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The Cancer Control Challenge:
Better Understanding It to Better Face It

CHARACTERIZATION OF REGULATORY GENOMIC REGIONS AND TRANSCRIPTION FACTORS IN PRIMARY COLON CANCER

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Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death worldwide, representing a significant public health challenge. Epigenetic DNA modifications play a critical role in the pathogenesis and progression of CRC. However, the underlying mechanisms driving these alterations remain poorly understood, highlighting the need for further investigation. **Objectives:** We aimed to perform an integrative epigenetic analysis to identify methylation signatures in primary colon cancer tissues. **Methods:** Data from the TCGA-COAD project were downloaded using the TCGAbiolinks package, including RNA-seq expression profiles from 481 tumor and 41 normal tissue samples, and DNA methylation data generated with the Illumina 450K platform, comprising 312 tumor and 38 normal samples. The ELMER tool was employed to identify differentially methylated distal regions, map nearby target genes, detect enriched DNA motifs, and associate them with potential transcription factors (TFs). Probes that presented an adjusted False Discovery Rate (FDR) < 0.01, minimum methylation difference (β-value) of 0.4, *p-value* < 0.01 and that were present in at least 20% of the samples of the evaluated group were considered as differentially methylated. Pairs between hypomethylated distal probes and nearby genes were identified using significance thresholds of raw *p-value* (< 0.05) and empirical *p-value* (Pe < 0.05). Motifs enriched in regulatory regions were identified based on an odds ratio (lower OR) > 1.1. **Results:** Regulatory TFs were identified by correlating their expression with motif methylation in an unsupervised analysis, considering a p-value < 0.05. A total of 154,053 probes showed significant hypomethylation in tumor samples from CRC patients, according to the defined thresholds. Among the highlighted pairs, there are genes such as *ASCL2*, *CYCSP6*, *AL009178.3*, *AL009178.3*, *SLC6A6* and *PPP2R2C* associated with probes cg11328661, cg14802742, cg10132767, cg03850936, cg07438246, cg10516057, respectively. Among the 331 enriched motifs, *FOSL2* emerged as one of the most frequently enriched in hypomethylated distal regions (953 probes and lower OR = 2.19), and further correlation analyses identified three potential regulatory transcription factors: *NKRF*, *FEZF1*, and *TLX1*, which were significantly associated with *FOSL2* motif methylation (p < 0.05). **Conclusion:** Our results revealed hypomethylation in six potentially regulatory regions and their respective target genes.

Furthermore, we identified a frequently enriched motif in these regions and their associated TFs, highlighting potential molecular targets in colon cancer patients.

Keywords: Epigenetic modification; Methylome; Molecular targets.