Epigenetic Dysregulation of Transposons in Pancreatic Neuroendocrine Tumors

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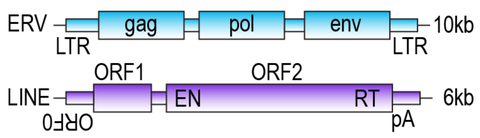
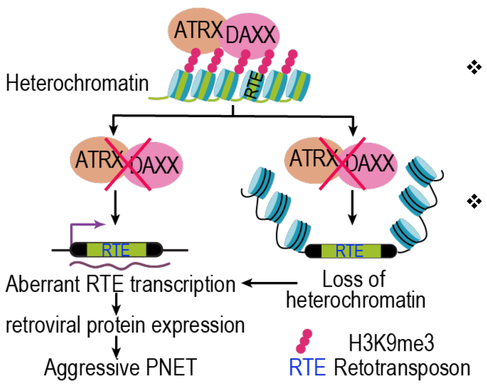
BACKGROUND

- Pancreatic neuroendocrine tumors (PNETs) are rare neoplasms and represent about 1-2% of all pancreatic tumors.
- *MEN1* (multiple endocrine neoplasia I), *DAXX* (death domain associated protein) and *ATRX (*ATRX chromatin remodeler) are the most frequently mutated genes occurring in up to 70% of PNETs[1,2].
- While *MEN1* mutations can be inherited or somatic, *ATRX* and *DAXX* mutations are exclusively somatic, detected in about 40% of PNETs[1,2].
- *ATRX* and *DAXX* loss-of-function (LOF) mutations in PNETs are associated with metastatic disease, increased risk of recurrence, and poorer survival[3-5].
- ATRX is a chromatin remodeling protein that interacts with the histone chaperone DAXX, to deposit histone H3.3 at heterochromatin regions.
- In embryonic stem cells, the ATRX/DAXX complex is essential for heterochromatin formation at RTEs, and knockdown of either ATRX or DAXX leads to aberrant transcription from retrotransposons (RTEs.
- RTEs are derived from ancient retroviruses that infected germ cells or germ cell progenitors.
- Once integrated, they duplicated and propagated themselves through reverse transcription of an RNA intermediate.
- RTE de-repression can play causal roles in cancer cells as they can function as promoters or enhancers leading to altered expression of oncogenes.
- They can code for proteins and splice into nearby genes that leads to chimeric transcripts or altered protein isoforms.



Types of retrotransposons that encode proteins. Intact human endogenous retroviruses contain three ORFs; *gag*, *pol* and *env*. LINE elements code for ORF1p, ORF2p in the sense and ORF0 in the anti-sense orientation. ORF2p contains the endonuclease (EN) and reverse transcriptase (RT) activities. pA: 3'-polyA tail.

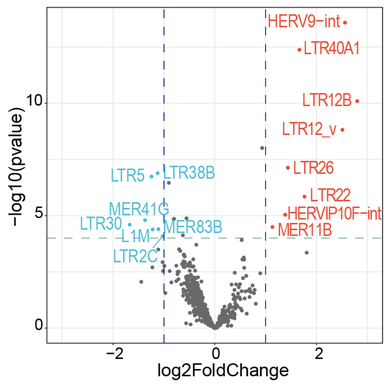


OVERALL AIMS

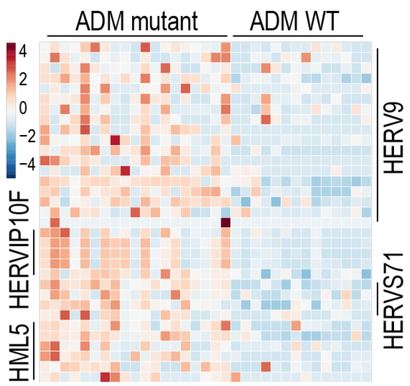
- To examine if ATRX/DAXX loss leads to disrupted heterochromatin and increased expression of retrotransposons in PNETs.
- To examine the extent of retrotransposon expression in PNETs with functional consequences for detection and/or therapeutic targeting.

RESULTS

Loss of function mutation of ATRX/DAXX leads to increased expression of full length HERV9 loci



Increased expression of HERV elements in ADM-mutant PNETs. Volcano plot showing log2(fold change) of HERVs in ADM-mutant PNETs (red) as compared to ADM WT. Each dot represents a HERV subfamily. p-value < 0.0001 by Wald's test and log2 fold change > 1 are highlighted in red.

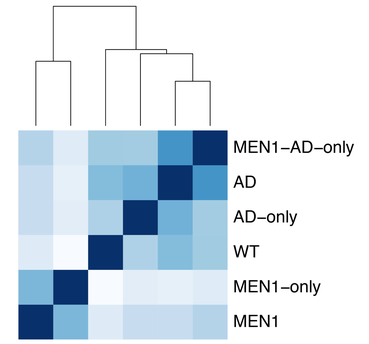


HERV families expressed in ADM-mutant PNETs. Heatmap showing HERV elements with increased expression in ADM-mutant PNETs (red shades).

ADM mutant-Mutation in ATRX or DAXX or MEN1. ADM WT-WT for all three genes
RNA-seq data from Chan et al., 2018.

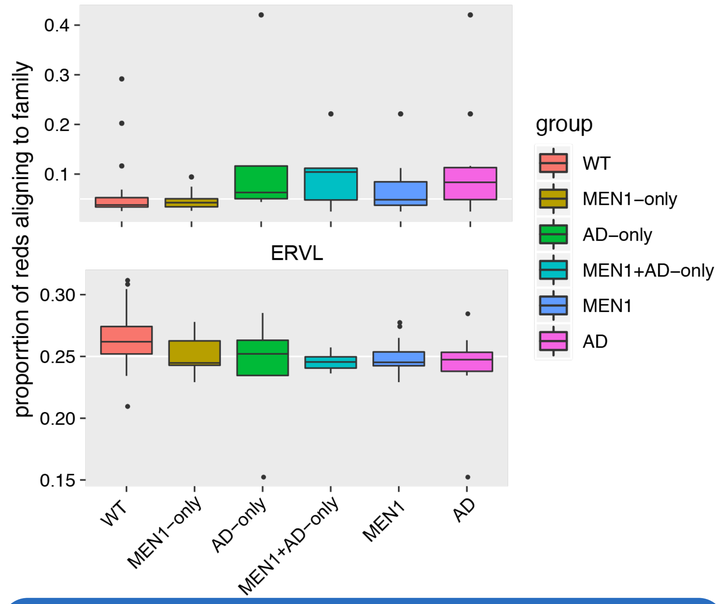
Group	*ATRX*	*DAXX*	*MEN1*
WT	WT	WT	WT
MEN1-only	WT	WT	LOF
AD-only	WT LOF	LOF WT	WT WT
MEN1+AD only	WT LOF	LOF WT	LOF LOF
MEN1 (MEN1-only +AD-only)	WT WT LOF	WT LOF WT	LOF LOF LOF
AD (AD-only+ MEN1+AD-only)	WT LOF WT LOF	LOF WT LOF WT	WT WT LOF LOF

Genotype of the various groups compared



Distance matrix based on similarity of HERV expression across groups

RNA-seq data from Scarpa et al., 2017 and ICGC (N=43)



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