Comparative transcriptomics of functional vs non-functional pituitary neuroendocrine tumours.

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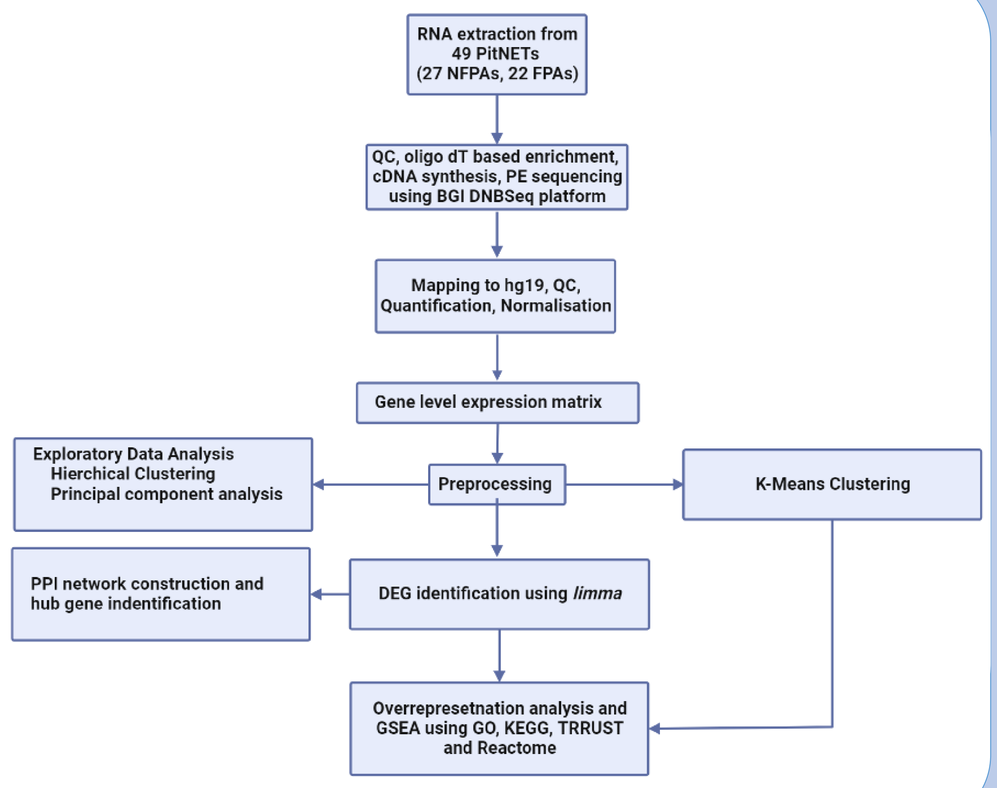
INTRODUCTION

- Pituitary neuroendocrine tumours (PitNETs) are a heterogeneous group of neoplasms. Their pathogenesis is poorly understood, and they exhibit extensive clinical and molecular heterogeneity that precludes the development of targeted therapeutic modalities.
- Their gene expression profile is highly heterogeneous, with a lack of a uniform molecular signature across tumour subtypes. While several genes have been consistently implicated across various transcriptomic studies, individual studies also identify novel DEGs with potential tumorigenic and therapeutic implications

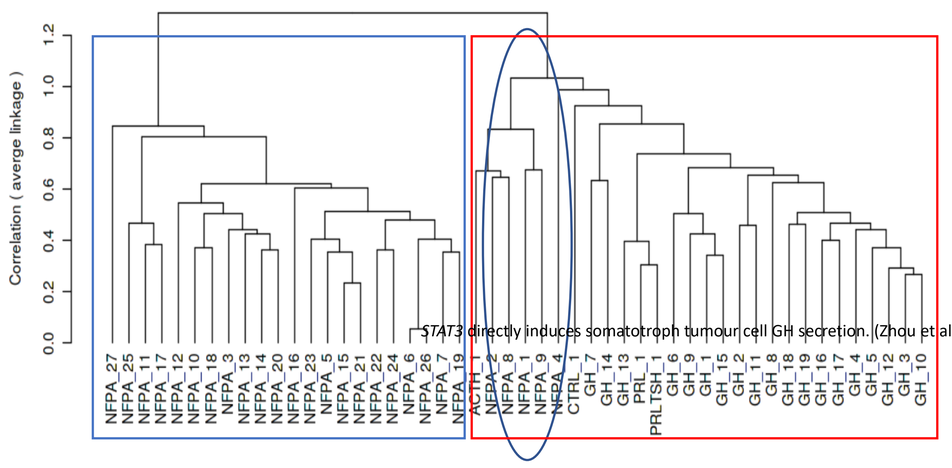
OBJECTIVES

To further characterise patterns of gene expression and de-regulated pathways in PitNETs

