HHS Public Access

Author manuscript

Epigenomics. Author manuscript; available in PMC 2017 May 11.

Published in final edited form as:

Epigenomics. 2013 ; 5(5): 469–471. doi:10.2217/epi.13.47.

Menin/PRMT5/Hedgehog signaling: A Potential Target for the Treatment of MEN1 Tumors

Buddha Gurung[1] and **Xianxin Hua**[1]

[1]Abramson Family Cancer Research Institute, Dept. of Cancer Biology, University of Pennsylvania Perelman School of Medicine, 421 Curie Blvd., BRB II/III, Philadelphia, PA-19104, USA

Multiple Endocrine Neoplasia Type I (MEN1) is an autosomal dominant inherited tumor syndrome characterized by development of tumors in multiple organs including parathyroid organs, pancreatic islets, pituitary gland, and some endocrine organs [1]. The gene mutated in this syndrome, *MEN1*, is located on chromosome 11q13 and encodes a nuclear protein, menin [2]. Menin is highly conserved from Drosophila to humans; however it does not exhibit any homology to known proteins [2]. A germline *MEN1* mutation leads to familial MEN1, and is also often detected in sporadic pancreatic neuroendocrine tumors [3]. The precise biochemical mechanisms underlying the function of menin in suppressing multiple endocrine tumors are not well understood,, and the recently solved crystal structure [4] indicates that it acts as a scaffold protein interacting with distinct partner proteins. For instance, menin interacts with JUND and represses its transcriptional activity by blocking JUN N-terminal kinase (JNK)-mediated JUND phosphorylation [4,5]. Menin also interacts with mixed lineage leukemia protein 1 (MLL1), a histone H3 lysine 4 methyltransferase and the chromatin-anchoring protein LEDGF and upregulates expression of cyclin-dependant kinase inhibitors, $p18^{Ink4c}$ and $p27^{Kip1}$, by increasing histone H3 lysine 4 tri-methylation (H3K4m3) [6,7]. Furthermore, menin acts as a central hub controlling mixed lineage leukemia by recruiting both wild type MLL and oncogenic MLL-fusion protein (MLL-FP) to the loci of their target genes in certain leukemias [8]. Thus epigenetic regulation of gene expression represents a key aspect of menin's function including disease, and the modulation of its epigenetic targets holds considerable therapeutic potential including the MEN1 syndrome.

Recently, we reported that menin interacts with another epigenetic modulator, protein arginine methyltransferase 5 (PRMT5). The interaction between endogenous menin, PRMT5 and its co-factor MEP50 was shown, and the menin-interacting domain was mapped to the N-terminus of PRMT5. PRMT5 symmetrically methylates histone H4 arginine 3 (H4R3m2s) and histone H3 arginine 8 (H3R8m2s), repressing gene transcription [9]. Menin recruits PRMT5 and its repressive histone methylation mark, H4R3m2s, to the promoter of the *Gas1* gene [10] and represses its expression. GAS1, along with two accessory protein CDO and BOC [11,12], facilitates binding of HH ligand to the cell surface receptor Patched (PTCH1), resulting in the release of PTCH1-mediated repression of the cell membrane

Correspondence: Xianxin Hua, Abramson Family Cancer Research Institute, Department of Cancer Biology, University of Pennsylvania Perelman School of Medicine, 421 Curie Blvd., 420 BRB II/III, Philadelphia, PA-19104, USA. Tel. 215-746-5567, Fax: 215-745-5525, huax@mail.med.upenn.edu.

protein Smoothened (SMO) [13] and subsequent activation of HH signaling. Excision of *Men1* in cells and in primary pancreatic islets isolated from *Men1*-excised mice demonstrates increased HH signaling as evidenced by elevated mRNA levels of *Gas1*, *Gli1* and *Ptch1*, targets of the HH signaling pathway. Similarly, PRMT5 knockdown in cells resulted in increased levels of *Gas1*, and enhanced HH ligand-mediated signaling. These findings suggest that the epigenetic repression of HH signaling by the menin-PRMT5 complex represents a novel means for menin-mediated repression of gene expression. Establishing a mouse model in which *Prmt5* can be conditionally excised in the pancreatic islets would be valuable for further validating these findings, and demonstrating unambiguously the role of repressive histone modifications and epigenetics in the pathogenesis of the MEN1 syndrome.

The HH signaling pathway is highly conserved, and is essential for embryonic development and tissue homeostasis in adults [13]. Aberrant regulation of the HH signaling pathway has been reported in many cancers including basal cell carcinoma (BCC) [14] and medulloblastoma [15]. Furthermore, HH ligand expression or HH signaling is enhanced in human gastrointestinal neuroendocrine tumors and in mouse small cell lung cancer [16,17]. Transgenic mouse embryos deficient in HH signaling display delayed pancreas formation resulting in reduction of the pancreatic epithelium and beta cell numbers [18]. However, the beta cell numbers recover by birth but the mice display glucose intolerance, increased insulin sensitivity and reduced production of total insulin [18], clearly indicating that the HH signaling plays an important role in the development and proper functioning of the endocrine pancreas. It has also been reported in genetically engineered mouse models of pancreatic ductal adenocarcinoma (PDAC) that inhibition of HH signaling results in depletion of the stroma surrounding the tumor, stimulating angiogenesis and enhancing delivery of chemotherapeutic drugs [19]. However, it was not previously known whether HH signaling plays a role in pancreatic neuroendocrine cells.

