Evaluation of long noncoding RNA LINC00941 mechanism of action and targets in pancreatic ductal adenocarcinoma by proteomic analysis

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a highly common neoplastic disease, representing 90% of all pancreatic malignancies. It is characterized by poor prognosis and absence of effective therapies leading to high lethality. Long non coding RNAs (lncRNAs) regulate a diversity of cellular processes and may support the tumoral phenotype, helping the comprehension of cancer molecular processes and being used as potential biomarkers. LINC00941 is an upregulated lncRNA in gastric cancer, lung adenocarcinoma, pancreatic adenocarcinoma, among others types of cancer and its expression level is often negatively correlated to patient's survival. The oncogenic role of LINC00941 has been demonstrated in vitro, where LINC00941' knockdown reduced migration, invasion and DNA repair and increased chemo sensibility to gemcitabine of PDAC cells. To gain insight into the molecular mechanisms of action of LINC00941, we generated proteomic profiles from PDAC cells after RNA silencing with siRNA and searched for protein levels affected by LINC00941 knockdown. Methods: AsPC-1 cells (1 x 105) were transfected with 50 nM siRNAs (siGENOME siRNA, Dharmacon) for LINC00941 and Lipofectamine 3000 (Invitrogen). Total RNA was extracted after 72 hours and quantified with Nanodrop. Reverse transcription with 2ug of RNA was performed and a RT-qPCR with the resultant cDNA and SYBR Green (Thermo Fisher) confirmed the transient lncRNA silencing. Transfected cells were homogenized in TCT buffer and protease inhibitor cocktail by sonication. Next, samples were incubated at 80°C for 20 minutes and 4°C for half an hour, followed by acidification. Centrifugation with a molecular exclusion filter allowed the obtention of a 10 kDa higher fraction which was dessalted with Amicon Ultra-0,5mL 3K-NMWL. Protein concentration was determined by SDS-PAGE and 10 ug were denatured with 10 uL of 0.2% RapiGest at 80°C. Cysteine reduction and alkylation were made with 6.25 mM DTT and 19.25 mM IAA, respectively. Protein digestion with Trypsin (Sequencing Grade, Promega) was performed overnight in 1:100 trypsin-protein proportion. Peptides were dessalted with C18 ZipTip and samples were air dried using SpeedVac. Peptides were suspended in formic acid for application in UPLC coupled with Q-TOF MS/MS. Mass spectra were analyzed against Homo sapiens' SWISSPROT. Results: 1681 proteins were detected in the PDAC proteome, 312 of them showed differential abundance when the proteomic profiles from LINC00941 knockdown and control samples were compared. 178 proteins were upregulated while 134 were downregulated. Functional enrichment with enrichGo, which performs an over representation analysis, showed that upregulated proteins were highly associated with cell and organelles membranes, but didn't show any significant association with any biological process or molecular function ($p < 0.05$). Downregulated proteins were associated with cytosol and nucleus in cellular component category and nitrogen compounds and macromolecules metabolic processes were the main subcategories in biological processes. Additional replicate samples will be processed and incorporated to strengthen the statistical power of the analysis and selection of candidates for experimental confirmation.

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