O20 m6A-regulated long non-coding RNAs in prostate cancer

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Introduction & Objectives: Prostate cancer (PCa) is a highly prevalent neoplasia in which deaths are mostly caused by advanced disease. Hence, further elucidation of the mechanisms involved in PCa aggressiveness is imperative. Methylation of the N6 position of adenosine (m^6A) is an RNA post-transcriptional modification involved in malignancy development. The main goals of this study are to assess the expression of proteins involved in m^6A establishment and to evaluate the impact of their deregulation on non-coding RNAs (ncRNAs) expression and consequently PCa aggressiveness.

Materials & Methods: To investigate the role and specific mechanisms, by which m^6A regulates PCa progression, in vitro experiments were performed. ELISA and western blot methods were used to characterize m^6A RNA methylation and the methyltransferase complex (MTC) expression in PCa cell lines. MTC stable silencing was established with CRISPR-Cas9 system. Long ncRNA (lncRNAs) profiler quantitative PCR array and bioinformatic analysis followed by m^6A/RNA co-immunoprecipitation were used to screen and validate the potential m^6A-regulated lncRNAs. Viability, wound-healing, BrdU cell proliferation and transwell invasion assays were performed to assess the m^6A downregulation's phenotypic impact in cells. LncRNAs expression was further tested in fresh-frozen prostatectomy specimens by qPCR and survival analysis was adopted to correlate with PCa patients' prognosis.

Results: Higher m^6A RNA methylation levels were found in metastatic PCa cells compared to primary and normal ones ($P<0.05$). Differential expression of the MTC components (VIRMA, METLL3, METLL14 and WTAP) was observed. VIRMA was overexpressed in the metastatic PC-3, cell line (Fold change=5; $P<0.05$). PC-3 VIRMA knockout significantly decreased m^6A levels ($P<0.05$) and suppressed the expression of several oncogenic lncRNAs involved in prostate tumorigenesis, including CCAT1, CCAT2 and PCAT1 (Fold-change>3; $P<0.00001$). Importantly, PC-3 VIRMA silencing attenuated malignant phenotype with significant reduction in cell viability, proliferation, invasion and migration capacity ($P<0.05$). In PCa patients, CCAT1 higher expression levels associated with advanced cancer stage ($P=0.039$) and clinical recurrence ($P=0.022$), whereas CCAT2 increased levels were linked with higher histological grade group ($P=0.048$). Moreover, higher CCAT1, CCAT2 and PCAT1 levels associated with shorter disease-free survival ($P=0.002$, $P=0.027$ and $P=0.021$, respectively), whereas CCAT1 and CCAT2 higher expression levels also associated with more frequent cancer-related deaths ($P=0.035$ and $P=0.042$, respectively), while PCAT1 expression levels associated with BCR-free survival ($P=0.032$) in the same cohort of PCa patients.

Conclusions: Herein, we show that VIRMA expression affects PCa aggressiveness in an m^6A-dependent manner through oncogenic lncRNA upregulation. Our findings may provide new insight to PCa biology and ultimately identify novel prognostic biomarkers for PCa.