Abstractsammlung zur 12. Deutschen Nebennierenkonferenz am 13./14.02.2016 in Rostock – orale und Posterpräsentationen

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O1 - Stephanie M. J. Fliedner (Lübeck) *et al*.

Classification of hypoxia inducible factor 2*α* mutation-related paragangliomas as sub-cluster 1C

Recently, activating mutations of hypoxia inducible factor 2α (HIF2A/ EPAS1) have been recognized to predispose to secondary polycythemia, multiple paragangliomas (PGLs), duodenal somatostatinomas, central nervous system hemangioblastomas, and ocular abnormalities. Previoulsy, mutations in the SDHA, SDHB, SDHC, SDHD, SDHAF1, VHL, FH, PHD1, and PHD2 genes have been associated with HIF-activation and the development of PGLs (i.e. cluster-1 or pseudohypoxic (phx) PGLs). In agreement with a common mechanisms of tumorigenesis, the gene expression profile of selected *HIF2A*-mutant PGLs have been shown to share features with phx PGLs. Nevertheless, despite overlap on the molecular level, tumor location, metastatic potential, syndromic presentation, and secretagogue production of phx PGL differ by genotype. Previously, we identified characteristic expression differences according to genotype and tumor location of phx PGLs. Here we aim to establish characteristic differences between *HIF2A* and non-*HIF2A* phx PGLs.
Six *HIF2A* PGLs were compared to normal adrenal medulla (n=8) and other hereditary phx PGLs (von Hippel-Lindau /VHL/: n=13, succinate dehydrogenase subunit B /SDHB/: n=18 and D /SDHD/: n=14).
Unsupervised hierarchical clustering showed that *HIF2A* PGLs make up a separate cluster from other phx PGLs. Significance analysis of microarray yielded 875 differentially expressed genes between *HIF2A* and other phx PGLs after normalization to adrenal medulla (false discovery rate 0.01).
Prediction analysis of microarray allowed correct classification of all *HIF2A* samples based on as little as 3 genes (*TRHDE*, *LRRC63*, *IGSF10*; error rate: 0.02). Genes with the highest expression difference between normal medulla and *HIF2A* PGLs were selected for confirmatory qRT-PCR.
In conclusion, *HIF2A*-PGLs show a characteristic expression signature that separates them from non-HIF2A phx PGLs. Unexpectedly, the most significantly differentially expressed genes have not been previously described as HIF target genes.

O2 – Guido Di Dalmazi (München) *et al*.

Plasma metabolomics profile in patients with Cushing's syndrome

Background: Cushing´s syndrome (CS) is a chronic disorder characterized by endogenous cortisol excess resulting in long-term metabolic and cardiovascular consequences. The identification of metabolic alterations occurring in patients with hypercortisolism could be beneficial in tailoring treatments of co-morbidities. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based assays have recently revolutionized the analysis of targeted metabolomics in biological fluids. So far, no studies investigating metabolomics profile in CS patients have been performed with this technique.
Aim: Our aim was to characterize the metabolic alterations occurring in patients with hypercortisolism by targeted plasma metabolomic profiling.
Methods: Subjects (n=149) were recruited from three German centers (Munich, Berlin, and Würzburg) belonging to the German Cushing registry (CUSTODES) and the European Network for the Study of Adrenal Tumors (ENSAT). Patients were classified in four groups, according to clinical characteristics and results of dexamethasone suppression test (DST): non-secreting adrenocortical adenomas (NS, post-DST cortisol <1.8µg/dL) (n=27), subclinical hypercortisolism (SCS, post-DST cortisol >1.8µg/dL without clinical signs of hypercortisolism, in presence of adrenal mass) (n=34), CS (post-DST cortisol >1.8µg/dL with signs of hypercortisolism) (n=46), and excluded Cushing (EC, post-DST cortisol <1.8µg/dL in patients with initial clinical suspicion of hypercortisolism, without adrenal tumor) (n=42). Plasma targeted metabolomics profiling was performed using AbsoluteIDQ-p180 kit (BIOCRATES AG, Austria). Internal standards served as reference for calculation of metabolite concentrations.
Results: Metabolic profile of CS was characterized by reduced carnitine levels (P=0.003) and increased activity of carnitine palmitoyl-transferase I (P<0.001), with respect to EC and SCS.
Polyamine levels (putrescine [P=0.018], spermidine [P<0.001], spermine [P<0.001]), and serotonin (P=0.013) were increased in CS patients, whereas tryptophan and kynurenine levels were decreased (P=0.002 and P=0.001, respectively) when compared to EC. Spermidine was progressively increased among NS, SCS, and CS patients, and showed positive correlation with post-DST cortisol (Coefficient=0.341, P<0.001). Logistic regression analysis, including age as a covariate, showed that the panel of significant metabolites among groups was able to correctly classify 84.6% of subjects (88.1% EC, 77.8% NS, 82.4% SCS, 87.0% CS). Using logistic regression and ROC curves we established two discriminating scores with sensitivity >95% and specificity >85% (area under the curve 0.971 and 0.978, respectively, P<0.001).
Conclusion: Metabolomic profile by LC-MS/MS revealed several metabolic disturbances in patients with hypercortisolism, mainly involving polyamine and tryptophan metabolism, and beta-oxidation. Metabolomic analysis showed also good accuracy in classifying patients with hypercortisolism according to their specific metabolic phenotype.

