The physiologically relevant functions of the Daxx/Atrx/H3.3 axis

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Background

Tumor sequencing studies have emphasized the role of epigenetics and altered chromatin homeostasis in cancer. Mutually exclusive mutations in *DAXX* and *ATRX*, a chaperone complex for the histone 3.3 (H3.3) variant, occur in approximately one third of pancreatic neuroendocrine tumors (PanNETs) implicating an important role in tumorigenesis.

Materials & Methods

To advance our understanding of physiological functions of this histone chaperone axis and gain insights to how mutations may contribute to tumor development, we have generated two new germline mouse models that specifically impair the interactions between Daxx and H3.3 ($Daxx^{S226A}$) and Daxx and Atrx ($Daxx^{Y130A}$). In the germline setting, these alleles allow us to study this importance of these protein interactions during embryonic development. Additionally, they can be combined with our conditional Daxx allele ($Daxx^{fl}$) to specifically interrogate the importance of these interactions in maintaining homeostasis and regulating endogenous retrovirus expression in the mouse pancreas.

Results

Germline deletion of *Daxx* is early embryonic lethal in mice. Surprisingly, Daxx mutant mice that are unable to interact with H3.3 ($Daxx^{S226A/S226A}$) survive to birth but are post-natal lethal. We have conducted a comprehensive transcriptome analysis from three embryonic tissues, with analysis of both the protein coding genes and transposable elements ongoing. Remarkably, Daxx mutant mice that are unable to interact with Atrx ($Daxx^{Y130A/Y130A}$) are both viable and fertile. Similar analysis is ongoing in embryonic tissues from these mutant mice to compare and contrast how the two mutants impact transcriptional states *in vivo*.

Conclusions/Next Steps

Combined, our results demonstrate that Daxx interactions with histone 3.3 and Atrx are not required for embryonic development. However, H3.3-dependent function(s) of Daxx are essential for post-natal survival. Current work is focused on using our genetically engineered mouse models to interrogate the Daxx/Atrx/H3.3 axis though comprehensive transcriptome and epigenome analysis. These studies contribute to our understanding of the physiologically relevant functions of these genes and inform the molecular underpinnings of pancreatic neuroendocrine tumors.

Lay Abstract

Cancers arise from DNA mutations that alter the properties of the cell, allowing for uncontrolled cell growth and tumor development. DNA mutations have been profiled in many different cancers, including pancreatic neuroendocrine tumors (PanNETs). Remarkably, the mutations in PanNETs are unique compared to other cancers and we currently do not understand how these mutations lead to disease. This knowledge is essential for development of new therapies. Studies that started in the Lozano laboratory and will continue in the Wasylishen laboratory focus specifically on *DAXX and ATRX*, genes that work together in the cell and are mutated in approximately one third of PanNETs. Mouse models provide an important and powerful platform to understand gene function and the consequences of gene mutation. To better understand Daxx and Atrx and how mutations may lead to cancer, we have generated two new mouse models. These models have provided surprising results and taught us that while Daxx and Atrx work together, they also have essential functions individually. Our current work couples these new models with state-of-the-art technologies to identify the cellular changes that are likely to be relevant to pancreatic neuroendocrine tumors. This comprehensive understanding of gene function is essential for future work to identify new treatments for tumors with *DAXX* or *ATRX* mutations.