Novel and classical molecular pathways identified in pituitary tumorigenesis using mRNA profiling.

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SCOPE

To identify novel molecular pathways involved in pituitary tumorigenesis using mRNA expression profiles.

METHODS

- RNA extracted from 8 pituitary tumours (5 NFPA, 2 GH-secreting tumours, 1 Prolactin/TSH- co-secreting) and pooled normal pituitary control RNA were used for microarray RNA expression analysis on Affymetrix HuGene 1.0 ST Chip.
- Microarray data was analyzed using GeneScript GX 11.0 and network analysis was carries out using the Ingenuity Pathway Analysis (IPA) software.
- Data from microarrays was verified using RNA from 30 pituitary tumours (20 NFPA, 6 GH-secreting, 2 PRL-secreting and 2 ACTH-secreting tumours) using quantitative PCR of key genes involved in the networks identified by the IPA.

RESULTS

1. Cluster Analysis

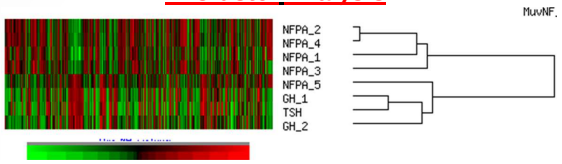


FIG. 1. Unsupervised cluster analysis of 8 pituitary tumours analysed by microarrays. Grouping clearly indicates that one NFPA(5) bears an RNA expression profile more similar to functional tumours than non-functional ones. NFPA; non –functional pituitary adenoma, GH- growth hormone-secreting tumour, TSH; thyroid stimulating hormone-secreting tumour

2. Verification by qPCR

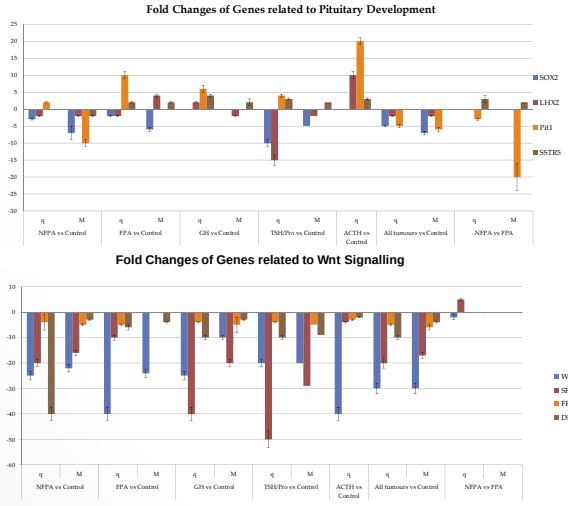
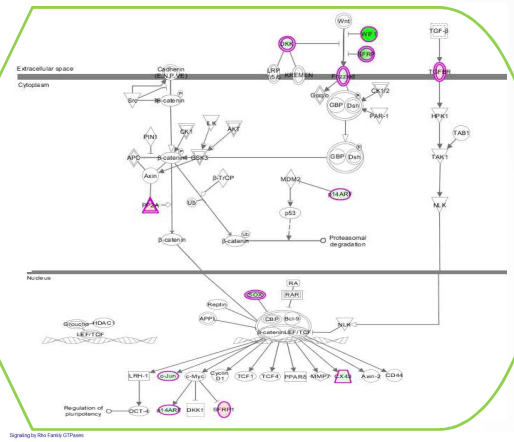


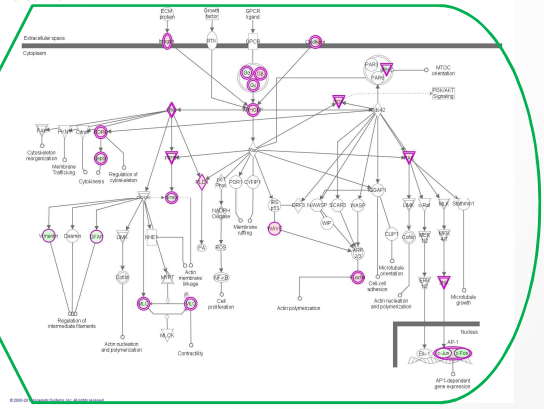
FIG. 2. Fold changes of 8 genes related to pituitary development and Wnt signalling analysed by qPCR (q) and Microarray analysis (M). Another 15 genes were used for verification related to cAMP signalling, hormone production and secretion and common oncogenes and tumour suppressor genes. qPCR results indicate relative quantification normalised with GAPDH and EMC7 housekeeping genes. FPA; functional pituitary adenomas, ACTH; adrenocortico tropic hormone. Error bars indicative of standard error

3. Canonical Pathways involved in Pituitary Adenomas

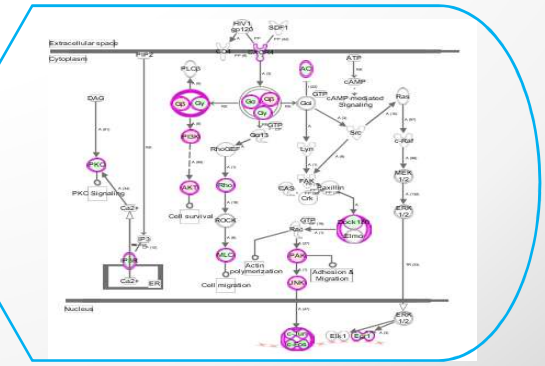
Wnt Pathway was involved in all pituitary adenomas types.



Rho/GTPase pathway significantly involved in NFPA.



CXCR4 Signalling significantly involved in NFPA & GH



CONCLUSIONS

- **Microarrays coupled with qPCR are a useful tool in characterizing tumour types.**
- **Classical pathways were confirmed by microarray and IPA , namely the cAMP signalling, PI3K cascade and Wnt signalling pathways [1,2,3]**
- **Wnt pathway inhibitors are greatly down-regulated, however, canonical signalling through β-catenin appears unaltered.**
- **Novel pathway identified include the AHR signalling, GABA receptor signalling and p53 signalling pathways among others.**

References

[1] Lania A, Mantovani G, Spada A. 2012. cAMP pathway and pituitary tumorigenesis. Ann Endocrinol (Paris). 73(2), 73-75.

[2] Elston MS, Gill AJ, Conaglen JV, Clarkson A, Shaw JM, Law AJ, Cook RJ, Little NS, Clifton-Bligh RJ, Robinson BG, McDonald KL. 2008. Wnt pathway inhibitors are strongly down-regulated in pituitary tumours.

[3] Rubinfeld H, Shimon I. 2012. PI3K/Akt/mTOR and Raf/MEK/ERK signalling pathway perturbations in non-functioning pituitary adenomas. Endocrine. 42(2), 285-291.

The Research was funded by the UoM Research Fund Committee Allocation (PHBRP07-02) and Faculty of Medicine and Surgery funds (MDSIN08-22)