O3 – Mirko Peitzsch (Dresden) *et al*.

Paediatric reference intervals of plasma free normetanephrine, metanephrine and 3-methoxytyramine: Application for diagnosis of neuroblastoma

Background: Neuroblastoma is the most common extracranial solid tumour of childhood representing 8.5% of all malignancies in paediatric patients. These neoplasms derive from neural crest progenitors and like phaeochromocytomas produce catecholamines. The tumours, however, have a limited capacity for catecholamine storage and secretion so that the catecholamines produced are largely metabolized within tumour cells. Catecholamine metabolites, not the parent amines, therefore provide the mainstay for diagnosis, but continue to rely on measurements in spot urine samples of homovanillic acid (HVA) and vanillylmandelic acid (VMA), tests first implemented 50 years ago, with limited diagnostic sensitivity. Measurements of free metanephrines, the O-methylated metabolites of catecholamines, are well established to provide superior sensitivity for diagnosis of phaeochromocytoma than the parent amines or downstream metabolites such as VMA. Improved understanding of catecholamine metabolism, specifically recognition of production of metanephrines within chromaffin cells and their tumour cell derivatives provides the basis for recognizing the diagnostic advantages of metanephrines, which we hypothesize, might also apply to neuroblastoma.
Methods: Plasma concentrations of normetanephrine, metanephrine and methoxytyramine were measured in two patient cohorts: 1. a patient cohort, without evidence of a catecholamine-producing neoplasm, including 228 girls and 212 boys, aged between 2 days and 18 years; and 2. additional 25 paediatric patients with confirmed diagnosis of neuroblastoma.
Results: Among the reference group cohort, concentrations of plasma free normetanephrine and 3-methoxytyramine were high in neonates up until six months of age, but thereafter decreased within the next several months to levels comparable to those of adults. In contrast, concentrations of plasma free metanephrine showed a reciprocal pattern with low concentrations in neonates, thereafter increasing during the first year to levels comparable to those of adults. Using this reference population to establish upper cut-offs, all children with neuroblastoma had increases in either or both methoxytyramine (96%) and normetanephrine (84%), indicating a diagnostic sensitivity of 100%. For the routinely used combination of VMA and HVA, diagnostic sensitivity was 94.4% (94.4% for HVA and 66.6% for VMA).
Conclusions: The dynamic reciprocal changes in plasma concentrations of normetanephrine and methoxytyramine compared to metanephrine during early childhood suggest underlying developmental changes in extra-adrenal and adrenal chromaffin tissue that must be considered in establishing paediatric reference intervals in catecholamine metabolites. With such reference intervals now at hand, the present results also indicate that measurements of plasma normetanephrine and methoxytyramine provide a potentially useful diagnostic test for identification of patients with neuroblastomas.

O4 – Cristina L. Ronchi (Würzburg) *et al*.

