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

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ABSTRACT

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.

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 the 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.

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 analyses are ongoing in embryonic tissues from these mutant mice to compare and contrast how the two mutants impact transcriptional states *in vivo*.

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.

INTRODUCTION

The DAXX/ATRX/H3.3 axis is mutated in several human cancers

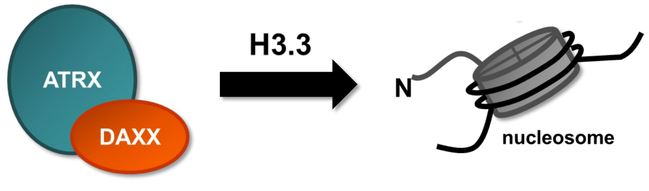


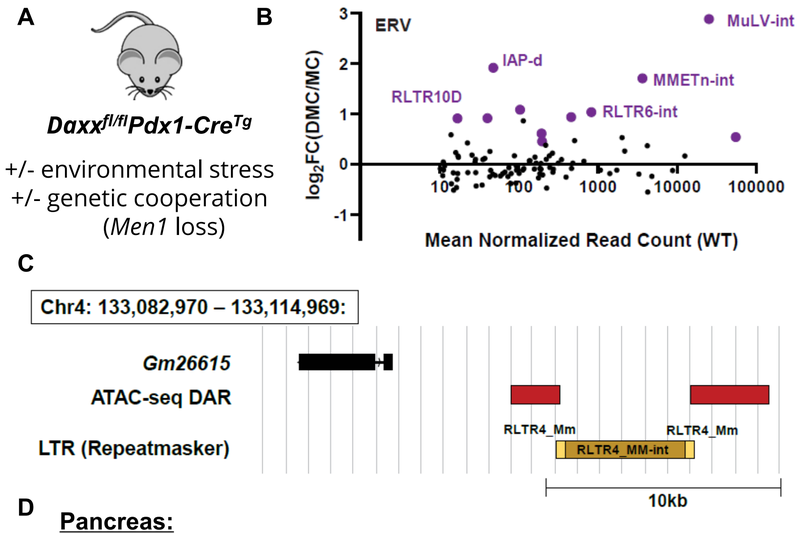
Table 1: Mutation frequencies in cancer

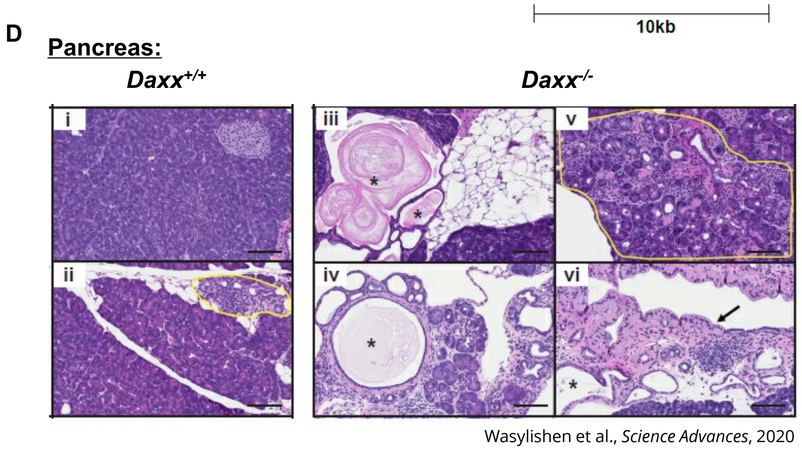
	DAXX	*ATRX*	*H3.3*
Pancreatic neuroendocrine tumors (PanNETs)	25%[1]	18%[1]	-
Glioblastoma (GBM)			
Pediatric (DIPG)	-	29%[2]	31%[2]
Adult (HGG)	-	6%[3]	-
Giant cell tumors of the bone	-	-	92%[4]
Chondroblastoma	-	-	95%[4]

[1]Jiao et al., *Science*, 2011; [2]Schwatzentruber et al., *Nature*, 2012; [3]Brennan et al., *Cell*, 2013; [4]Behjati et al., *Nature Genetics*, 2013

