Thymidylate Synthase Overexpression Accelerates MEN1-Mediated Pancreatic Neuroendocrine Tumor Development; A New Target for AAV-Based Therapy

HYPOTHESIS: The incidence of pancreatic neuroendocrine tumors (PanNET) is increasing and few therapeutic options are available. We hypothesize that human thymidylate synthase (hTS) accelerates development of MEN1-driven PanNET and that new strategies to incorporate TS inhibition within current cancer treatment will prevent development and progression of PanNET.

BACKGROUND/AIMS: Elevated level of Thymidylate Synthase (TS) plays a direct causal role in tumorigenesis in vitro and overexpression of human TS (hTS) in transgenic mice promotes development of adenomas in the endocrine pancreas in vivo. Pancreatic islet tumor formation in hTS transgenic mice occurred with a long latency period, suggesting that additional somatic events are required to promote PanNET formation and progression. Men1 was recently shown to be the most commonly mutated tumor suppressor gene in sporadic PanNETs (44% of PanNET patients have MEN1 mutations). Mice with conditional knockouts of Men1 gene in the pancreatic islets develop pancreatic islet tumors with long latency after homozygous inactivation of Men1 gene, suggesting that further sequential somatic events are required for tumor formation. The lack of suitable animal models that recapitulate human disease has limited development and testing of new treatments for PanNET. We established a mouse model designated hTS/Men1−/−, where hTS is overexpressed in pancreatic islet cells carrying a conditional Men1 null allele. Since hTS overexpression accelerates PanNET development in Men1 null mice our research goals focus on defining the mechanism underlying the ability of high levels of hTS to accelerate tumor growth and to develop new treatment strategies.

METHODS: We crossed hTS transgenic mice with conditional Men1 null mice that normally develop pancreatic islet carcinoma with long latency. We compared the effect of hTS overexpression on the lifespan of Men1−/− mice vs. hTS/Men1−/− mice. To test whether high levels of hTS results in earlier PanNET development, we sacrificed hTS/Men1−/− and control Men1−/− mice at 5, 6.5 and 8 months of age (n=16 for each age group) and compared tumor development. To determine whether hTS increase mutation frequency, we crossed hTS/Men1−/− mice with Big Blue® transgenic mouse that serves as a mutation detection system. We analyzed mutation frequency in tumors isolated from hTS/Men1−/− and Men1−/− mice. We also used AAV vectors as delivery of TS shRNA and measured TS levels, tumor progression and survival of hTS/Men1−/− mice.

RESULTS & CONCLUSIONS: Our newly established hTS/Men1−/− mice model developed aggressive PanNET with 100% penetrance associated with overexpressed TS in Men1 null mice. TS expression induced islet carcinoma with shortened latency as compared to Men1−/− mice. The hTS/Men1−/− mice develop islet carcinoma as early as 6 month of age whereas Men1−/− mice develop islet carcinoma at 8 to 10 months of age. We also observed significant decrease of overall survival in hTS/Men1−/− mice as compared to Men1−/− mice (p<0.001). In addition, we observed that overexpression of TS results in the increase of mutational frequency in tumors derived from the hTS/Men1−/− as compared to control Men1−/− mice. Mutations such as transitions, transversions, insertions and deletions were 3.2 fold higher in tumors isolated from hTS/Men1−/− as compared to control Men1−/− mice. We showed that high levels of TS increase mutational frequency that may accelerate PanNET progression in hTS/Men1−/− mice. To evaluate the effect of TS inhibition in PanNET progression, we delivered AAV-TS shRNA to the endocrine pancreas of hTS/Men1−/− mice and showed that AAV-TS shRNA, specifically targeted pancreatic islet cells, decreased TS expression, significantly decreased PanNET progression and increased survival of hTS/Men1−/− mice.