Systemic Administration of Bifunctional shRNA Lipoplex Targeting PDX-1 for Murine Model of Insulinoma and Pancreatic Cancer.

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Despite current multimodality therapy, patients with metastatic pancreatic cancer (PC) survive 4-6 months. New therapeutic strategies for PC are urgently needed. The transcription factor pancreatic duodenal homeobox transcription factor (PDX-1) is a master regulator of embryologic pancreatic development and regulates insulin expression and other islet hormones in the adult pancreas. We and others found PDX-1 is overexpressed in pancreatic tumor and may play a key role in proliferation and invasion of human PC. Additionally, overexpression of PDX-1 is a well established hallmark for Insulinoma resulting in hyperinsulinemia with uncontrollable hypoglycemia. PDX-1 is a rational target gene for expression knockdown by RNA interference (RNAi) for the treatment of PC and Insulinoma. Our previous study has shown silencing PDX-1 expression in pancreatic cancer cell line PANC-1 inhibited cell proliferation *in vitro* and suppressed tumor growth *in vivo* with increased tumor cell apoptosis. We have recently developed a novel bifunctional shRNA strategy with high efficiency, specificity, durability and required lower dose for target gene knockdown. The bifunctional shRNA for PDX-1 (bi-sh-PDX-1) achieved knockdown 90% of PDX-1 protein within 48 hours. This study is designed to demonstrate efficacy of bi-sh-PDX-1 *in vivo* with a murine model for Insulinoma and PC. Methods: In the insulinoma model SCID mice were implanted with 1×10^6 β-TC-6 cells by intraperitoneal (IP) injection. In the PC model SCID mice were implanted with 5×10^5 PANC-1 cells by IP injection. Two weeks later, mice were randomly divided into two groups for each model; one group was given lipoplex (DOTAP:Cholesterol liposome plasmid complex) containing 35 μg of control vector (plasmids without shRNA insert) and the second group was given lipoplex containing 35 μg of either mouse specific bi-msh-PDX-1 (for insulinoma model) or human specific bi-hsh-PDX-1 (for PC model) via tail vein injection. Two repeated deliveries were made every 2 weeks for a total of 3 injections. Blood insulin and glucose levels were monitored at regularly scheduled intervals. At 2 weeks, 10 weeks and 18 weeks after the third injection, 6 mice from each group were killed for pathology and tumor evaluation. Three cycles of treatment were well tolerated. Bi-sh-PDX-1 treated mice showed significant elevation of fasting glucose levels in contrast to the gradual depression in the vehicle treated mice for the insulinoma model ($P<0.05$) at 171.4±10.6 vs 129.4±16.5, 171.8±11.5 vs 116.8±14 and 170.4±21.0 vs 73.0±2.6 on day 7 after 1st, 2nd and 3rd treatment cycle, respectively. However, there is no significant change of glucose levels on PANC-1 bearing tumor models between treatment with bi-hsh-PDX-1 and empty vehicle at each treatment cycle. Kaplan-Meier survival analysis, pathology and tumor evaluation will be reported.