RESULTS

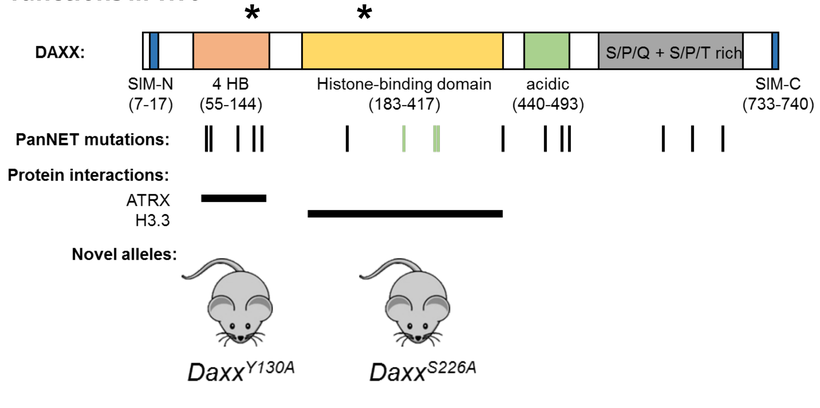
Daxx maintains endogenous retroviral silencing and restricts cellular plasticity *in vivo*





Wasylishen et al., *Science Advances*, 2020

New germline mutant alleles to understand specific Daxx functions *in vivo*



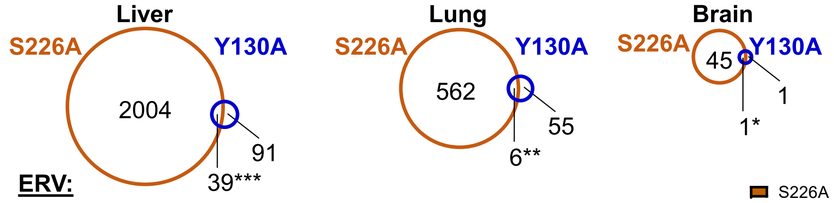
Daxx histone chaperone function is not required for embryonic development. But Daxx:H3.3 is essential for post-natal viability

Table 2: Mendelian ratios

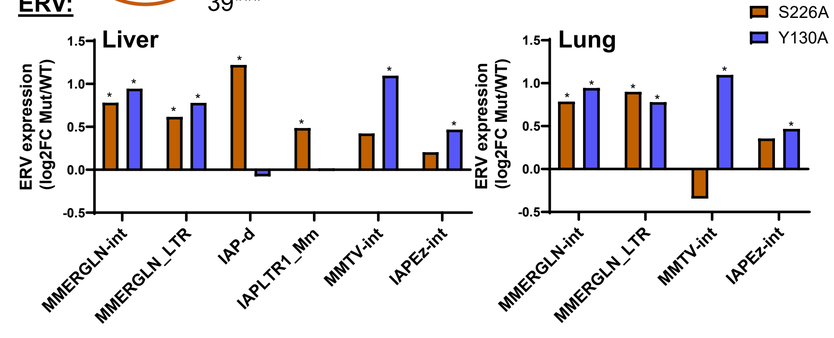
	P21		E18.5	
	Expected (%)	Observed (%)	Expected (%)	Observed (%)
+/+	21.5 (25)	30 (35)	15.5 (25)	21 (34)
S226A/+	43 (50)	54 (63)	21 (50)	28 (45)
S226A/S226A	21.5 (25)	**2 (2)**	15.5 (25)	13 (21)
+/+	17.75 (25)	14 (20)		
Y130A/+	35.5 (50)	39 (55)		
Y130A/Y130A	17.75 (25)	18 (25)		

RNA-seq reveals distinct patterns of transcriptional changes in embryonic tissues between *Daxx*^{S226A} and *Daxx*^{Y130A} mice

PROTEIN CODING:



ERV:



SUMMARY

Daxx^{S226A}

- **Survives embryonic development**
- **Post-natal lethal**
- **Significant transcriptome changes**
- **Derepresses ERVs**

Daxx^{Y130A}

- **Viable and fertile**
- **Limited transcriptome changes**
- **Derepresses ERVs**

ACKNOWLEDGEMENTS

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