METHODOLOGY

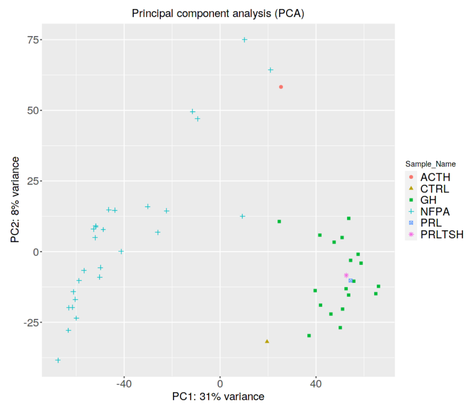


RESULTS

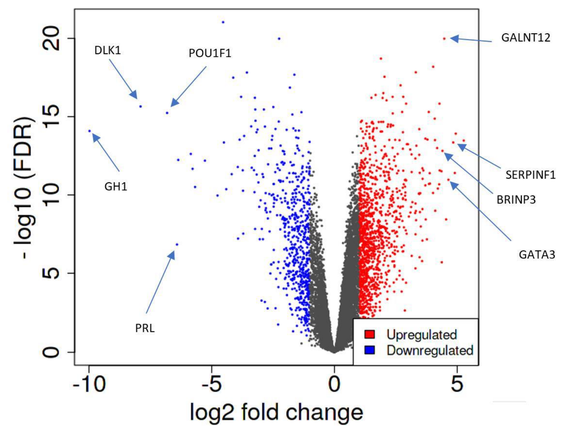


STAT3 directly induces somatotroph tumour cell GH secretion. (Zhou et al., 2015).

Dendrogram showing the result of hierarchical clustering of the individual samples according to differences in gene expression, using the top 75% of genes regarding expression levels.
Samples with similar patterns of gene expression are grouped together.
This analysis shows two main groups: one composed solely of NFPAs and the other made up of all functioning pituitary adenomas (GH-secreting, PRL-secreting, PRL/TSH co-secreting, ACTH-secreting), the control and 5 NFPAs.



Principal component analysis (PCA) showing variability in gene expression across all samples included in the project. PC1 and PC2 define 31% and 8% of the variance, respectively. The different biological groups form separate clusters, indicating that biological replicates resemble each other with regard to their expression profile. However, the NFPA cluster on the left is more broad, indicating greater within group heterogeneity, compared to the FPA cluster on the right, which is more compact.



Volcano plot showing DEGs between NFPAs and FPAs. Upregulated and downregulated genes in NFPAs compared to FPAs that pass the thresholds of $\log_2$FC ≥2 and FDR <0.1 are coloured as red and blue, respectively. Some of the DEGs exhibiting a large fold change are also labelled.

Ion-related events were upregulated in NFPAs whereas functional subtypes revealed enrichment in **GH signalling, JAK-STAT signalling** and **PI3K-AKT signalling**.
Some genes previously associated with pituitary adenomas were found to be differentially expressed:

CACNA2D4, FOLR1 Potential therapeutic targets
(Taniguchi-Ponciano et al., 2021; Liu et al., 2013)

DLK1, SERPINF1 General tumourigenic mechanisms
(Evans et al., 2018; Tebani et al., 2021)

GATA3 Potential marker to supplement pituitary adenoma classification (Mete et al., 2019)

Direction of differential expression in NFPAs vs FPAs	Pathway Name	Adjusted *p* value
Upregulated	Cardiac muscle contraction	0.001
Downregulated	Growth hormone synthesis, secretion and action	0.00240
Downregulated	JAK-STAT signalling pathway	0.00240
Downregulated	PI3K-Akt signalling pathway	0.00310

