Enhancer Signatures Stratify and Predict Outcomes of Non-functional Pancreatic Neuroendocrine Tumors

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In Brief

Nonfunctioning pancreatic neuroendocrine tumors (NF-PNETs) are rare pancreatic tumors of neuroendocrine origin that do not produce an excess of hormones and oftentimes are detected incidentally. NF-PNETs have a wide spectrum of clinical behaviors ranging from indolent and localized to aggressive with distant metastases, most commonly to the liver. Tumor size has served as a strong prognostic factor in predicting malignancy, where tumors <2cm have excellent prognosis with survival rates up to 100% at 15 years.[1,2] Thus, current guidelines recommend observation for asymptomatic patients with tumors <1cm and either surgery versus observation for tumors between 1 to 2 cm; tumors >2cm are recommended for resection.[3] The World Health Organization (WHO) classification distinguishes PNETs as well-differentiated or poorly-differentiated tumors histopathologically. Well-differentiated tumors are further divided into Grade 1, 2 or 3 based on Ki-67 proliferation indices of <3%, 3 to 20%, and >20%, respectively; all poorly-differentiated PNETs are considered Grade 3.[4] These factors are most reliably determined after surgery and are strong predictors of overall survival and disease-free survival.[5] Recent genomics studies have revealed that PNETs are commonly driven by mutations in tumor suppressor genes (MEN1, ATRX, DAXX), with the latter two being associated with alternative lengthening of telomeres (ALT) and recurrence. However, there is currently a paucity of clinically-available definitive biomarkers to help guide peri-operative management of these tumors.

The primary purpose of this study published by Cejas and colleagues was to identify novel epigenetic markers of NF-PNET cellular origin, and correlate these findings with clinical outcomes. They used enhancer mapping to identify transcription factors (TFs) at active chromatin signatures to elucidate lineage-specific gene regulatory programs, specifically searching for markers of alpha or beta islet cell origin. The authors found that most PNETs fall into two major epigenomic and transcriptomic subtypes based on H3K27ac and H3K4me2 chromatin signatures. Specifically, chromatin immunoprecipitation sequencing (ChIP-seq)-derived profiling of H3K27ac-associated enhancers was able to identify three distinct subtypes of PNETs. Across 8 non-functional PNETs, Type A was characterized by a two-fold higher H3K27ac signal in 288 enhancers, whereas Type B was characterized by increased H3K27ac

signal at 104 unique regions. Analyzing these data from a cell-lineage perspective revealed that Type A tumors expressed strong H3K27ac signals at α-cell-specific loci (ARX and IRX2) and Type B had strong signals at β-cell TFs (PDX1); type C had variable signal and mRNA expression at these loci. ChIP-seq profiling for H3K4me2 in this cohort yielded similar results. RNA-sequencing of these PNETs also confirmed differential ARX and PDX1 expression between A- and B- subtypes.

As shown in prior mouse models, ARX is necessary to drive α-cell fate while PDX1 is a maker of β-cell differentiation. This was confirmed in this study where the authors compared ChIP-Seq and RNA-seq profiles between PNET subtypes and normal pancreatic islets – this analysis showed that Type A and Type B tumors were highly enriched for α-cell-specific and β-cell-specific markers, respectively. These results were validated in an additional cohort of 13 PNETs. Interestingly, this differential transcriptome profiling was more accurate in distinguishing PNET subtypes than glucagon and insulin mRNA levels.

An additional Dutch cohort was then evaluated by tissue microarray immunohistochemistry (IHC) consisting of 77 PNETs, of which 61 had a germline MEN1 mutation and 13 were insulinomas. There were 34 (31%) Type A / ARX+, 31 (37%) Type B / PDX1+, 6 (8%) double negative, and 6 (8%) double positive tumors. These findings were notable for variable expression of ARX in Type A tumors, whereas PDX1 was strong in all Type B tumors and insulinomas. Selected tumors underwent ChIP-Seq and RNA-seq, which revealed concordant results as compared to IHC. These data suggest that the authors have identified a unique marker that can differentiate tumor subtypes on conventional IHC.

