Enhancing Diagnostic Precision Through Combined Genomic and Histopathological Analysis of Pancreatic Neuroendocrine Tumours

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The NETwork! Project (New Zealand) began with creation of the NETwork! registry, which catalogues all Neuroendocrine tumour patients diagnosed between 1995-2012 in the Auckland region and 2008-2012 across New Zealand. With strong support from NET patient support group (Unicorn Foundation New Zealand), we have ethical approval to retrospectively access clinical formalin-fixed paraffin embedded (FFPE) tissue blocks belonging to patients in the NETwork! Registry. We aim to conduct genomics on these annotated samples to understand NET biology, as it pertains to clinical management.

Methods: 69 sporadic well-differentiated pancreatic neuroendocrine tumours (pNETs) from 60 individuals alongside matched normal tissues underwent targeted DNA sequencing (NimbleGen SeqCap), RNA expression analysis (Affymetrix Microarrays) and immunohistochemistry (IHC) alongside pathological examination, to search for molecular drivers; incidentally providing valuable evidence for re-diagnosis in three patients. Cases selected had a clinical and pathological diagnosis of well-differentiated pNET, expressed at least one of the three neuroendocrine IHC markers (chromogranin A, synaptophysin or CD56) and were surgically resectable at initial diagnosis. This poster describes 3 cases that were excluded from the main analysis because genomic analyses allowed us to recognise that they were not pNETs (for pNET analysis see ENETs 2018 Poster B10).

In tumours initially diagnosed as pNETs, DNA, RNA and histopathological evidence contributed to re-diagnosis as SPN or miNEN

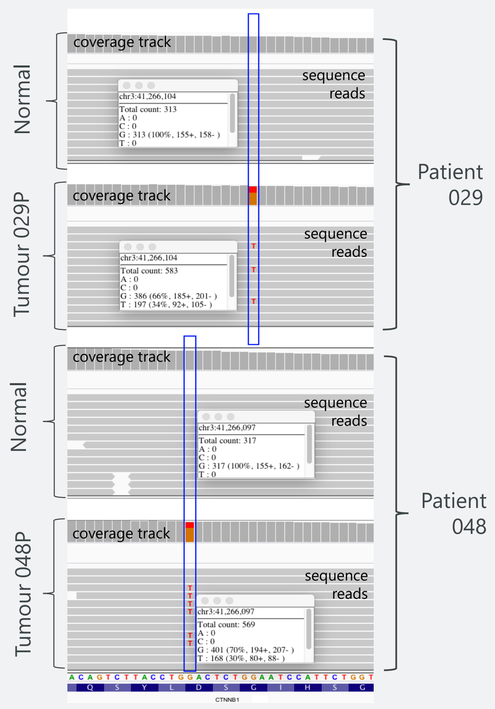
Solid Pseudopapillary Neoplasm (SPN)

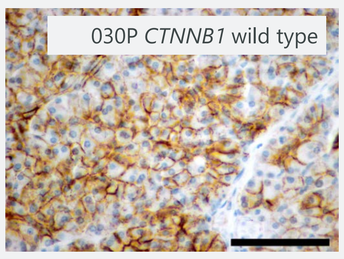
In two cases (029 and 048), tumours were re-diagnosed as pancreatic SPNs; originally diagnosed as pNETs by morphological and IHC but noted uncertainty due to some variable SPN-like features.

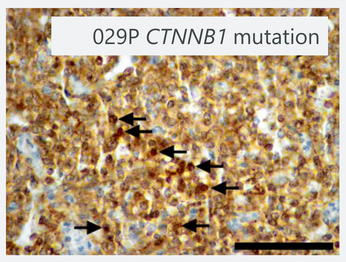
Alongside deep pathological examination, evidence for re-diagnosis came from β-catenin mutations, RNA expression patterns, IHC (β-catenin localisation)

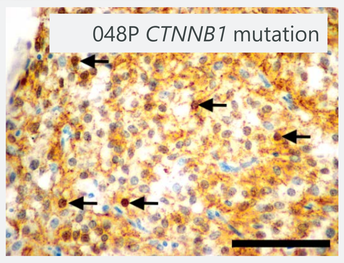
Deep targeted DNA-seq

Revealed activating mutations[1] in *CTNNB1* encoding B-catenin, pathognomonic for SPNs - present in 90% of all SPN cases[2].







