been suggested that HPA axis dysregulation is a potential risk factor for the development of depression. In line with this, several studies reported that L-Dopa treatment may alter the serum levels of ACTH, PRL, and glucocorticoids in parkinsonian patients and Parkinson's disease animal models. In the present study, we determined whether the chronic treatment with L-Dopa altered the stress response inducing depressive-like behaviours. Adult male Wistar rats were treated orally during 24 days with LEBOCAR® - commercial formulation of L-Dopa (75 mg/day) and Carbidopa (7.5 mg/day) - in drinking water. Animals were stressed by immobilization during the last 9 days of treatment and depressive-like behaviours were assessed by the sucrose intake and forced swimming tests. Behavioural tests showed no signs of depressive-like behaviours in the LEBOCAR®-treated and/or stressed rats. We next explored the SAM axis reactivity. Circulating noradrenaline and adrenaline increased in rats treated with LEBOCAR® ($p<0.05$; HPLC). Also, the adrenals from stressed animals showed higher content of adrenaline ($p<0.05$). Then, we studied the HPA axis activity. Chronically stressed rats displayed a lower ACTH secretion (ELISA) and a downregulation of POMC expression (qPCR) in the anterior pituitary ($p<0.05$). In addition, LEBOCAR® treatment induced a reduction in serum ACTH and POMC levels ($p<0.05$). As expected, serum corticosterone (ELISA) enhanced under chronic stress, an effect that was inhibited by treatment with LEBOCAR® ($p<0.05$). Finally, pituitary PRL gene expression (qPCR) was downregulated by LEBOCAR® treatment with a more pronounced effect when rats were also stressed ($p<0.05$). Our results suggest that L-Dopa alters the neuroendocrine stress response enhancing SAM axis reactivity and reducing HPA axis activity and PRL expression.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Evaluation of the Molecular Pathogenesis of Adrenocortical Tumors by Whole-Genome Sequencing

Kerstin Neininger, PhD[1], Patrick May, PhD[2], Barbara Altieri, MD, PhD[3], Juliane L. Lippert, PhD[3], Kirsten Roomp, PhD[2], Guido Di Dalmazi, MD[4], Letizia Canu, MD, PhD[5], Filippo Ceccato, MD[6], Anna Riester, MD, PhD[7], Sabine L. Herterich, PhD[3], Martin Fassnacht, MD[3], Jochen G. Schneider, MD[8], Cristina L. Ronchi, MD[9].
[1]Information Technology for Translational Medicine, Luxembourg, Luxembourg, [2]University of Luxembourg, Luxembourg, Luxembourg, [3]University Hospital of Wuerzburg, Wuerzburg, Germany, [4]University of Bologna, Bologna, Italy, [5]Dipartimento di Scienze Biomediche Sperimentali e Cliniche, AOU Careggi, Prato, Italy, [6]Endocrinology Unit, Padova, Italy, [7]LMU Munich, Munich, Germany, [8]University of Luxembourg, Saarlouis, Germany, [9]University of Birmingham, Birmingham, United Kingdom.

Pathogenesis of autonomous steroid secretion and adrenocortical tumorigenesis remains partially obscure. Our aim was to identify novel genetic alterations in adrenocortical adenomas (ACA) without somatic mutations in known driver genes. Whole-genome sequencing was performed on 26 ACA/blood-derived DNA pairs without driver mutations in *PRKACA*, *GNAS* and *CTNNB1* genes at previous WES (ENSAT study JCEM 2016). These included 12 cortisol-producing adenomas with Cushing syndrome (CS-CPAs), 7 with mild autonomous cortisol secretion (MACS-CPAs), and 7 endocrine-inactive ACAs (EIAs). Seven adrenocortical carcinomas (ACC) were added to the cohort. We developed a bioinformatics pipeline for a comprehensive genome analysis and to reveal differences in variant distribution. Strelka, VarScan2 and ANNOVAR software and an in-house confidence score were used for variant calling and functional annotation. Combined Annotation-Dependent-Depletion (CADD) values were used to prioritize pathogenic variants. Additional focus relied on variants in pathogenically known pathways (Wnt/β-catenin, cAMP/PKA pathway). NovoBreak algorithm was applied to discover structural variations. Two hypermutated CS-CPA samples were excluded from further analysis. Using different filters, we detected variants in driver genes not observed at WES (one p.S45P in *CTNNB1* and one p.R206L in *PRKACA* in two different CS-CPAs). In total, we report 179,830 variations (179,598 SNVs; 232 indels) throughout all samples, being more abundant in ACC (88,954) compared to ACA (CS-CPAs: 31,821; MACS-CPAs: 35,008; EIAs: 29,963). Most alterations were in intergenic (>50%), followed by intronic and ncRNA intronic regions. A total of 32 predicted pathogenic variants were found in both coding (CADD values ≥ 15) and non-coding (CADD values ≥ 5) regions. We found 3,301 possibly damaging and recurrent variants (intergenic mutations removed) (CS-CPAs: 1,463; MACS-CPAs: 1,549; EIAs: 1,268; ACC: 1,660), mostly accumulated in intronic regions. Some of these were detected in members of the Wnt/β-catenin (CS-CPAs: 6; MACS-CPAs: 2; EIA: 1) and cAMP/PKA (CS-CPAs: 6; MACS-CPAs: 7; EIA: 4) pathways (e.g. *ADCY1, ADCY2, GNA13, PDE11A*). We also found a slightly higher number of structural variations in EIA (3,620) and ACC (3,486) compared to CS-CPAs (977) and MACS-CPAs (2,119). In conclusion, still unrevealed genetic alterations, especially in intronic regions, may accompany early adrenal tumorigenesis and/or autonomous cortisol secretion.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Extracellular Vesicles From SDHB Deficient hPheo1 Cells Activate STAT3 in Wild-Type Cells

Hans K. Ghayee, DO[1], Sujal Patel, BS[2], Kubra M. Tuna, MD[2], Lauren Liu, BS[2], Yiling Xu, BS[2], Abdel A. Alli, PhD, MPH[2].
[1]University of Florida and Malcom Randall VA Medical Center, Gainesville, FL, USA, [2]University of Florida, Gainesville, FL, USA.

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors that originate from the adrenal medulla and extra-adrenal paraganglia, respectively. Inactivating mutations in succinate dehydrogenase (*SDHx*) genes leads to succinate accumulation, increased HIF1-α levels, and uncontrollable growth of PPGLs. We hypothesized that small extracellular vesicles (EVs) released from progenitor cells derived from pheochromocytoma (hPheo1) with a shRNA mediated knockdown of *SDHB* are enriched in succinate metabolites that play a key role in the activation of various tyrosine dependent