To determine whether aberrant HH signaling induced upon *Men1* excision plays an important role in MEN1, we examined the expression levels of target genes in an established MEN1 mouse model which largely phenocopies the human MEN1 tumor syndrome [20]. It is recently reported that *Men1*-excised mice have elevated levels of HH signaling as determined by increased mRNA levels of *Gas1*, *Gli1* and *Ptch1* in isolated pancreatic islets compared to their wild type (WT) littermates. Furthermore, inhibition of the HH signaling pathway with GDC-0449, a SMO antagonist [21], resulted in significant reduction in the proliferation of insulinoma cells and reduced insulin secretion. GDC-0449 also known as vismodegib and currently marketed as Erivedge, is an FDA-approved drug used for treating metastatic or locally advanced BCC in adults where surgery and radiation are not applicable, and according to the clinical trials database at the National Institute of Health (NIH) is undergoing clinical trials for various cancers including metastatic colorectal cancer, medulloblastoma, ovarian cancer and metastatic pancreatic cancer (ClinicalTrials.gov Identifier NCT00959647, NCT00878163). The potential use of an existing FDA-approved drug for treating a subset of MEN1 patients with elevated levels of HH signaling thus holds considerable promise.

The relevance of menin-PRMT5 interaction and MEN1 syndrome was examined *in vitro* by complementing menin-null cells with either WT or two physiologically relevant point mutants, A242V and L22R. The interaction between menin and PRMT5, and histone methyltransferase (HMT) activity towards recombinant histone H4 are considerably decreased in the two MEN1 disease-related menin point mutants. Additionally, menin-null cells complemented with the menin mutants fail to repress *Gas1* mRNA levels indicating that loss of menin-mediated repressive histone methylation, H4R3m2s, at the *Gas1* promoter and aberrant HH signaling plays a role, at least in part, towards pathogenesis of the MEN1 syndrome.

To further examine whether HH signaling is aberrantly upregulated in MEN1 patients, the levels of *GLI1* in tumor and normal tissues from pancreatic sections needs to be quantitated, and correlated with the expression levels of menin. Elevated levels of *GLI1* mRNA and its correlation with loss of menin expression in MEN1 tumors from patients will further reinforce the notion of treating MEN1, and sporadic neuroendocrine tumors harboring *MEN1* mutation with enhanced HH signaling with Erivedge.

Currently, no curative treatment for insulinomas in MEN1 patients exists. Surgical removal of part of the pancreas (distal pancreatectomy) to combat insulin imbalance due to excessive insulin secretion by insulinomas in benign tumors as well as malignant tumors, and nearby lymph nodes appear to be the only prevalent options [22]. However, surgical approach to pancreatic endocrine tumors in MEN1 patients is controversial with relatively high rates of recurrence with some treatment-related morbidity and mortality [23]. Thus novel, non-surgical means for treating specific symptoms in MEN1 patients harboring insulinomas is currently lacking and highly desirable.

MEN1 is an autosomal inherited tumor syndrome, and genetic testing is currently readily available for detecting *MEN1* mutations, particularly for the population prone to the disease. Thus, pro-active measures including treatment with GDC-0449 to delay or even prevent the onset of tumorigenesis in MEN1 patients with upregulated HH signaling is very intriguing and remains to be tested. Inhibition of HH signaling by administration of GDC-0449 to MEN1 mouse models prior to development of insulinomas will yield clues as to whether overt activation of HH signaling in the pancreatic islets represents a necessary step for initiation and onset of the MEN1 disease. Long-term administration of GDC-0449 might be tolerated or acceptable especially for adults as the adverse side effects in clinical trials were relatively mild events including muscle spasms, alopecia, taste disturbance and fatigue [24]. Additionally, treatment of wild-type mice twice daily with GDC-0449 for four weeks did not result in gross abnormalities or changes to the pancreatic islet morphology. Given that inhibition of HH signaling reduced proliferation of insulinoma cells in a MEN1 mouse model, and insulin production was decreased in adult mice lacking SMO and HH signaling [18], administration of GDC-0449 in MEN1 patients with aberrant HH signaling may improve the therapy for this group of patients.

An important aspect of menin's function is defined by its interactions with epigenetic modulators including MLL and PRMT5. Further understanding of menin-mediated epigenetic modifications and characterization of the altered signaling pathways is garnering

considerable interest, particularly as it pertains to several malignancies including leukemia, MEN1 and Type 2 Diabetes (T2D).