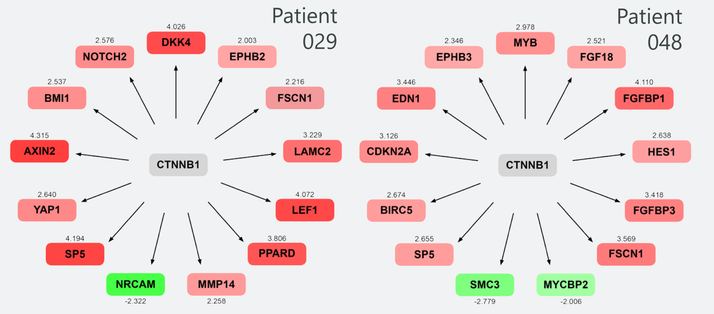


Histopathological analysis

Revealed cellular relocalisation of β-catenin (brown) to the nucleus, concordant with SPN status and transcription factor function.

RNA expression

RNAs up-regulated by β-catenin[3] were highly expressed, in accord with the mutation's known activating effect.

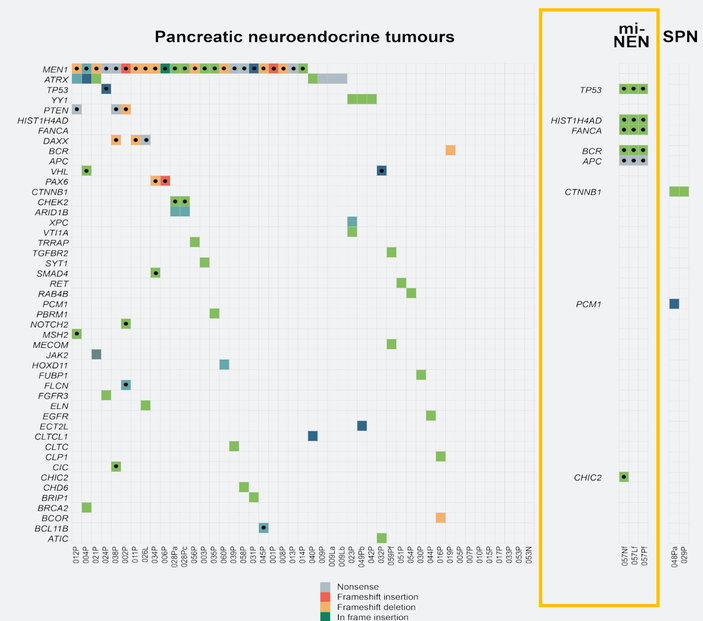


RNAs known to be up-regulated by β-catenin activity in colorectal cell lines were intersected with RNAs with significantly high or low expression (≥2 SD above / below the mean of expression of the RNA in our pNETs). Colours map RNA expression from maximally low expression (green) through mean (white) to maximally high expression (red) across pNETs. Numbers indicate Z-transformed expression.

Mixed Neuroendocrine Non-Neuroendocrine Neoplasm (miNEN)