Genetic landscape of sporadic unilateral adrenocortical adenomas without PRKACA p.Leu206Arg mutation

Genetic alterations affecting the PKA/cAMP pathway are commonly found in cortisol-producing adrenocortical adenomas (ACAs), while activating mutations in the gene coding for beta-catenin (*CTNNB1*) are reported in both adenomas and carcinomas. However, the molecular pathogenesis of most ACAs is still unclear. Aim of the study was a comprehensive genetic characterization of sporadic ACAs.
Whole-exome sequencing was performed on DNA of ACAs and corresponding blood samples of 99 patients (39 with overt Cushing´s syndrome, 35 with subclinical hypercortisolism and 25 endocrine inactive) negative for the p.Leu206Arg *PRKACA* mutation.
In total, 706 protein-altering somatic mutations were detected in 88/99 ACAs (median: 6 mutations per sample, range: 0-55). Several altered genes could be recognized as part of the Wnt/β-catenin pathway (*CTNNB1, APC, APC2, PKP2*, and different members of the protocadherin superfamily), being associated with larger tumor size and endocrine inactivity. Moreover, many components of the cAMP/PKA pathway were affected by somatic mutations, including three new mutations in the *PRKACA* gene, mutations in metabotropic glutamate receptors (*GRM3, GRM4, GRM6*) and others (*GNAS, PRKAR1A, CREB1, CREBBP, ADCY3*), being associated with female gender and overt Cushing's syndrome. Finally, more surprisingly, we also observed several alterations in genes involved in the Ca2+ signaling.
In conclusion, this study represents the most comprehensive genetic characterization of unilateral ACAs, including inactive adenomas. We thereby identified somatic alterations affecting signaling pathways known or potentially involved in the adrenal tumorigenesis.

O5 – Constanze Hantel (München) *et al*.

Small non-coding RNAs as markers for therapeutic response in a preclinical *in vivo* model for adrenocortical carcinoma

The vault complex, consisting of three vault proteins and four small non-coding RNAs is considered the largest intracellular ribonucleoprotein particle. Although in the past vaults were believed to be involved in multidrug resistance, the exact function of this complex has remained uncertain.

Recently, we investigated the therapeutic applicability of a Tumor-Vascular-Disrupting Agent in preclinical models for endocrine tumors. Subsequent analyses identified vault RNAs 1 - 3 as the most pronounced regulated transcripts in a neuroendocrine tumor model showing therapeutic responsiveness while no such changes were detectable in therapy-resistant adrenal NCI-H295R xenografts. Subsequently, we investigated NCI-H295R xenografts, which had been treated and shown to be responsive to two different chemotherapeutic regimens (EDP-M and LEDP-M). In this therapeutic setting treatment-dependent upregulation of vault RNAs in NCI-H295R tumors was also evident (% of controls; vault1: EDP-M 296.3±44%, $p<0.001$; LEDP-M 243.5±16%, $p<0.001$; vault2: EDP-M 157.2±20%, $p>0.05$; LEDP-M 160.7±20%, $p>0.05$; vault3: EDP-M 241.2±55%, $p<0.001$; LEDP-M 118.9±16%, $p>0.05$). Moreover, we investigated expression of various microRNAs in these xenografts. While expression of miR-195, miR-483-3p, miR-483-5p and miR-503 were not significantly altered, miR-210 was inhibited by EDP-M (39.69±26%, $p<0.05$) and LEDP-M (37.86±23%, $p<0.05$) compared to controls. miR-210, also known as master hypoxamir, is known to be highly upregulated at hypoxic conditions, contributing to aberrant regulation of cell proliferation, DNA stability and angiogenesis. Moreover, elevated miR-210 levels were recently shown to be associated with tumor aggressiveness and poor prognosis in ACC. In summary, small non-coding RNAs might have potential to improve and monitor efficacy of anticancer treatment against ACC.

O6 – Christian Adolf (München) *et al*.

Paradoxical worsening of lipid metabolism after successful treatment of primary aldosteronism.

Context: Primary aldosteronism (PA) describes the most frequent cause of secondary arterial hypertension. Aldosterone itself represents a BP-independent cardiovascular risk factor associated with increased rates of morbidity and mortality. Recently a worsening of lipid metabolism after successful treatment has been described.

