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Title: EHMT1 activity in prostate cancer is regulated by dual lysine-methylations

Background: Lysine methylation is an important post-translational regulation on histones, but its molecular functions on non-histone proteins remain largely unknown. In a pilot study, we identified novel lysine methylations at non-histone proteins in prostate cancer (PCa) cells through an affinity pull-down assay. EHMT1/GLP, containing monomethylated K450 and K451, was one of these proteins, and it was known to repress transcription through methylating H3K9. EHMT1 and EHMT2/G9a are members of the REST repressor complex, that includes LSD1/CoREST/HDACs and represses neuronal genes in non-neuronal cells. Loss of function for this complex has been suggested as a mechanism to drive neuroendocrine (NE) transition of PCa. We hypothesize that the K450/451 methylations may inhibit the activity of EHMT1 and that LSD1 may demethylate these lysines and thus enhance EHMT1 activity.

Methods: LNCaP stable cell lines overexpressing doxycycline-regulated V5-tagged EHMT1-WT, K450R, K451R, and K450/451R were generated. We performed ChIP-seq analyses of V5 and H3K9me2, and RNA-seq analyses in all four cell lines treated with/out doxycycline. We also conducted *in vitro* demethylation assays to examine whether LSD1 directly demethylates EHMT1. Moreover, we performed co-immunoprecipitation assays to determine the effect of LSD1 inhibition on the interaction of LSD1 with EHMT1.

Results: The K450/451R mutant had a significant increase of chromatin binding in V5 and elevated levels of H3K9me2 compared to the weak chromatin binding seen in WT, K450R and K451R**.** Analyses in RNA-Seq revealed that the K450/K451R mutant repressed genes enriched for neuronal pathways, which was not seen in WT, K450R or K451R. The demethylation assays indicated that methylated K450 but not K451 is a potential substrate of LSD1. Furthermore, we confirmed the interactions of LSD1 with EHMT1 and found that LSD1 inhibition repressed the interaction of LSD1 with EHMT1.

Conclusions: Our studies suggest that K450 of EHMT1 is specifically demethylated by LSD1, but this alone is not sufficient to induce the chromatin recruitment of EHMT1. However, demethylation at both K450/K451 can dramatically increase the chromatin binding of EHMT1, which subsequently represses neuronal pathways. This mechanism may be particularly important for maintaining the prostate epithelial cell lineage and loss of this demethylation might lead to the emergence of NE-like PCa.