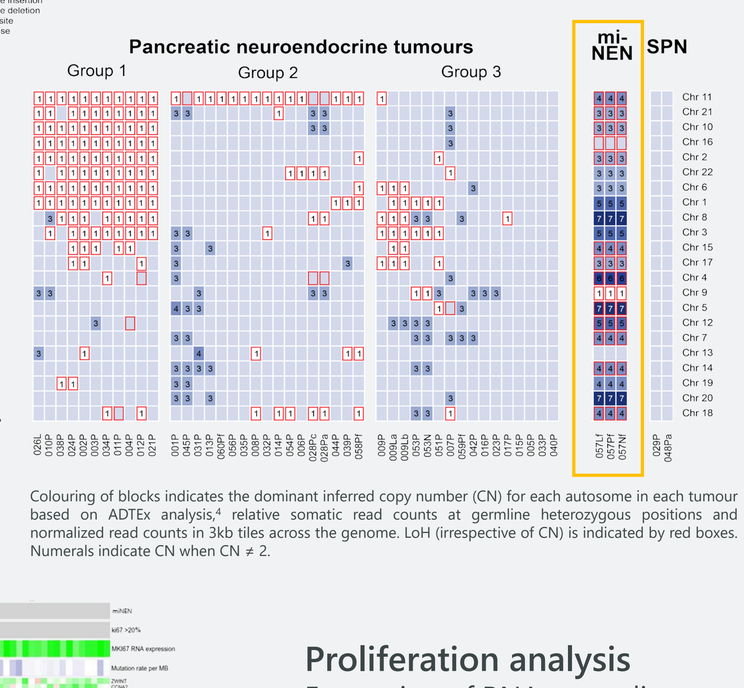
In one case (057), three tumour samples from the same patient showed possible features of mixed neuroendocrine non-neuroendocrine neoplasm (miNEN).

Alongside deep pathological examination, evidence for re-diagnosis came from mutational landscape, aneuploidy analysis, and proliferation analysis.



Mutational landscape

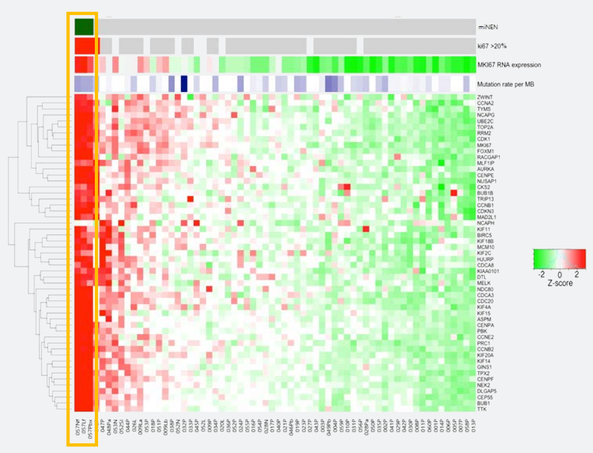
Revealed mutations in *APC*, *TP53* and *FANCA*, in contrast to mutations found in pNETs in this study.



Aneuploidy analysis

Revealed extensive aneuploidy in tumours from patient 057 in contrast to the degree and patterns of aneuploidy seen in pNETs in this study.

Colouring of blocks indicates the dominant inferred copy number (CN) for each autosome in each tumour based on ADTEx analysis,[4] relative somatic read counts at germline heterozygous positions and normalized read counts in 3kb tiles across the genome. LoH (irrespective of CN) is indicated by red boxes. Numerals indicate CN when CN ≠ 2.



Proliferation analysis

Expression of RNAs encoding cellular proliferation proteins were distinctly elevated in tumours from patient 057 in contrast to pNETs in this study. Concordantly, these tumours had very high MKI67 RNA expression and ki-67 positive tumour cells.

Heatmap shows Z-transformed expression of RNAs encoding cellular proliferation proteins[5] sorted by 1st principal component, highlighting the highly proliferative nature of Case 057. Using unsupervised clustering, Case 057 fell to the very left of the heatmap.

Conclusions: Homing in on precise diagnoses

- SPNs and miNENs share some cytological features with pNETs but are genomically distinct.
- β-catenin IHC should be conducted more frequently when diagnosing pNETs.
- While differentiation of uncommon pancreatic malignancies from pNETs can be challenging, it is important as the diagnosis has different prognostic implications. Genomic analysis provides a further tool for making this critical distinction.
- We believe that combining genomic information with traditional pathological information is likely to generate more precise diagnoses for many tumour types[6]

1. Moreno-Bueno et al. (2001) PMID 11703283
2. Abraham et al. (2002) PMID 11943721
3. Herbst et al. (2014) PMID 24467841
4. Amarasinghe et al. (2014) PMID 25167919
5. Nagalla et al. (2013) PMID 23618380
6. Harris et al. 2017 PMID 27556576

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