Objective: Our aim was to analyse changes in lipid parameters according to treatment outcome in a large prospective cohort of PA patients. Data of 215 consecutive PA patients from 4 centers with documented unilateral aldosterone-producing adenoma (APA, n=144) or bilateral idiopathic adrenal hyperplasia (IHA, n=71) were extracted from the database of the German Conn's Registry.

To assess the metabolic outcome, they were investigated before, one year and three years after successful treatment by adrenalectomy (ADX) or by MR-antagonist (MRA).

Methods: Creatinin (Cr), glomerular filtration rate (GFR), fasting plasma glucose (FPG) and components of lipid metabolism including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured at 8.00 after a 12-hour fasting period.

Results: One year after initiation of treatment (either ADX or MRA treatment) potassium had been normalized in all patients. HDL-C and triglycerides changed inversely. HDL-C was significantly lower in patients with APA (53.00 ± 16.81 *vs*. 52.00 ± 16.88 [mg/dl], p=.03) and IHA (52.00 ± 14.64 *vs*. 48.00 ± 14.55 [mg/dl], p=.00) after treatment.

Triglycerides were significantly higher in both subgroups at follow-up (APA 103.50 ± 76.40 *vs*. 111.00 ± 77.12 [mg/dl], p=.00; IHA 111.00 ± 57.85 *vs*. 129.00 ± 89.45 [mg/dl], p=.02), whereas BMI remained unchanged and FPG even improved in follow-up patients with APA (99.00 ± 19.37 *vs*. 95.00 ± 17.53 [mg/dl], p=.00).

Furthermore, there was a significant decrease in GFR, as well as an increase in Cr and urea in both subgroups at one year follow-up (p=.00). Changes in HDL-C and TG correlated with decrease in GFR. Three years after initiation of treatment there was a slight further decline in GFR (82.15 ± 21.76 vs. 80.49 ± 23.37 [ml/min/1,73 m²]; p=.01), whereas HDL-C remained stable.

Conclusion: Our results show that treatment of PA is associated with a worsening of lipid parameters despite improved glucose parameters and stable BMI. This paradoxical effect could be explained by renal dysfunction following adrenalectomy or spironolactone therapy caused by a decrease in glomerular hyperfiltration. In view of stable LDL-C, reduced albuminuria, FPG and blood pressure, however, a higher risk for atherosclerosis in patients with APA and IHA after successful treatment seems unlikely.

O7 – Sara Jung (München) *et al*.

Investigation of a novel liposomal chemotherapy protocol in different preclinical models for adrenocortical carcinoma *in vivo*.

Recently, we demonstrated for adrenocortical carcinoma (ACC) in NCIh295 xenografts promising antitumoral effects for LEDP-M (etoposide, liposomal doxorubicin, liposomal cisplatin, mitotane) a liposomal variant of the classical EDP-M protocol (etoposide, doxorubicin, cisplatin, mitotane).

However, clinical translation of novel therapeutic regimens remains challenging due to high tumor heterogeneity. Thus, to obtain preclinical results with more clinically predictive power we investigated for the first time LEDP-M comparatively in two further xenograft models for ACC *in-vivo*: SW-13 and the novel pediatric tumor model SJ-ACC3. Furthermore, we included liposomal etoposide resulting in a novel treatment scheme called L(l)EDP-M. In short-term experiments, after one treatment cycle, tumors were immunohistochemically [cells/high-power fields (HPF)] analysed regarding total cell number [Ki67 positive and negative/HPF] and apoptosis [TUNEL positive cells/HPF]. The number of tumor cells decreased for SW-13 in all treatment groups with highest therapeutic efficacy for the liposomal variants (EDP-M: 20.5±1.6 $p<0.01$; LEDP-M: 17.2±1.3 $p<0.001$; L(l)EDP-M: 14.7±0.9 $p<0.001$) vs. controls (28.9±2.2). In contrast, in SJ-ACC3 xenografts only EDP-M (30.3±1.2 vs. controls 35.9±1.3, $p<0.05$) led to short-term therapeutic effects. Long-term experiments confirmed for SW-13 antitumoral efficacy upon all treatments compared with controls ($p<0.001$) and for L(l)EDP-M furthermore improved overall survival compared to EDP-M ($p<0.003$). Moreover, while H&E-stainings revealed strong nephrotoxic effects upon repeated treatments with EDP-M, L(l)EDP-M was associated with improved off-target profiles. For SJ-ACC3 xenografts, long-term experiments confirmed poor antitumoral efficacy of free and liposomal treatment schemes.

