IDENTIFYING AND CHARACTERIZING PROTEINS THAT BIND TO LONG NON-CODING RNAS ASSOCIATED WITH PANCREATIC CANCER STEM CELLS

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal neoplasms due to its aggressive metastatic dissemination. Metastatic capability is associated with the acquisition of a stem-like phenotype by cancer stem cells (CSCs), a process that is still poorly understood in PDAC, which limits therapeutic advances in PDAC treatment. Based on the hypothesis that long non-coding RNAs (lncRNAs) are involved in the CSC phenotype, we aimed to identify CSC-enriched lncRNAs and determine their mechanism of action through the identification of their protein binding partners. Publicly available RNA-seq data of matched tumorsphere (CSC-enriched) and adherent (non-CSC-enriched) cultures from PDAC patient-derived xenograft (PDX) tumors were analyzed to identify CSC-enriched lncRNAs. Promising lncRNAs were selected for RNA pulldown followed by mass spectrometry-based proteomic analysis to identify CSC-enriched lncRNA protein binding partners, using the PVT1 lncRNA and its protein binding partner EZH2 as a positive control. We identified 53 lincRNAs enriched in PDAC CSCs and designed RNA pulldown probes for 3 promising candidates (LINC01559, LINC01948, LINC02432). Sofar RNA pulldown assays confirmed efficient lncRNA pulldown by qRT-PCR. PVT1-EZH2 interaction was detected by Western blotting. In conclusion, we have identified PDAC CSC-enriched lncRNAs and successfully implemented a RNA pulldown protocol capable of capturing specific lncRNAs of interest along with their associated protein partners. Proteomics analysis by mass spectrometry will be used to identify proteins associated with CSC-enriched lncRNAs, which will contribute to elucidate the mechanism of action of these lncRNAs in cancer stemness and may contribute to the identification of relevant therapeutic targets for PDAC.

Agradecimentos: