‘Invasion signature’ revealed by the analysis of AIP positive and AIP mutation negative human pituitary adenomas

S Barry1, J A Saleh1, E Gadaleta2, C Chelala2 and M Korbonits1

1Department of Endocrinology, William Harvey Research Institute
2Centre for Molecular Oncology, Barts Cancer Institute,
Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK

1. Introduction

Familial isolated pituitary adenoma (FIPA) is an autosomal dominant condition with incomplete penetrance. Heterozygote mutations have been identified in the aryl-hydrocarbon receptor interacting protein (AIP) gene in 20% of FIPA families. In AIP positive patients, the disease is occurring at a younger age and have larger, more aggressive tumours than AIP negative patients and often show invasion at the time of diagnosis as well as poor response to somatostatin analogues than sporadic tumours3.

2. Aims

The aim of this study was to perform comparative gene expression microarray analysis of familial AIP positive and AIP negative adenomas and compare them to sporadic tumours and normal pituitary to discover novel genes and pathways responsible for familial pituitary tumorigenesis.

3. Methods

We have performed gene expression analysis on normal pituitary, sporadic GH-secreting adenomas, AIP positive and AIP negative familial somatotroph adenomas (five samples of each category) using the Affymetrix human Gene Chip HG-U133 Plus 2.0 array. Data analysis was carried out in the statistical ‘R’ environment. Ingenuity Pathway Analysis (IPA) tool was used for pathway analysis. Expression of the ten selected genes from microarray analysis was validated by quantitative reverse transcriptase PCR. Functional assays were performed using BioCoat-Matrigel invasion chambers.

4. Unsupervised hierarchical clustering

5. Identification of differentially expressed genes

6. Ingenuity Pathway Analysis

Ingenuity pathway analysis shows the top functional modules and top canonical pathways

A consensus transcriptome signature of ‘AIPpos tumours’

7. Validation by RT-qPCR

Five up (S100P, NTS, SP1, CRTAC1, ZEB1) and five down-regulated (CTNNB1, CDH1, ESRP1, PERP, EPCAM) genes were validated

8. Invasion assay

AIP knockdown leads to increased invasion of BxPC3 cells

9. Conclusions

These results indicate that our invasion signature is enriched for the epithelial-to-mesenchymal (EMT) markers (CDH1, ESRP1, EPCAM, PERP, CTNNB1, ZEB1) and genes involved in invasion pathway (S100P, SP1, NTS).

Therefore, in pituitary tumorigenesis EMT likely occurs within a specific genetic context and may be related to their increased local invasion and aggressive phenotype which may contribute to treatment resistance.

We have also demonstrated that lack of AIP plays a critical role in cellular invasion; these changes may recapitulate the in vivo situation.

This study has important implications for our understanding of the molecular basis of AIPpos pituitary tumorigenesis. Identified genes may predict the invasive potential of these tumours and provide new opportunity to develop therapies.

10. References


11. Acknowledgement

We are grateful for the financial support from Pfizer to our studies related to FIPA.