In summary, liposomal regimens hold promise for clinical translation mainly for adult, but less so for pediatric ACC. However, our results also show that tumor heterogeneity should be taken more into account in preclinical studies.

O8 – Constanze Hantel (München) *et al*.

MUC-1 – update on a newly established tumor model for adrenocortical carcinoma

Only two human cell lines (NCI-H295R and SW-13) and one pediatric xenograft model (SJ-ACC3) are available for adrenocortical carcinoma (ACC). Furthermore, SW-13 is at least suggested to be not derived from an ACC, but from a small cell carcinoma metastasized in the adrenal gland. In an attempt, to overcome this lack of preclinical models we recently aimed at the development of patient-individual tumor models for ACC. During these studies one xenograft (MUC-1), derived from a neck metastasis of an ACC, showed extraordinary engraftment properties and sustained tumor growth over several passages in the murine host. Immunohistochemical analyses of explanted tumors revealed highly vascularized, proliferating and SF-1 positive MUC-1 xenografts. Various samples have been collected during different passages to allow further characterization and future

utilization as a new preclinical tumor model for ACC. During ongoing studies we investigated tumor samples of all currently available xenograft models for ACC by quantitative real time PCR and immunohistochemistry. Thereby, we found compared to NCI-H295R significantly lower IGF-1-receptor ($p<0.01$) and elevated EGF-receptor expression ($p<0.01$) for MUC-1 while SJ-ACC3 was characterized by extraordinary high levels of SF-1 ($p<0.001$) and IGF-2 ($p<0.001$). Moreover, we determined the Ki67 indices [%] for all xenograft models *in vivo* (NCI-H295R: 50.3±1.3, SW-13: 60.8±10.3, SJ-ACC3: 33.7±3.6 and MUC-1: 30.3±3.3; all $p>0.05$ vs. NCI-H295R). *In vitro*, we established a primary culture based on explanted MUC-1 xenograft pieces. A first try of cell culture establishment failed after several passages due to a massive contamination by murine fibroblasts.

Thus, we initiated a second round of culturing involving continuous and highly specific murine and human fibroblast removal. The resulting multi-clonal cell suspension is now viable in passage 13.

Cross-contamination by murine fibroblasts could be excluded on the basis of a Universal-Primer Probe-Assay using ApoE as specific target gene and representative pictures of passages 4, 7 and 10 demonstrate specific stainings for Ki67, SF-1 and 3ßHSD. Moreover, genetic characteristics of MUC-1 cells were investigated by PCR-Single-Locus Technology and the appropriate genomic DNA reveals a distinct marker profile different from that of NCI-H295R and SW-13 cells. Thereby, MUC-1 cells were recently authentified and certified as a novel adrenocortical cell line of human origin.

P1 - Christina M. Berr (München) *et al*.

Muscle strength in Cushing's syndrome: cross-sectional evaluation of the German Cushing's Registry

