PD100648: Integrating genomic and epigenomic alterations in tumours of endocrine system
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Publications funded by the current proposal

1. Banlaki Z, Szabo JA, Szilagyi A, **Patocs A**, Prohaszka Z, Fust G, Doleschall M: *Intraspecific evolution of human RCCX copy number variation traced by haplotypes of the CYP21A2 gene.* **GENOME BIOLOGY AND EVOLUTION** 5:(1) pp. 98-112. (2013), 2013 **IF: 4,759**
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Objectives 1 and 2: A. Evaluation *of the first hit* in tumorigenesis of endocrine glands

During the past three year we successfully identified novel mutations of menin, RET, VHL, SDHB, SDHC and SDHD genes in patients with hereditary endocrine tumor syndromes. The most clinically relevant findings were published within the frame of large international cooperations. By collecting and studying data of more 1200 patients with MEN2 syndrome we were able to demonstrate that adrenal sparing surgery is the most adequate therapeutical approach for removing pheochromocytoma in these patients. Our results were published in the very prestigious **journal The Lancet Oncology** (1). Other study performed by the same international group showed that the aggressive behavior of **pheochromocytoma presented in childhood** was linked to germline mutation of SDHB and VHL genes (2).

Parallel with these studies I collected and reevaluated clinical, laboratory and genetic data of 100 patients with pheochromocytoma/paraganglioma syndromes. In 21 cases mutations were detected. Seven novel mutations and interesting genetic associations were also observed. The manuscript containing data from this part will be submitted this year.

In addition, my group started to use whole genome sequencing approach for identification of germline mutations in patients with monogenic endocrine tumor syndromes. First we used exome sequencing by Illumina and Complete genomics workflows. For data analysis we set up a group composed from informatician, molecular biologist and mathematician for mapping and evaluation of

sequencing data. 17 patients with already known genetic mutations were re-evaluated with two aims: i.) to compare and to test the applicability of exome sequencing in routine genetic practice and ii.) to identify novel genetic modifiers in these syndromes. Our data confirm that **exome sequencing** is able to identify cost-effectively genetic mutations. The cross-validation showed 100 % coincidence between the results obtained from these two platforms. The second aim is still under evaluation, by analyzing SDHB mutation carriers we identified couple hundreds genetic variants between exomes of patients with benign and malignant diseases, but the clear cause for malignancy was not revealed. Increasing sample size and by studying tumor tissue we may be able to detect the different variants. A lecture at National Genetic Meeting was presented from these data (3).

Frequently overseen tumor is a neuroendocrine pancreas tumor. Together with my collaborators we wrote a review about the prevalence, pathogenesis and therapy of pancreas neuroendocrine tumors in von Hippel-Lindau syndrome in order to draw the attention of clinicians to this rare but important manifestation (4). I wrote a chapter about hereditary head and neck cancer for a book for dentistry students (5).

During the last three years another important topic was **the complex evaluation of *CYP21A2* gene**. This gene is located in an interesting chromosomal location frequently presenting as a copy number alteration. Together with Marton Doleschall we showed that the ***CYP21A2*** gene is very polymorph mainly due to its intron 2 (6, 7). During evolution *CYP21A2* was altered by both positive and negative selection mechanisms (7). We were able to describe 33 different *CYP21A2* haplotypes and also by studying the hormone levels of patients with adrenal incidentalomas we demonstrated that **two frequent haplotypes cause different hormone response after ACTH stimulation.** This later finding suggests that individual **sensitivity against everyday stress may be linked to *CYP21A2* haplotypes** (8).

Together with my PhD student, Vince Grolmusz a complex genetic study in patients with **polycystic ovary syndrome** was performed. Our data demonstrated that the functional polymorphisms of Fatty-acid hydroxylase enzyme C385A not (9) but the **polymorphisms of *HSD11B1*** gene may protect against disease (10).

We introduced the genetic testing for Hyperparathyroidism-jaw tumor syndrome. Four clinically diagnosed patients were tested in two of them we successfully identified the pathogenetic alteration (11).

