Friday, November 17, 2023, 2:00 p.m.
UT MD Anderson Cancer Center
Z7.1003A (Conference Room in Zayed building, 7th floor)
and via Zoom
Meeting ID: 850 2222 8045 | Password: 469903

Histone Lysine Methyltransferase NSD3 Governs Transcriptional Programs that Drive Pancreatic Neuroendocrine Tumors (PanNETs)

Mary Esmeralda Fuentes, BS (Advisor: Pawel K. Mazur, PhD)

Pancreatic Neuroendocrine Tumors (PanNETs) are the most common and lethal neuroendocrine malignancies. For decades, standard chemotherapeutic regimens and targeted therapeutics have been used with limited efficacy, adverse side effects, and treatment resistance. Thus, there is a critical need to uncover novel precision therapeutics for PanNET patients. Additionally, pre-clinical models that accurately represent disease are urgently necessary for translational studies.

This dissertation directly addresses these challenges by identifying histone lysine methyltransferase (KMT) NSD3 as a critical oncogenic driver and druggable therapeutic target. Briefly, NSD3 is known to canonically di-methylate Histone H3 lysine residue 36 (H3K36me2) resulting in global chromatin changes that select for gene expression programs with potential cancer implications. Thus, we hypothesized that NSD3, ***via* regulation of H3K36 di-methylation dynamics, governs transcriptional programs that drive PanNET tumorigenesis.**

We identified NSD3 expression to be elevated in human PanNETs and mouse models of disease. Thus, we first explored the role of NSD3 in PanNETs *in vitro*. These initial findings indicate that loss of NSD3 using the CRISPR-Cas9 system significantly reduces cell growth and decreases levels of H3K36me2, suggesting that NSD3 has a potential oncogenic function. Additionally, we generated a novel genetically engineered mouse model (GEMM) focusing on the pancreatic-islet-specific loss of conditional *Men1*$^{Lox/Lox}$, *Atrx*$^{Lox/Lox}$, and *Pten*$^{Lox/Lox}$ (*MAP*) alleles which are the top inactivating mutations observed in human disease. Our initial studies indicate that loss of *MAP* led to an increase in tumor burden and proliferation, as quantified by Ki67. In parallel, to assess the role of NSD3 *in vivo*, we generated and characterized a GEMM with conditional loss of *MAP* and *Nsd3*$^{Lox/Lox}$. Results from this model indicate that loss of *Nsd3* significantly attenuates tumor growth and proliferation *in vivo*. Overall, these findings indicate that NSD3 is an important regulator of PanNET tumorigenesis. However, questions regarding the enzymatic function and molecular mechanisms of action governed by NSD3 remain.

Therefore, to understand the role of the enzymatic activity of NSD3 in PanNET tumorigenesis, we performed studies *in vitro* using enzymatic mutants of NSD3. Briefly, we reconstituted the

following mutants in NSD3-depleted cells: NSD3 wild-type ($NSD3_{WT}$), enzymatic-deficient NSD3 ($NSD3_{Y1174A}$), and a hyperactive mutant to leverage overexpression of NSD3 ($NSD3_{T1232A}$). Findings from this portion of testing indicate that $NSD3_{T1232A}$ increases H3K36me2 and significantly increases proliferation *in vitro* and *in vivo* using xenografts, while $NSD3_{WT}$ rescues H3K36me2 and proliferation in NSD3-depleted cells. Interestingly, the enzymatic-deficient form of $NSD3_{Y1174A}$ did not rescue H3K36me2 or the phenotype observed. These results indicate that the catalytic activity of NSD3 is critical for PanNET tumor progression. Additionally, to leverage NSD3 hyperactivity *in vivo*, we generated a PanNET GEMM with conditional loss of *MAP* and expression of $NSD3_{T1242A}$ ($Rosa26^{LSL}$-$NSD3_{T1242A}$). Here, $NSD3_{T1242A}$ corresponds to human $NSD3_{T1232A}$. These results demonstrated that $NSD3_{T1242A}$ significantly decreases survival, accelerates tumor burden, and proliferation when compared to time-matched control and *Nsd3*-deficient mice. Overall, these results provide insights that NSD3 not only has a role in tumor progression, but that NSD3 regulates tumorigenesis via the H3K36me2 axis.

While we have investigated the role of NSD3, therapeutic targets continue to be unexplored, and its mechanism of action remains unclear. Thus, we address these questions in a twofold approach: by identifying amplified NSD3 ($NSD3_{T1232A}$) vulnerabilities using a characterized inhibitor library screen and by performing RNA-Seq to identify transcription factors regulated by NSD3. From this, we found several inhibitors that rendered sensitivities to $NSD3_{T1232A}$, of interest, AZD5153, a bivalent inhibitor of bromodomain and extraterminal (BET) domain/BRD4 proteins. Our findings indicate that treatment at low concentrations of AZD5153 decreased proliferation in human and primary cells with amplifications of NSD3 ($NSD3_{T1232A/T1242A}$). Lastly, RNA-sequencing revealed that expression of $NSD3_{T1232A}$ results in the upregulation of hallmark genes with respect to cell cycle pathways (G2M Checkpoint, E2F Targets), Myc Targets, and MTORC1 signaling. Taken together, these results correlate with previous findings that NSD3, while elevating MYC expression and having oncogenic properties in PanNETs, is therapeutically vulnerable to BET/BRD4 inhibition.

Taken together, this dissertation explores the epigenetic role of KMT NSD3 and establishes its oncogenic capacity in PanNETs while identifying actionable therapeutic targets.

Advisory Committee:
Pawel Mazur, PhD, Chair
Anirban Maitra, MBBS
Junjie Chen, PhD
Jae-Il Park, PhD
Michael Kim, MD
Di Zhao, PhD