Background: Endogenous Cushing's syndrome (CS) is rare with an estimated yearly incidence of 1-3 patients/million. CS describes a group of diseases that have in common an excess secretion of glucocorticoids which results in a characteristic clinical phenotype. Severe courses of Cushing's syndrome are characterized by a break-down of protein catabolism translating into clinical consequences including muscle weakness and extremely thin skin with impaired wound healing. While remission of CS is achievable by surgical removal of the ACTH- or cortisol-producing tumor, the effect of biochemical cure on muscular function is yet unclear.
Objective: The aim was to analyze parameters of muscular function in Cushing's syndrome Methods: We performed a cross-sectional, prospective study (as part of the German Cushing's registry) analysing 285 consecutive patients in 4 centres of the Cushing's registry. Patients with CS were studied during the active phase of the disease or after successful treatment. Rule-out CS patients were used as controls. The following parameters were analysed: hand grip strength using a hand grip dynamometer and the chair rising test as measure of proximal muscular function. Hand grip was standardized to age and sex.
Results: We included 44 patients with active Cushing's syndrome (ACS, 64 % female), 149 with Cushing's syndrome in clinical and biochemical remission (CSiR, 82 % female, remission time 2 to 53 yrs.), and 92 patients with ruled-out Cushing's syndrome (RO, 70 % female). The age and gender corrected normally distributed hand grip strength was significantly lower in ACS compared to the RO group (right hand p=0.007, left hand p=0.003). Similarly, lower limb muscular function was impaired in ACS (p=0.004). We did not find a correlation between parameters of muscular function and biochemical parameters of glucocorticoid excess in the ACS group. The CSiR group showed age and gender corrected reduced hand grip strength (94 % for non-dominant hand, p=0.007; 92 % for dominant hand p<0.001 compared to normal reference values). One third of patients in the remission group performed a chair rising test of ≥ 10 seconds independent of time elapsed since successful treatment (p=0.18).
Conclusion: Cushing's syndrome affects muscle strength in the acute phase, but functional impairment remains detectable also in the long term.

P2 – Marcus Quinkler (Berlin) *et al*.

The cardiovascular markers Copeptin and hsCRP in Primary Aldosteronism – Data from the German Conn's Registry and the KORA F4 Study

Context: Copeptin and high-sensitive CRP (hsCRP) are biomarkers associated with increased mortality in patients with cardiovascular and cerebrovascular disease as well as in the general population. No data exists regarding these markers in patients with primary aldosteronism (PA).
Objective: To evaluate copeptin and hsCRP levels as cardiovascular risk markers in PA patients.
Methods: 113 PA patients (64% male) from two centers of the prospective German Conn's Registry were identified, for whom a full data set and blood samples at baseline and follow-up (14±3.4 months) after initiation of specific PA treatment were available. These cases were matched 1:3 (n=339) for sex, renal function, BMI, age and systolic blood pressure with participants from the KORA F4 survey. Copeptin and hsCRP were determined by sandwich fluoroimmunoassay.
Results: HsCRP but not copeptin levels, were significantly higher in PA patients at baseline compared to matched controls. Following specific therapy, copeptin and hsCRP decreased significantly in PA patients (7.8 (4.6, 13.5) to 5.0 (3.1, 8.9) pmol/L, p<0.001; 1.6 (0.8, 3.4) to 1.2 (0.6, 2.1) mg/L, p<0.001 respectively). Men had higher copeptin and hsCRP levels at baseline and at follow-up compared to women. The combination of sex, hypokalemia, lateralization index and blood pressure were the best predictors of outcome. However, copeptin and hsCRP had no predictive value despite the association of lower copeptin levels with better outcome regarding cure of PA.
Conclusion: Copeptin and hsCRP levels decrease following specific PA therapy reflecting successful cardiovascular risk reduction. However, they are no independent predictors regarding cure of PA.

P3 – Ulrike Heise (Rostock) *et al*.

The aldosterone to renin and potassium ratio compared to the aldosterone to renin ratio

Background: The aldosterone to renin ratio (ARR) is a widely employed screening tool for primary aldosteronism (PA). Because aldosterone secretion also depends on serum potassium concentrations it is recommended to supplement potassium when hypokalaemia is present. However, this procedure is frequently impracticable because the potassium concentrations may still differ from the target value of approximately 4.0 mmol/l. Therefore, we asked if correcting the ARR for serum potassium values benefits patients to avoid potassium supplementation applying the ARR and the new formula of aldosterone to renin and potassium ratio (ARP).
Methods: In a retrospective design, we studied 136 subjects (mean age 54.8 yrs., 48.5 % women), 43 with PA and 93 with essential hypertension (EH). PA patients had by definition an elevated ARR and positive confirmatory test. EH was diagnosed when PA and other secondary forms of hypertension were excluded. To analyse the sensitivity and specify of the ARR and ARP formulas, we employed the receiver-operating curve (ROC) and calculated Youden's indices.
Results: The ROC analysis shows almost similar curves for ARR and ARP with the equivalent area under the curve (99.4 %). The best cut-off value for the ARR was 27 (sensitivity 100 %, specificity 93.5 %, PPV 86 %, NPV 100 %, Youden's index 0.935) and for ARP was 10 liter per mmol (sensitivity 97.7 %, specificity 95.7 %, PPV 90.7 %, NPV 98.9 %, Youden's index 0.934).
Conclusion: The statistic tests show that the ARP has a comparable screening power as the ARR. This indicates that the ARP can be used just as well as the ARR to screen for primary aldosteronism and it may improve the screening process.