From a translational perspective, ARX and PDX1 status was correlated to clinical outcomes in 61 NF-PNETs. In this cohort, there were no pathologic differences between ARX+ and PDX1+ tumors, such as tumor size or grade. However, at a median of 24 months follow-up, relapses (all occurring in the liver) were almost exclusively associated with ARX+ or double negative groups. Notably, no PDX1+ or double positive tumors recurred. These findings were confirmed in an additional cohort of NF-PNETs from Massachusetts (n=56). With a longer median follow-up of 66 months, most relapses again occurred in the ARX+ or double negative tumors; instead, PDX1+ and double positive tumors were more indolent, accounting for three total relapses.

While these data suggest that PDX1+ expression is correlated with a more favorable prognosis, the authors sought to analyze the prognostic impact of ALT status given its association with ATRX and DAXX mutations and known risk of recurrence. Telomere-specific FISH analysis revealed that ALT positivity was more commonly found in ARX+ and double negative tumors (N=13/27, 48%) as compared to PDX1+ tumors (N=5/35, 14%). As expected, ALT was associated with disease recurrence, however, these results were more striking when combined with PNET subtypes where relapses occurred in all ARX+/ALT+ tumors, only 9% of ARX+/ALT- tumors, and one PDX1+/ALT- tumor. A final multi-variable analysis of a combined cohort including 83 NF-PNETs with 15 relapses demonstrated that only ALT positivity and absence of PDX1 expression both independently predicted relapse, while controlling for tumor size and WHO grade.

In summary, this study concluded that enhancer profiles of non-functional PNETs subtypes uniquely resemble islet α and β-cell profiles. Identification of several of these markers, namely ARX and PDX1, can be performed readily on IHC and subsequently used as prognostic markers for disease recurrence. Specifically, ARX+ tumors are at risk for relapse especially in the setting of ALT positivity, while PDX1 tumors appear more indolent. In this study, these

markers were better predictors than tumor size and WHO grade, thus, adaptation into clinical practice could help guide management of NF-PNET, particularly for tumors <2-3cm.

Critique and Future Directions

ARX and PDX1 expression identified in NF-PNETs represent a novel clinically-significant prognostic marker that can be potentially tested preoperatively to guide surveillance versus surgery decision-making, as well as perhaps alter surveillance strategies post-operatively. Given the significant difference in clinical course based on ARX and PDX1 expression, the reliability of signal detection is important – ARX strength on IHC was shown to be variable, thus there may be overlap between a staining failure and true ARX+ tumors with low expression. Further investigation of the clinical implications between potential staining failures in double negative samples versus weak signal strength will be important moving forward. Additionally, the double positive (ARX+ PDX1+) tumors need to be further investigated regarding the possibility of a dual cell lineage, and the resultant oncogenic implications. Evaluating how varying signal strengths correlate with prognosis may shed light on a tumor's state of differentiation and ability to metastasize. In the Dutch cohort, the double positive group correlated with a good prognosis, a finding that was not clearly confirmed in the multivariable analysis of the combined cohort. Mechanistic studies are warranted to determine how exactly ARX positivity is driving metastatic potential independent of ALT positivity, and why PDX1 positivity appears to protect against it.

A clinical limitation of this study is the relatively small number of relapses observed, which limits a more comprehensive multivariable analysis. Future studies with larger cohorts of relapsed patients can also investigate associations between ARX and PDX1 positivity with response to adjuvant therapy (everolimus, lutetium Lu 177 dotatate, etc.) Lastly, from a pre-operative management perspective, this study's tumor analyses were performed on resected PNET specimens. It was not described whether this stratification can be reliably performed on preoperative FNA cytology. While one follow up study with a small sample size correlated ARX and PDX1 expression between cytologic and pathologic samples, an investigation in a larger cohort is needed to more accurately determine the reliability in expression from samples obtained by endoscopic FNA specimen.[6] Overall, this research identifies novel preoperative prognostic markers to predict aggressive behavior in NF-PNETs with the potential benefit of guiding treatment algorithms.

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