ROLE OF PUTATIVE STEM CELL MARKER DCAMKL-1 IN PANCREATIC CANCER AND EMT

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Background and Aim: The stem cell origin of pancreatic cancer has generated great interest in the identification of novel markers of cancer stem cells (CSCs). Reports indicate the role of Notch signaling in various cancers. Epithelial-mesenchymal transition (EMT) plays a key role in cancer invasion/metastasis, and there is a gain of stem cell properties by the cells undergoing EMT. We sought to determine the expression patterns and involvement of the intestinal and pancreatic stem cell marker DCAMKL-1 in pancreatic neoplasia and EMT. Additionally, we have utilized Nanoparticle (NP) technology to deliver DCAMKL-1 specific siRNA.

Methods: Human pancreatic tumors were immunostained for DCAMKL-1, 14-3-3σ and vimentin. AsPC1, human pancreatic adenocarcinoma cells were transfected with DCAMKL-1 siRNA and tumor xenografts were treated with NP-based siRNA against DCAMKL-1 and analyzed for DCAMKL-1, c-Myc, Notch-1 (using immunoblot and real-time reverse-transcription polymerase chain reaction [RT-PCR]); KRAS, ZEB1, ZEB2, Snail, Slug and Twist mRNA and microRNAs pri-let-7a, pri-miR-144 and pri-miR-200a (using real-time RT-PCR) levels. A luciferase reporter assay, with two separate plasmids (let-7a and miR-144) with binding sites at the 3' UTR, was utilized to measure let-7a and miR-144 in AsPC1 cells.

Results: Increased expression of DCAMKL-1 was found in human pancreatic adenocarcinoma compared to uninvolved human pancreas. Increased expression and nuclear localization of 14-3-3σ within DCAMKL-1+ cells was observed in human pancreatic adenocarcinoma. We also observed fibrillar DCAMKL-1 to co-localize with vimentin. Knockdown of DCAMKL-1 in pancreatic cancer cells resulted in downregulation of proto-oncogene c-Myc and Notch-1 via let-7a and miR-144 miRNA dependent mechanism, respectively. Furthermore, siDCAMKL-1 treatment resulted in reduction in Snail, Slug and Twist mRNA following induction of miR-200a (EMT inhibitor), indicating its role in EMT. Administration of NP-siDCAMKL-1 into AsPC-1 xenografts resulted in tumor growth arrest, downregulation of proto-oncogenes c-Myc and KRAS via let-7a miRNA-dependent mechanisms. Furthermore, siRNA-mediated knockdown of DCAMKL-1 in AsPC-1 tumor xenografts resulted in downregulation of ZEB1, ZEB2, Snail, Slug and Twist following induction of miR-200a.

Conclusion: These findings illustrate direct regulatory links between DCAMKL-1, microRNAs and EMT in pancreatic cancer. Furthermore, these data also suggests that NP-based delivery of siRNAs directed against critical target such as DCAMKL-1 may provide a novel approach to treat cancer through the regulation of endogenous miRNAs. This may represent a novel target for anti-stem cell based therapies for pancreatic cancer.

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