Loss of epigenetic repression of retrotransposons in Pancreatic Neuroendocrine Tumors

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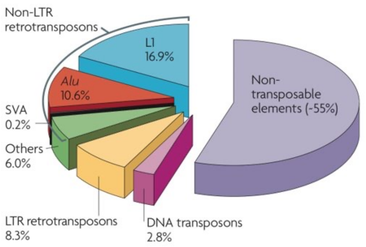
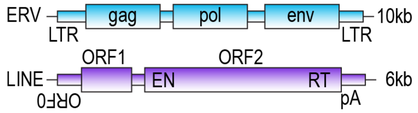
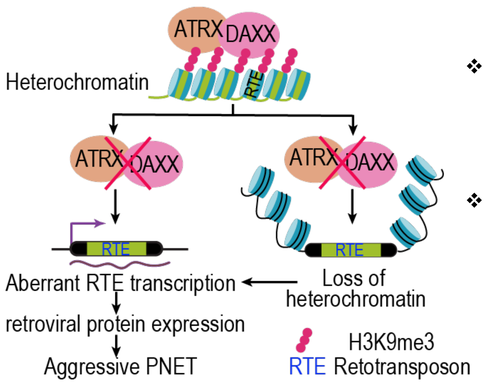
BACKGROUND



- Roughly half of the human genome is derived from transposons that still pose threats to its integrity.
- Most transposons are silenced in normal cells by various epigenetic mechanisms.
- Faithful silencing of transposons is sufficiently compromised in disease contexts, especially in the cancers leading to their aberrant expression and activity.
- *MEN1* (multiple endocrine neoplasia I), *DAXX* (death domain associated protein) and *ATRX (*ATRX chromatin remodeler) are the most frequently mutated genes reported in pancreatic neuroendocrine tumors (PNETs).
- All three proteins have roles in chromatin remodeling.
- ATRX/DAXX complex is essential for heterochromatin formation at retrotransposons (RTEs).
- RTEs are derived from ancient retroviruses 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.

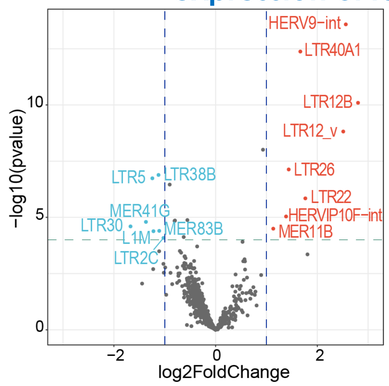


OVERALL AIMS

- 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

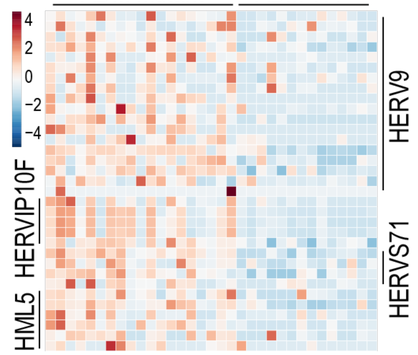
PNETs with ATRX/DAXX mutations exhibit increased expression of full length HERV9 loci



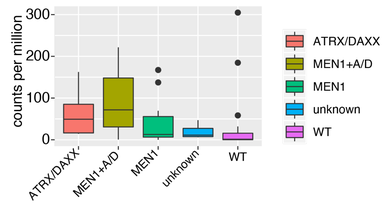
Increased expression of HERV elements in ADM-mutant PNETs. Volcano plot showing log2(fold change) of HERVs in ADM-mutant PNETs (red) as compared to ADM WT. Each dot represents a HERV subfamily. p-value < 0.0001 by Wald's test and log2 fold change > 1 are highlighted in red.

Group	*ATRX*	*DAXX*	*MEN1*
WT	WT	WT	WT
MEN1	WT	WT	LOF
ATRX/DAXX	WT LOF	LOF WT	WT WT
MEN1+A/D	WT LOF	LOF WT	LOF LOF

Genotype of the various groups compared

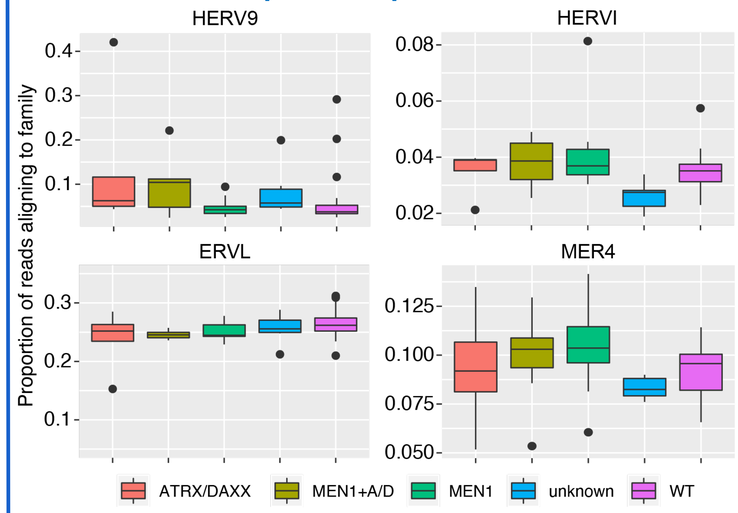


HERV families expressed in ADM-mutant PNETs. Heatmap showing HERV elements with increased expression in ADM-mutant PNETs (red shades). ADM mutant: Mutation in ATRX or DAXX or MEN1. ADM WT: WT for all three genes. RNA-seq data from Chan et al., 2018.



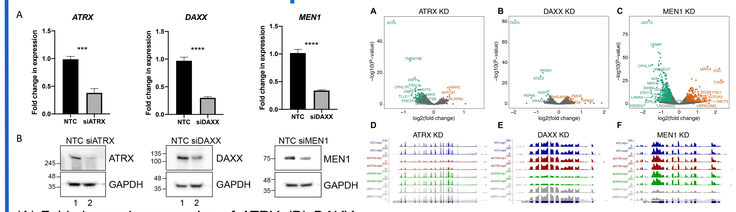
Boxplots show expression (counts per million mapped reads) of a HERV9 locus (HERV9_5p13.3a) across PNETs grouped by genotype. RNA-seq data from Scarpa et al., 2017 and ICGC (N=49)

HERV expression profile of PNETs



Boxplots show proportion of HERV mapped reads that are aligned to various families as indicated across PNETs grouped by genotype. RNA-seq data from Scarpa et al., 2017 and ICGC (N=49)

Knockdown experiments in QGP1 cell line



(A) Fold change in expression of *ATRX*, (B) *DAXX* and *MEN1* mRNA levels 96 hrs after transfection of 30nM respective siRNAs in QGP1 cell line. NTC: Non-targeting control. (B) Western blot analysis shows the protein levels of ATRX, DAXX and MEN1 proteins 96 hrs after transfection of the 30 nM respective siRNAs in the QGP1 cell line.

A) Volcano plot showing the gene expression changes observed after siRNA-mediated knockdown of *ATRX*, (B) *DAXX* and (C) *MEN1* gene in QGP1 cell line. NTC: Non-targeting control. (D) Genome browser tracks showing levels of *ATRX*, (E) *DAXX* and (F) *MEN1* mRNA in all RNA-seq samples.

Generation of CRISPR knockout cell lines

Sequencing confirmation of the ATRX and DAXX knockout clones in QGP1

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