

## Aberrant Activation of DNA Methyltransferase 1 (DNMT1) Mediated Global DNA Methylation and Implicates Sox/Wnt/ $\beta$ -Catenin Signaling Pathway in the Multiple Endocrine Neoplasm Type 1 (MEN1) Syndrome

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**Background:** MEN1 is caused by mutations in the *MEN1* gene which encodes menin. However, little is known about their pathogenesis. The present study is to identify important pathways involved in MEN1 tumorigenesis using our developed a global epigenetic approach as well as established human endocrine tumor tissue banks, *Men1* conditional knockout (KO) animals, and cell lines.

**Methods:** Human endocrine tissues, *Men1* KO mice and mouse *Men1* null cell lines were used for this study. DNA methylation patterns were demonstrated by HpaII tiny fragment enrichment by ligation-mediated PCR (HELP)-tagging genome-wide analysis. Validation of the findings for HELP-tagging was carried out by Prosequence and Sequenome. RNA and protein were measured by real-time PCR and immunofluorescence assays. Enzyme activity of DNMT1 was measured by a functional assay.

**Results:** Global DNA methylation was identified in MEN1 parathyroid hyperplasia. Rbbp5, a complex with menin was identified that it interacted with DNMT1 by ChIP-CHIP analysis and the expression and activity of DNMT1 were increased in tumor tissues of parathyroid and pancreas from MEN1 patients and *Men1* KO mice. The expression and activity of DNMT1 was upregulated by knockdown of menin with menin siRNA in mouse *Men1* wild type cell line. The expression and activity of DNMT1 was downregulated by inducing menin and/or knockdown of Rbbp5 with Rbbp5 siRNA in mouse null cell line. Bioinformatics and Ingenuity Pathway analysis showed that Sox genes in the Wnt/ $\beta$ -Catenin signaling pathway were involved. The hypermethylation of these Sox genes resulted in the downregulation of Sox genes and increased  $\beta$ -Catenin expression in MEN1 tumor tissues.

**Conclusion:** These data suggest that the inactivation of menin enhanced the activity of DNMT1 by Rbbp5 activation in MEN1 tumor tissues. The activity of DNMT1 further mediated global DNA methylation which involved aberrant activation of Sox/Wnt/ $\beta$ -Catenin signaling pathway in the promotion of MEN1 tumor development.