Acknowledgments

Grant Support: We appreciate support from AACR Care for Carcinoid Foundation (11-60-33XH) and the NIH (RO1-CA-113962 ND RO1-DK085121 to XH).

References

1. Marx, Sj. Molecular genetics of multiple endocrine neoplasia types 1 and 2. Nat Rev Cancer. 2005; 5(5):367–375. [PubMed: 15864278]
2. Chandrasekharappa, Sc, Guru, Sc, Manickam, P., et al. Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science. 1997; 276(5311):404–407. [PubMed: 9103196]
3. Lemos, Mc, Thakker, Rv. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. Hum Mutat. 2008; 29(1):22–32. [PubMed: 17879353]
4. Huang J, Gurung B, Wan B, et al. The same pocket in menin binds both MLL and JUND but has opposite effects on transcription. Nature. 2012; 482(7386):542–546. [PubMed: 22327296]
5. Agarwal, Sk, Guru, Sc, Heppner, C., et al. Menin interacts with the AP1 transcription factor JunD and represses JunD-activated transcription. Cell. 1999; 96(1):143–152. [PubMed: 9989505]
6. Milne, Ta, Hughes, Cm, Lloyd, R., et al. Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. Proc Natl Acad Sci U S A. 2005; 102(3):749–754. [PubMed: 15640349]
7. Karnik, Sk, Hughes, Cm, Gu, X., et al. Menin regulates pancreatic islet growth by promoting histone methylation and expression of genes encoding p27Kip1 and p18INK4c. Proc Natl Acad Sci U S A. 2005; 102(41):14659–14664. [PubMed: 16195383]
8. Thiel, At, Blessington, P., Zou, T., et al. MLL-AF9-induced leukemogenesis requires coexpression of the wild-type Mll allele. Cancer Cell. 2010; 17(2):148–159. [PubMed: 20159607]
9. Pal S, Vishwanath Sn, Erdjument-Bromage H, Tempst P, Sif S. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. Mol Cell Biol. 2004; 24(21):9630–9645. [PubMed: 15485929]
10. Gurung B, Feng Z, Iwamoto Dv, et al. Menin epigenetically represses Hedgehog signaling in MEN1 tumor syndrome. Cancer Res. 2013; 73(8):2650–2658. [PubMed: 23580576]
11. Allen, Bl, Song, Jy, Izzi, L., et al. Overlapping roles and collective requirement for the coreceptors GAS1, CDO, and BOC in SHH pathway function. Dev Cell. 2011; 20(6):775–787. [PubMed: 21664576]
12. Martinelli, Dc, Fan, Cm. Gas1 extends the range of Hedgehog action by facilitating its signaling. Genes Dev. 2007; 21(10):1231–1243. [PubMed: 17504940]
13. Jiang J, Hui Cc. Hedgehog signaling in development and cancer. Dev Cell. 2008; 15(6):801–812. [PubMed: 19081070]
14. Xie J, Murone M, Luoh Sm, et al. Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature. 1998; 391(6662):90–92. [PubMed: 9422511]
15. Thompson, Mc, Fuller, C., Hogg, Tl, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. J Clin Oncol. 2006; 24(12):1924–1931. [PubMed: 16567768]
16. Shida T, Furuya M, Nikaido T, et al. Sonic Hedgehog-Gli1 signaling pathway might become an effective therapeutic target in gastrointestinal neuroendocrine carcinomas. Cancer Biol Ther. 2006; 5(11):1530–1538. [PubMed: 17102592]
17. Park, Ks, Martelotto, Lg, Peifer, M., et al. A crucial requirement for Hedgehog signaling in small cell lung cancer. Nat Med. 2011
18. Lau J, Hebrok M. Hedgehog signaling in pancreas epithelium regulates embryonic organ formation and adult beta-cell function. Diabetes. 2010; 59(5):1211–1221. [PubMed: 20185815]

19. Olive, Kp, Jacobetz, Ma, Davidson, Cj, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009; 324(5933):1457–1461. [PubMed: 19460966]

20. Crabtree, Js, Scacheri, Pc, Ward, Jm, et al. Of mice and MEN1: Insulinomas in a conditional mouse knockout. Mol Cell Biol. 2003; 23(17):6075–6085. [PubMed: 12917331]

21. Rudin, Cm, Hann, Cl, Laterra, J., et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. N Engl J Med. 2009; 361(12):1173–1178. [PubMed: 19726761]

22. Tucker, On, Crotty, Pl, Conlon, Kc. The management of insulinoma. Br J Surg. 2006; 93(3):264–275. [PubMed: 16498592]

23. Hausman, Ms, Jr, Thompson, Nw, Gauger, Pg, Doherty, Gm. The surgical management of MEN-1 pancreatoduodenal neuroendocrine disease. Surgery. 2004; 136(6):1205–1211. [PubMed: 15657577]

24. Sekulic A, Migden Mr, Oro Ae, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med. 2012; 366(23):2171–2179. [PubMed: 22670903]