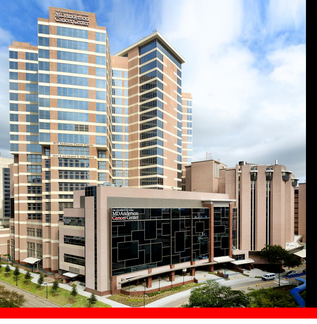

KMT2D Loss Promotes Head and Neck Squamous Cell Carcinoma Through Modulation of Immune Microenvironment

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world. Recent data from the field of Epigenetics has shown that in cancer cells, there is reprogramming of all components of the epigenetic machinery. Common epigenetic mechanisms include DNA methylation and histone modifications.

KMT2D is a major mammalian histone 3 lysine mono-methyltransferase that functions as a scaffold protein. It regulates transcription by binding to enhancers and promotors. It plays a role in development, metabolism, and differentiation. KMT2D has been shown to alter the tumor microenvironment (TME) and to act as a tumor suppressor in various cancer settings.

TME consists of diverse cell types, including immune components, whose interaction with tumor cells is dictated by communication between the two compartments in the form of cytokines, signaling molecules, and antigen presentation.

TAP2 and ERAP1 are two proteins involved in antigen presentation. TAP2 translocates short peptides to the endoplasmic reticulum, where it is broken down by ERAP1 into smaller fragments. These fragments are then presented on the cell membrane via MHC1.

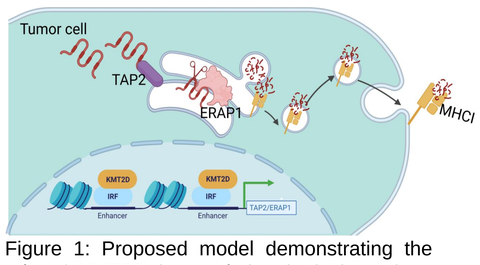

Figure 1: Proposed model demonstrating the role of KMT2D in regulating intrinsic antigen presentation machinery in HNSCC

Objective

Study KMT2D as a tumor suppressor factor in HNSCC and its role in regulating antigen processing and presentation machinery.

Methods

•Cell culture and generation of KMT2D knockdown (KD) HN30 cell lines: HN30 cell lines were cultured in DMEM supplemented with 10% FBS, L-glutamine, sodium pyruvate, non-essential amino acids, vitamins and 1% penicillin-streptomycin. KD cell lines were generated with short hairpin RNAs (shRNAs) and lentiviral system.
•TCGA data analysis: For TGCA analysis and WES, clinical data were downloaded from firebrowse, samples with evidence of HPV infection were excluded. Analysis of mutations were carried out using the Maftools R package.
•RNA-seq on cell lines: RNA extraction was performed using Rneasy Mini Kit. Isolation of mRNA was performed using NEBNext Poly(A) mRNA Magnetic Isolation Module and libraries were prepared using NEBNexet Ultra II Directional RNA Library prep kit and utilized for subsequent NGS.
•WES analysis: All mutations were binarized to 1 (mutated) or 0 (non-mutated) . The jacquared distance matrix was computed, the resulting matrix was clustered using wards minimum variance method.
•*In vivo* mouse studies: 4 - 6 week old female mice on C57BL/6 background were injected into tongue with doxycycline inducible shKMT2D KD and shNT MOC1 cell lines. Subsequently doxycycline containing water was administered.
•Western Blot: Western blot was performed after protein isolation following a standard protocol from BioRad.

Results

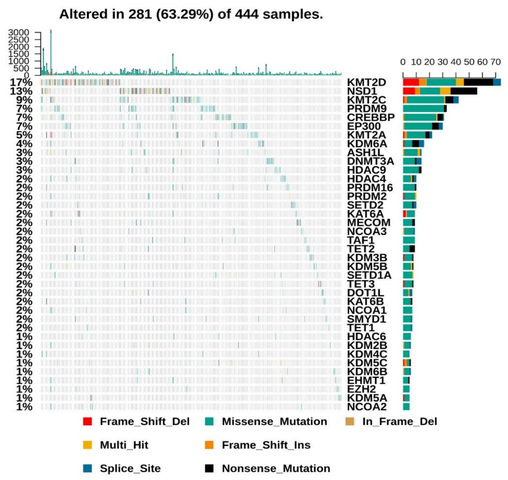

Figure 2: Oncoplot of epigenetic modifiers (histone acetyltransferases and deacetylases, histone methyltransferases and demethylases, and DNA modifiers) in TCGA HNSCC.

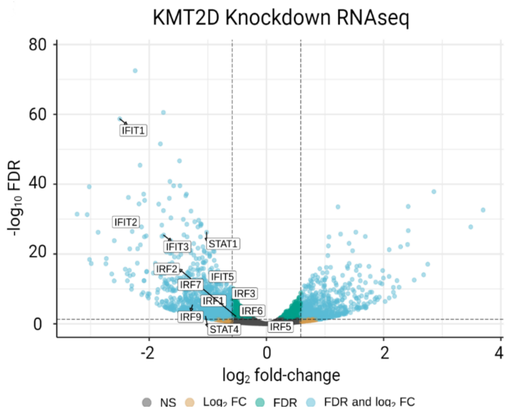

Figure 3: Volcano plot demonstrating differentially expressed genes in the HN30 cell line after knockdown with shKMT2D.

