# Assessing the immune gene and cellular networks within human non-functioning and growth-hormone secreting pituitary adenomas via transcriptomic and pathway analysis

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## INTRODUCTION

The **tumour microenvironment** (TME) is an active promoter of cancer progression due to its decisive role in tumour proliferation and immune evasion in numerous cancers. Its composition varies between tumour types, but hallmark features include **cells** (**neoplastic, immune,** and **stromal**) and other **non-cellular elements** such as **cytokines** and **growth factors**. Understanding the TME enhances understanding of cancer resistance. However, despite advancements in research, little is known about the TME in **pituitary neuroendocrine tumours** (PitNETs). Furthermore, the link between the immune gene and cellular networks within these types of tumours has not been previously, comprehensively explored. This could hinder the development of novel therapies such as immunotherapy.

## OBJECTIVES

- Obtain a list of differentially expressed genes (DGEs) sourced from growth-hormone secreting (GHPAs) and non-functioning pituitary adenomas (NFPAs)
- Select and process a cohort of immune system genes via pathway analysis
- Observe the baseline expression data of these genes within a subset of immune cells

## METHODS

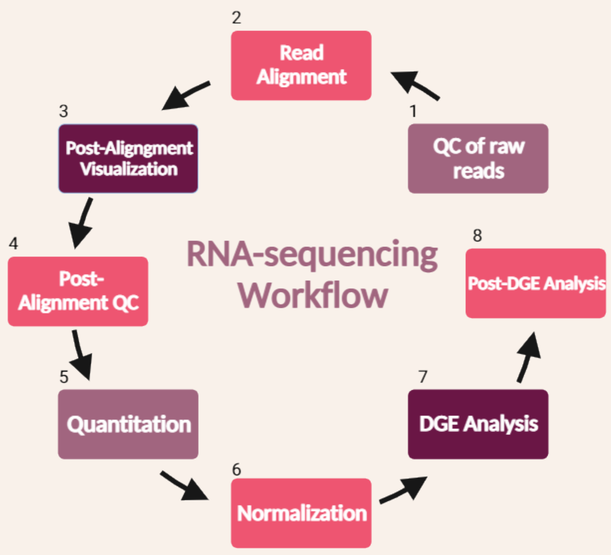


**Figure 1**: 8-stage RNA-sequencing workflow. The tools utilised were FastQC, STAR for alignment, SeqMonk & DeSeq2 for differential gene expression (DGE) analysis

RNA-sequencing technology (**Figure 1**) was implemented to obtain a list of DEGs sourced from NFPAs (n=7) and GHPAs (n=3) RNA-sequencing data. A cohort of DEGs, all having an immune function, was then selected for further processing . This was done through an observation of baseline expression data for these genes within a subset of immune cells via cellular network analysis (i.e. B-cells, abT-cells, gdT-cells, innate lymphocytes, dendritic cells, macrophages, and monocytes); and pathway analysis (**Figure 2**)

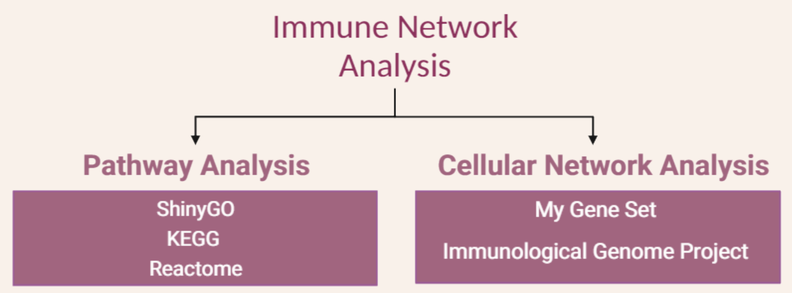


**Figure 2**: List of the software utilised throughout the pathway and cellular network analyses. All software is web-based with the exception of KEGG (used via Cytoscape v3.9.1)

## RESULTS

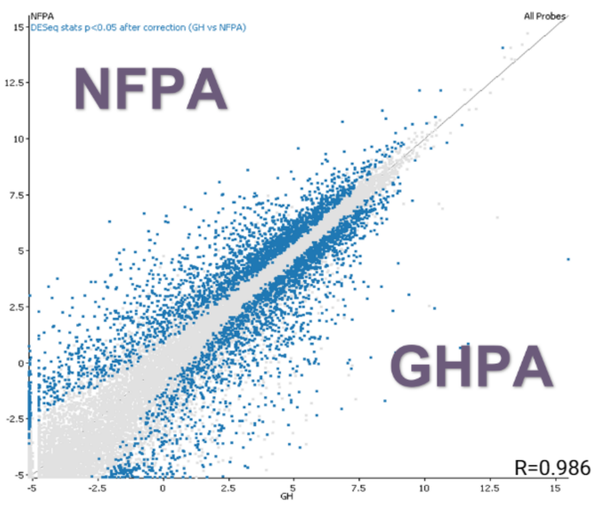


**Figure 3**: Distribution of differentially expressed genes for the 'NFPA vs GHPA' Maltese cohort. This data revealed a total of 4,039 genes (2,274 were upregulated and 1,765 were downregulated). Obtained via Seqmonk v1.48.1. R-value 0.986