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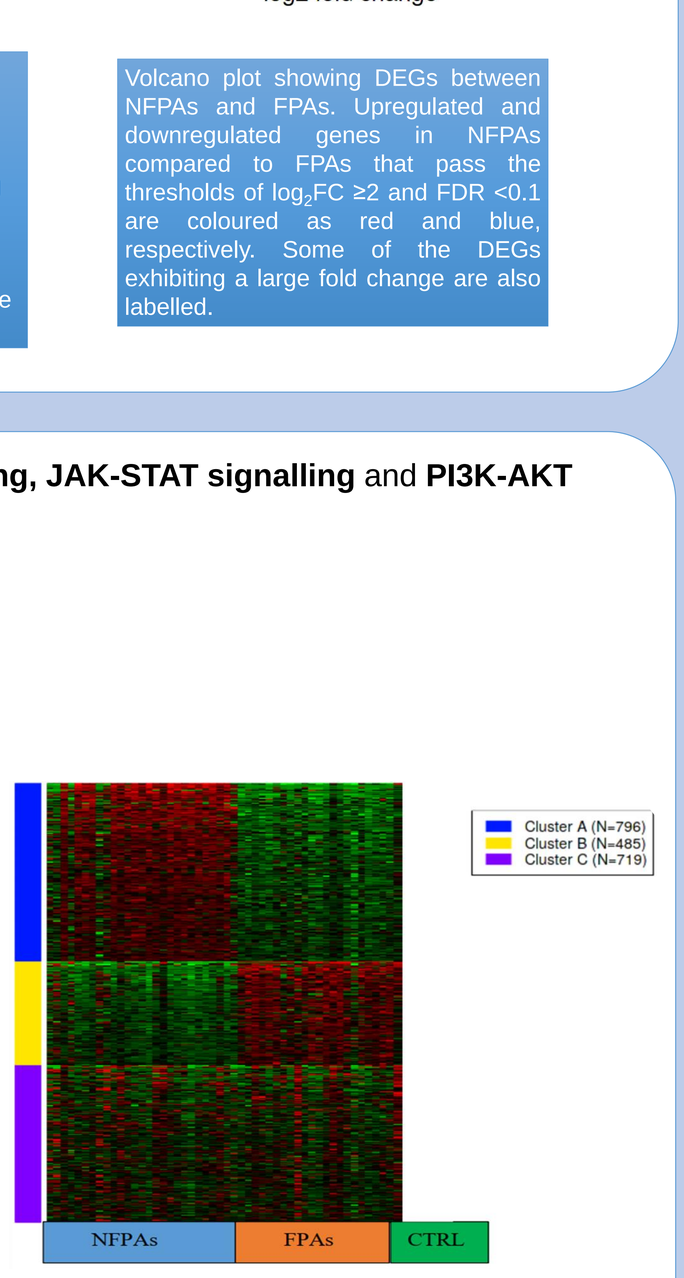
Implicated in other human cancers. Studies have investigated the therapeutic effects of targeting the PI3K/AKT/mTOR pathway in pituitary adenomas (Cerovac *et al*. 2010).

Cluster A
Cardiac conduction
Voltage-gated potassium channels

Cluster B
Growth hormone receptor signalling
Signalling by Retinoic Acid



- **NFPAs show heterogeneity in their transcriptomic profile**. Clustering analysis segregated the pituitary adenoma samples into two main clusters, in accordance with the clinical classification as functioning and non-functioning tumours. Since most NFPAs are silent gonadotrophinomas belonging to the gonadotroph lineage, driven by *SF1*, while most FPAs in this study were GH-secreting somatotrophinomas driven by *POU1F1*, it could be postulated that the clustering was according to the transcription factors driving terminal differentiation.
- **Differential gene expression analysis reveals alterations in genes closely related to pituitary adenoma development.** The top up-regulated genes in NFPAs included several genes which have previously been linked to NFPA development such as BRINP3, GALNT12, *SERPINF1*, *GATA3* and *FOLR1*.
- **NFPAs exhibit upregulation of ion-related events.** Over-representation analysis of the identified DEGs using Gene Ontology terms, revealed the enrichment of terms such as metal ion transmembrane transporter activity and ion channel activity, and also from GSEA analysis using the KEGG pathway database, which revealed the cardiac muscle contraction pathway, a pathway which is replete with ion channels, as the most significantly upregulated pathway. The alterations in genes involved in the regulation of ions such as calcium and potassium is in line with results from previous pituitary adenoma transcriptomic studies. Taniguchi-Ponciano et al. (2020) also identified alterations in calcium signalling pathways in NFPAs. Additionally, *CACNA2D4*, one of the most significantly upregulated genes that formed part of the enriched cardiac muscle contraction pathway in NFPAs in our analysis, was proposed as a potential therapeutic target in NFPAs.
- **FPAs demonstrate upregulation of tumourigenesis-related pathways.** The most upregulated genes in FPAs, most of which were GH-secreting tumours, included genes coding for hormones that form part of the growth hormone/prolactin family such as *GH1*, *GH2*, *CSH2* and *PRL*. Also as expected, *POU1F1*, the gene that encodes the transcription factor responsible for the differentiation of GH-, PRL- and TSH-producing cells (Lopes, 2017), was upregulated in FPAs. Pathway analysis identified the GH synthesis, secretion and action pathway as the most upregulated pathway in FPAs. Additionally, the PI3K-Akt and JAK-STAT signalling pathways were also found to be altered in these tumours.