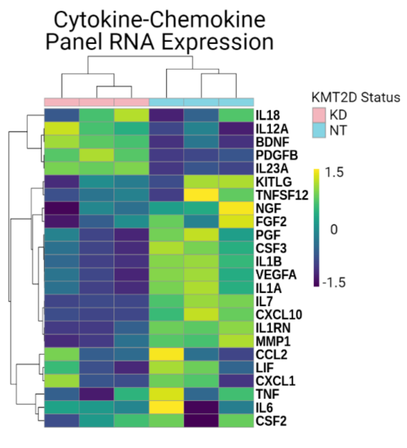

Figure 4: Hierarchical clustering of cytokine-chemokine gene panels based on HN30 shKMT2D RNA-seq data performed using Ward's method

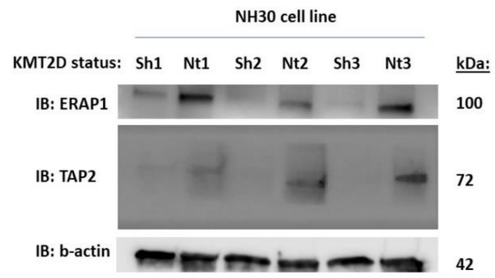

Figure 5: Western blot demonstrating the level of ERAP1 and TAP2 in KMT2D KD and KMT2D NT HN30 cell lines.

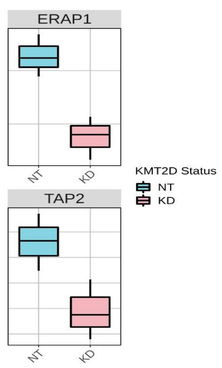

Figure 6: Boxplot showing the level of TAP2 and ERAP1 RNA expression using GO biological process.

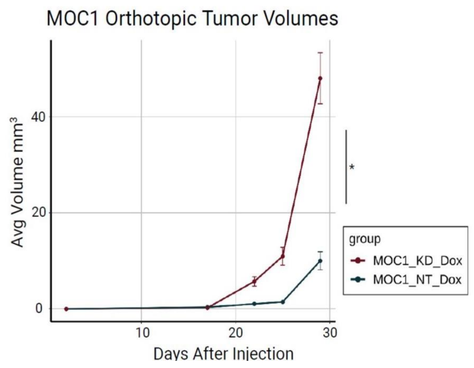

Figure 7: Tumor growth curve demonstrating the tumor size after injection of KMT2D KD and KMT2D NT MOC1 cell lines.

Discussion

KMT2D mutation is the most frequent epigenomic mutation in head and neck squamous cell carcinoma. KMT2D functions as a tumor suppressor and loss of function mutation drives tumorigenesis. This notion is supported by our in vivo studies: KMT2D KD tumors had larger volumes compared to NT tumors in our model. Moreover, analysis of TCGA deposited data demonstrates that patients harboring KMT2D loss-of-function mutations have lower two-year-recurrence free survival compared to KMT2D WT counterparts (Figure 8).

Our data shows that when KMT2D is knocked down in tumor cells, gene expression for interferon regulatory factors (IRF) as well as the level of chemokines and cytokines responsible for recruitment of immune cells into TME, are down-regulated. IRFs are a family of transcription factors that play pivotal roles in innate and adaptive immune responses.

Additionally, we observe a down-regulation of terms associated with antigen processing when KMT2D is deleted in tumor cells. RNA-seq and WB results demonstrate reduced levels of TAP2 and ERAP1 in knock-downed systems. Further studies are required to demonstrate the role of IRF factors in regulation of terms associated with antigen presentation and processing in HNSCC models.

Together, these findings show that KMT2D is a major epigenetic factor controlling HNSCC onset and give opportunities to explore novel treatment plans with immunomodulatory approaches for patients harboring KMT2D loss-of-function mutations.

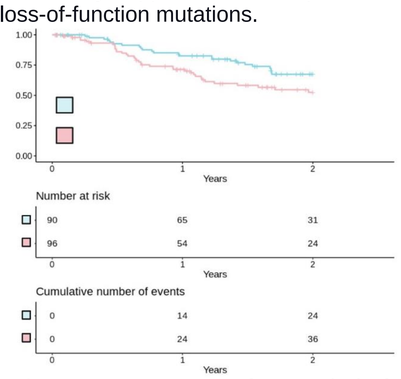

Figure 8: Two-year-recurrence free survival of KMT2D low expressing (pink) and KMT2D high expressing (light blue) patients in TCGA HNSCC (p = 0.031, log-rank test)

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