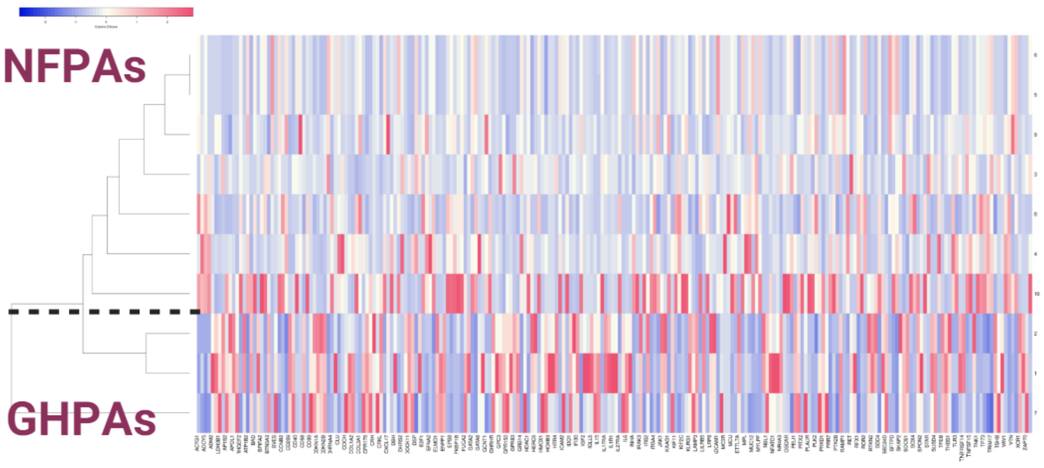


**Figure 4**: The expression profile of the immune system gene cohort in the GHPA and NFPA samples. The y-axis represents therespective samples and the x-axis represents the immune functioning genes. **The main clusters separated depending on the different subtypes as expected**. Obtained via 'Heatmapper'. The Pearson Correlation Coefficient was used for the cluster analysis.

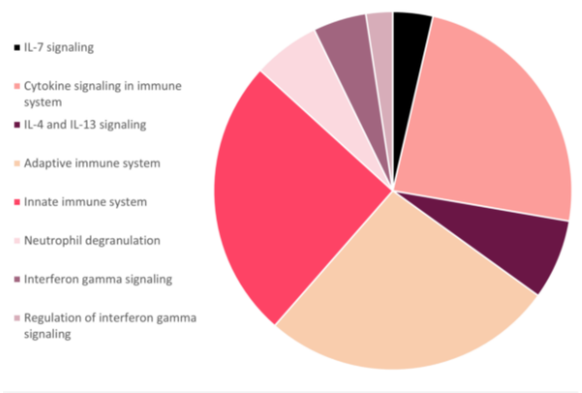


**Figure 5**: The top 8 most significant immune system pathways determined via Reactome

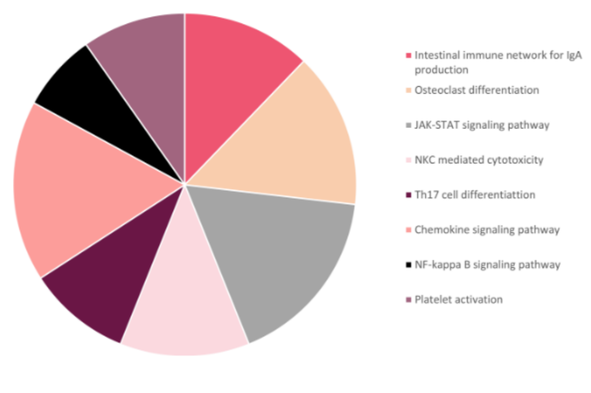


**Figure 6**: The top 8 most significant immune system pathways determined via KEGG

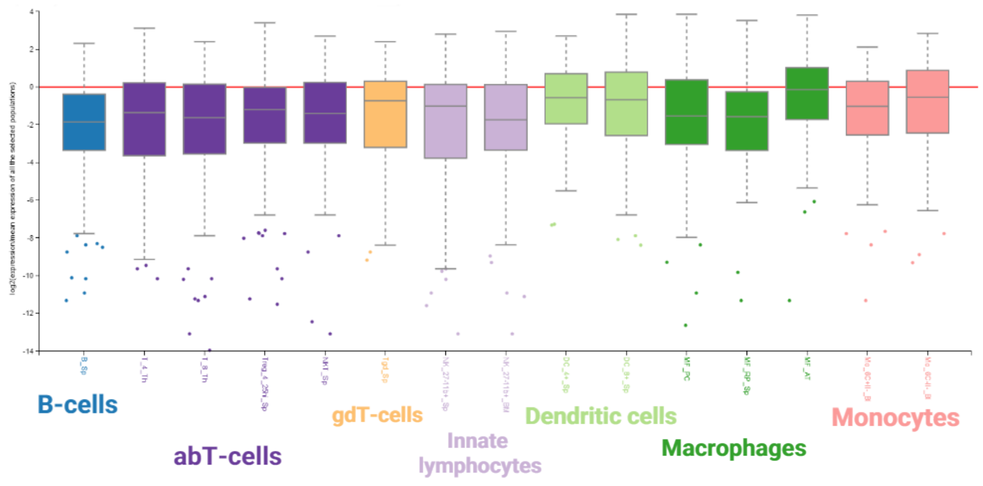


**Figure 7**: The overall expression of the immune system gene cohort within a selection of immune cells using baseline gene expression data. Overall, **macrophages dendritic cells, and monocytes had the highest overall expression values for the DEGs**. The remaining immune cell types assessed appeared to have a similar trend in gene expression. Additionally, in most cell types, ***KLRG1*** and ***THBS1*** were expressed the least. Obtained via 'My Gene Set - The Immunological Genome Project'

## CONCLUSIONS AND FURTHER WORK

- Our findings suggest that there are well-known and novel DEGs and pathways within immune gene and cellular networks that could have a probable role in PitNET functioning
- Currently, we are performing gene prioritization to select a subset of genes to be used as candidatets for subsequent validatiton using a variety of techniques

## ACKNOWLEDGEMENTS

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