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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Activating *NSD2* Mutations Drive Oncogenic Reprogramming By Disturbing Epigenetic Landscape in Mantle Cell Lymphoma

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Background: NSD2/MMSET/WHSC1, a histone lysine methyltransferase (HMT), is an oncoprotein first characterized by its overexpression in multiple myeloma (MM). *NSD2* mutations within the catalytic SET domain are frequently identified in lymphoid malignancies including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and mantle cell lymphoma (MCL). We previously demonstrated that *NSD2* p.E1099K mutation caused an imbalance of H3K36me2/H3K27me3 and drove glucocorticoid resistance in pediatric ALL. In MCL, *NSD2* mutations, especially p.E1099K and p.T1150A, are found in 10-15% of cases of MCL and are enriched in MCL patients who relapse from targeted therapies such as ibrutinib, an inhibitor of B cell signaling. However, the activity of *NSD2* mutations in MCL remains unexplored. We hypothesize that *NSD2* mutations contribute to disease progression and therapy resistance due to aberrant chromatin modification.
Aim: To demonstrate the role of *NSD2* mutations in MCL progression due to aberrant chromatin modification.
Methods: We created isogenic MCL cell lines by knock-in *NSD2* p.E1099K and *NSD2* p.T1150A mutation into Z138 and Jeko-1 cell lines using CRISPR/Cas9 gene editing. We then determined H3K36me2/H3K27me3/H3K36me3 using immunoblot and the biological activities including cell growth (IncuCyte), apoptosis (Annexin V/PI staining), cell cycle (BrdU incorporation), and *in vivo* experiments using luciferase-tagged cell lines in NOD-SCID mice. We determined alteration of gene transcriptome using RNA-Seq and histone modification using CUT&RUN (H3K36me2, H3K27me3 and H3K4me3) and ChIP-Seq (H3K27ac) in isogenic Z138 cell lines. Finally, we integrated multi-omics analysis of our MCL cell lines and human patient samples and to comprehensively disclose the epigenetic landscape in MCL with *NSD2* mutations.
Results: We successfully created isogenic Z138 cell lines with *NSD2* p.E1099K mutation or p.T1150A mutation, and isogenic Jeko-1 cell line with *NSD2* p.E1099K mutation. Insertion of either *NSD2* p.E1099K mutation or p.T1150A mutation by CRISPR/Cas9 gene editing led to a striking increase of H3K36me2, a decrease of H3K27me3 but no change of H3K36me3. *NSD2* p.E1099K mutation significantly increased cellular growth and enhanced S phase entry while decreased G0/G1 phase and apoptosis. In NOD-SCID mouse xenografts, *NSD2* p.E1099K mutation resulted in a higher tumor burden and shorter survival lifespan in mice. RNA-Seq analysis demonstrated striking changes in gene expression with 1794 genes upregulated and 1492 genes downregulated in *NSD2* mutant MCL cells. Among them, anti-apoptotic genes *BCL2* and *BCL2L2* were upregulated while pro-apoptotic genes *BAX*, *BID*, and *BIK* were downregulated in *NSD2* mutant cells, which was consistent with the biological phenotypes. Surprisingly, *CCND1*, *CCNE1*, *CCNE2*, *TP53*, and *CDKN2C* (*p21)* were downregulated while *CDKN2D*, *CDKL3*, and *CDKL5* were upregulated in *NSD2* mutant cells. Altered gene expression in isogenic Z138 cells was compared with patient expression profiles, with gene ontology revealing significant overlap and enrichment of cell adhesion, neural pathways, and evidence of activated signaling. *NSD2* mutation drove these oncogenic reprogramming by disturbing epigenetic landscape as increased levels of H3K36me2, H3K4me3 and H3K27ac but decreased levels of H3K27me3, which contributed to aberrant gene expression patterns.
Conclusions: The *NSD2* mutation led to increased tumor cell growth but decreased apoptosis due to the dysregulation of epigenetic landscape and gene expression, suggesting an oncogenic reprogramming driven by activated *NSD2* mutations in MCL.

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