

Tissue-Specific Tumorigenesis in Multiple Endocrine Neoplasia Type I. Juliet C. Gardiner, Sunita K. Agarwal, Asha Adem, Carmen Sánchez Claros, Sylvia Lee, Daniel Slegowski, Arnold J. Levine, Ziqiang Yuan, Steven K. Libutti. Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey.

Background/Significance to NETs: Multiple Endocrine Neoplasia type 1 (MEN1) is an inherited cancer syndrome caused by a germline mutation in the *MEN1* gene. About 90% of MEN1 patients inherit a heterozygous germline mutation in the *MEN1* gene followed by loss of heterozygosity of the remaining *MEN1* allele, defining *MEN1* as a tumor suppressor gene. MEN1 is a rare disease having a prevalence of 1 in 30,000 people, with about 95% penetrance by 50 years of age. Tumors caused by MEN1 syndrome pose a challenge due to their metastatic potential, growth rate and resistance to treatment. While a germline heterozygous mutation in the Multiple Endocrine Neoplasia type 1 (MEN1) gene predisposes tumor formation in specific tissues such as the endocrine pancreas, parathyroid glands and anterior pituitary, this tissue-specific tumorigenesis is not dependent on *MEN1* mutations alone. In fact, a homozygous deletion of *Men1* in mouse pancreatic exocrine tissue does not result in tumor formation, suggesting a tissue-specific mechanism. Loss of menin activates a menin-interacting protein retinoblastoma-binding-protein 5 (RBBP5). Since RBBP5 transcriptionally regulates DNA methyltransferase 1 (*DNMT1*), this causes global DNA hypermethylation and subsequent tumorigenesis in MEN1-target endocrine tissues. We hypothesize that while RBBP5 is ubiquitously expressed, it exclusively binds to the *DNMT1* promoter in MEN1-target-tissues through its recruitment by tissue-specific factors.

Materials and Methods/Experimental Approach: CHIP-PCR was used to determine whether Rbbp5 exhibits tissue-specific occupancy at the *Dnmt1* promoter in various MEN1-target and non-target mouse tissues. To investigate the tissue-specific occupancy of Rbbp5 at the *Dnmt1* promoter, a CHIP-Seq analysis was carried out on DNA purified from pancreatic endocrine islet cells and pancreatic exocrine cells from wild type (WT) mice. These DNA samples were analyzed to identify differentially occupied DNA binding sites of menin and Rbbp5 by a high-throughput tissue-specific CHIP-seq assay. This analysis yielded a list of endocrine-specific genes regulated by both menin and Rbbp5. In order to further identify the tissue-specific factors that may alter menin-Rbbp5 binding to the *Dnmt1* promoter, we performed tissue-specific RNA-Seq on endocrine and exocrine pancreas tissues from WT mice. Integrating tissue-specific CHIP-Seq and RNA-seq data, we identified 20 endocrine tissue-specific factors that may alter the interaction of the menin-Rbbp5 complex with the *Dnmt1* promoter. By carrying out an unbiased genome-wide screen, we identified endocrine-specific candidate factors that may interact with the menin-Rbbp5 complex at the *Dnmt1* promoter. Of these 20 candidate factors, several of the top candidates were screened for their expression in MEN1-target tissues versus non-MEN1-target tissues and the stand-out candidates were identified for further validation. IHC was carried out to determine tissue-specific expression of these factors and co-IP was carried out to determine whether the factors form a protein complex with Rbbp5. **Results:** Using CHIP-PCR, we demonstrated that Rbbp5 is bound to the *Dnmt1* promoter in MEN1-target-tissues, while not in non-target tissues. Following a high-throughput genome-wide approach, we identified candidate factors, Nkx2.2 and Pax5, that may recruit Rbbp5 to the *Dnmt1* promoter. Immunohistochemistry revealed MEN1-target-tissue-specific expression of the candidate factors and co-IP revealed MEN1-target-tissue-specific binding of Rbbp5 to the factors. **Conclusions/next steps:** In conclusion, Rbbp5 binds the *Dnmt1* promoter in MEN1-target-tissues and we have identified candidates for Rbbp5 recruitment to the *Dnmt1* promoter that must be tested further to determine their role in the observed tissue specificity of MEN1-related tumorigenesis. We intend to further investigate the role of these tissue-specific factors *in vitro* and *in vivo* as the next step in understanding their role in MEN1 tumorigenesis. First, *in-vitro* knock-out of the candidate factors will be performed in a mouse β -cell line to determine their role in the recruitment of Rbbp5 to the *Dnmt1* promoter. Then, *in-vivo* tissue-specific KO of the candidate factors in the pancreatic β -cells of a *Men1* KO mouse

followed by an evaluation of tumor formation and blood insulin levels will help to further elucidate the role of the candidate factors in MEN1 tumorigenesis. We hypothesize that the inhibition of these Rbbp5 recruitment factors in MEN1-target tissues *in vivo* may reveal a significant target for therapeutic intervention. Expanding on the tissue-specific mechanism of MEN1 tumorigenesis is vital in developing a targeted therapeutic approach for treating MEN1 syndrome and will lay a groundwork for other tissue-specific diseases.