Development of a Novel, RNAi-Based Therapeutic Targeting Pancreatic Duodenal Homebox-1 (PDX-1) for Insulinoma and Pancreatic Cancer

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Insulinoma is the most common type of islet cell tumor. Malignant insulinomas are devastating from hyperinsulinemia and result in uncontrollable hypoglycemia. Pancreatic duodenal homebox-1 (PDX-1) belongs to a homeodomain-containing transcription factor family and plays a primary role in pancreatic organogenesis. PDX-1 maintains beta-cell function by regulating transcription of insulin, glucokinase and glucose transporter type 2. PDX-1 is overexpressed in insulinomas resulting in hyperinsulinemia. PDX-1 is also found to be commonly overexpressed in pancreatic tumors. Metastatic pancreatic cancer has a 4-6 month survival from diagnosis. Silencing of PDX-1 expression represents an attractive approach to inhibit tumor growth. A proprietary tandem "bi-functional" short hairpin RNA (shRNA) was designed to silence gene expression of PDX-1. The bi-functional shRNA cassette has been demonstrated to be highly effective.

To investigate the efficacy and specificity of PDX-1 bi-shRNA in insulinoma and pancreatic cancer, miR30-based bi-functional shRNA cassettes against either human PDX-1 or mouse PDX-1 were cloned into pUMVC3 vector (currently used in clinical studies of the TAG and FANG cancer vaccines). Bi-functional shRNAs were electroporated into either a mouse insulinoma cell line with significant endogenous expression of mouse PDX-1 or a human colon cancer cell line with overexpressed PDX-1. Stem-loop PCR was used to detect processed mature shRNAs, while RACE-PCR was employed to examine potential cleavage products of human and mouse PDX1 mRNA. RT-QPCR and immunoblotting were used to examine the knockdown of expression of either human PDX-1 or mouse PDX-1.

The processed mature shRNAs were detected 24 hours after transfection and sequence confirmed. RACE-PCR analysis showed that both human PDX1 mRNA and mouse PDX1 mRNA were precisely cleaved in the center of target region as predicted by corresponding bi-functional shRNAs. In addition, the bi-functional shRNA targeting human PDX-1 did not cause the cleavage of the mouse PDX-1 mRNA and vice versa. The silencing effect of bi-functional shRNA on human PDX-1 was observed 24 hours after transfection and lasted for at least 96 hours. The maximum silencing effect, 80% knockdown of human PDX-1, was achieved 72 hours after transfection. Moreover, bi-functional shRNA targeting mouse PDX-1 did not affect the expression of human PDX-1. Similarly, expression of mouse PDX-1 was silenced 24 hours after transfection and the silencing effects lasted for at least for 96 hours. The maximum silencing effect, 95% of mouse PDX-1 expression, was observed 48 hours after transfection. Moreover, bi-functional shRNAs targeting human PDX-1 did not alter the expression of mouse PDX-1 either.

This study demonstrates the efficacy and species specificity of bi-functional shRNAs targeting either mouse or human PDX-1.