Objective 3: Analysis *of the second hit,* integrating miR's role into the tumorigenesis process

In the beginning of our work we faced that many, valuable data are available in various biorepositories measured in tumor samples obtained from patients with endocrine tumors. Our pioneer work presented an approach about using these data (12). By **integrating data** from various high throughput platforms (genomic data, gene expression and microRNA expression studies) we were able to identify novel **pathomechanisms for adrenocortical cancer** (13), and together with a Canadian group in **renal cell cancer** (14). MicroRNAs showing differential expression between malignant and benign tumors were revealed.

Significantly different expressed microRNAs in adrenal and hormonally non-functioning pituitary tumor revealed that miss-regulation of **cell cycle through microRNAs** may have an impact in the pathogenesis of these tumors. Our data was presented at a scientific meeting (15) and manuscript containing these data is under submission.

Our group was also interested in evaluation of **circulating microRNAs in detection of malignant adrenocortical tumor**. We identified five circulating microRNAs which expression was significantly higher in blood samples obtained from patients with cancer compared to patients with benign disease. These microRNAs can function as biomarkers with good diagnostic power (16, 17).

Objective 4. Functional characterization of miRs identified using in vitro models

Based on integrative data analysis both in pituitary and adrenal tumors involvement of microRNAs in cell cycle was identified as a potential mechanism for tumorigenesis. Together with my PhD student (Vince Grolmusz) we evaluated the dynamism of microRNAs expression during cell cycle progression. A flow cytometry method was developed for sorting of cells based on their DNA content,

and after cell isolation total RNA and protein were purified. Whole genome microRNA and gene expression studies were performed in order to detect those microRNAs and genes which expression show alteration during cell cycle progression. Validation of expression studies was performed with quantitative real time PCR. Our result confirmed that the flow cytometry method valid, cells can be separated and further studied. Gene expression profile revealed 20 genes showing dynamic changes through cell cycle progression. However, no microRNA changed significantly during cell cycle. This surprising result had to be confirmed therefore small RNA sequencing was also used for identification of significantly changing microRNAs, but the results were the same. This data is under validation, a manuscript will be submitted later this year.

Results of functional studies may give us further insights into tumor biochemistry. The impact of miRs on gene and protein expression levels will clarify the related pathomechanism. Our results may open a completely new research area which may have not only diagnostic, but also therapeutic consequences.

Other results:
A systematic review about the role of **glucocorticoids in regulation of clock genes** (19) in peripherial tissues and about the **interplay between local glucocorticoid signaling immune system** (18) was made. These two studies demonstrated that glucocorticoid receptor-mediated signaling is well controlled at multiple levels. Our aims are to further characterize the interaction between GR and clock genes and also between microRNA machinery.

Results and significance

Objective 1-2. Identification of genetic modifiers of MEN1, MEN2, VHL and hereditary pheochromocytoma/paraganglioma syndromes
Sequencing of *Menin, RET, vhl, SDHx and TMEM128* genes represent the first step in genetic diagnosis of hereditary tumor syndromes. Gene and even codon specific genetic counseling can be provided for mutation carriers. Specific therapeutic procedures are available for these patients contributing for individualized therapy. Efficacy of exome sequencing in molecular genetic screening will further help us in obtaining a more rapid diagnosis.

Variants of CYP21A2 may contribute to identify those frequent haplotypes which associate with over stimulation of hypothalamic-pituitary-adrenal axis. Common disorders including obesity and hypertension are also linked to HPA axis disturbances. Therefore we plan to extend this part of our work by studying larger cohorts of patients suffering from these morbidities.

Objective 3-4

By identification of biomarker microRNAs our data will help us in better prediction of outcome of adrenal and pituitary tumors and renal cell cancer. Circulating miRs are also good biomarker candidates, but further validations are needed.

Our integrated algorithm has been successfully used by other groups in obtaining important novel findings in various cancers and pathophysiological conditions.

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