses to be differentially active in rectal cancer. Promoter A encodes for a truncated protein-coding transcript and is down-regulated in rectal cancer. Promoter B encodes for a non-coding transcript up-regulated in rectal cancer identified as lnc-RNMT-2:5. The aim of this study was to characterize the two alternative promoters *in silico* in order to explain their differential activity and to investigate the profile of LDLRAD4 transcripts in colon cell lines. Nucleotide sequences used in the analyses were downloaded from the Ensemble genome database (reference GRCh37). Three bioinformatics tools were used for core promoter element prediction: GPMiner, YAPP and CNNPromoter. Four bioinformatics tools were used for transcription factor binding site prediction: PROMO, TFBIND, CiiiDER and Tfsitescan. Only the predictions made by two or more tools were considered. Primer extension followed by fragment analysis was used to characterize LDLRAD4 transcripts present in colon cell lines. The promoter element predictions showed that the promoter A is typical, while promoter B has most typical elements and lacks GC boxes. The transcription binding site predictions indicate that three different transcription factors bind only to the promoter A (NF-kB, EGR1 and IRF-7), while four different transcription factors bind only to the promoter B (HNF1, POU2F1, POU2F2 and PTF1). The predicted transcription factors are mostly involved in regulation of cell differentiation and proliferation. The primer extension experiment performed with primer specific for exon 2-exon 3 junction produced multiple signals of relatively low intensity, indicating the presence of multiple LDLRAD4 transcripts in colon cell lines. The results obtained by *in silico* analysis may explain promoter B activation in rectal cancer. However, based on the results of primer extension, neither of the LDLRAD4 transcripts is dominant in colon cell lines. Considering that promoter B generates long non-coding RNA that can exert its function even at low expression level, it can serve as potential colorectal cancer biomarker and its potential role in carcinogenesis should be investigated.
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