P4 – Hanno Funk (Düsseldorf) *et al*.

Calculation Models to determine lateralization of Aldosterone Secretion in Adrenal Venous Sampling

Background: Adrenal venous sampling (AVS) is the method of choice to distinguish unilateral from bilateral excess aldosterone secretion. Aldosterone to cortisol (A/F) ratios are determined in each of the adrenal veins and compared to each other for calculation of the lateralization index (LI). However, the concentration of steroid hormones in the adrenal venous blood is the sum of hormone distributed to the respective adrenal gland in addition to the secreted amount of hormone. We studied the hypothesis that subtraction of the arterial inflow from the adrenal venous outflow may render results superior to the classically calculated LI.
Methods: We retrospectively analyzed 70 patients with definite primary aldosteronism who all underwent computed tomography or magnetic resonance imaging and altogether 83 AVS studies. 34 patients underwent unilateral adrenalectomy (ADRx). We applied four different calculation models and related the results to the information from imaging in all cases and to follow-up and histology in the ADRx patients.
Results: The overall AVS success rate was 90 % when both F and A were analyzed. Classical models for calculating the LI revealed a lower matching rate with imaging studies (65 %) compared to two calculation models respecting A and F concentrations distributed to the adrenal glands (68 %) when peripheral blood was drawn and analyzed in parallel to each of the two adrenal vein cannulations. This
was also seen in the subset of ADRx-patients (71 % *vs*. 73 % for imaging and 94 % vs. 97 % for histology).
Conclusion: Calculation models that respect peripheral hormone concentrations seem to better characterize the amount of A and F secreted by the adrenal glands. This seems to be only relevant for AVS studies when the adrenal venous blood is diluted. For such cases, current models of determining the LI may have to be revised.

P5 – Constanze Hantel (München) *et al*.

IGF1-R inhibition and liposomal doxorubicin: progress in preclinical evaluation for the treatment of adrenocortical carcinoma

Adrenocortical carcinoma (ACC) is a tumor with poor prognosis and limited therapeutic options. In the past, IGF1 receptor (IGF1-R) dependent signaling has been shown to promote tumorigenesis and preclinical studies have provided evidence for therapeutic applicability of IGF-1 receptor directed approaches against ACC. Based on these findings, different IGF-1-R targeting therapies have been investigated in recent years and some of them have led to promising results in preclinical and early clinical studies. However, subsequent clinical trials were disappointing and it remains uncertain whether inhibition of IGF1-R alone is sufficient to mediate sustained therapeutic effects. Recently, we provided evidence for short-term-therapeutic efficacy of IGF-1R inhibition in combination with liposomal doxorubicin (L) as well as of anti IGF1-receptor antibody (Ab) coupled, doxorubicin loaded immunoliposomes (IL) against NCI-H295R xenografts.

In the current study, we extend our findings by long-term treatments in this classical adrenocortical xenograft model as well as by short-term experiments in two novel ACC xenograft models (SJ-ACC3 and MUC-1) which all display different IGF-1R and IGF-2 expression levels. Assuming direct relevance of these factors for therapeutic efficacy our expression analyses suggested NCI-H295R (with high IGF-1R levels) and SJ-ACC3 (with high IGF-1R and IGF2 levels) as tumor models with presumed good therapeutic responsiveness to IGF-1R inhibiting approaches, while for MUC-1 (with low IGF-1R and IGF2 levels) this therapeutic strategy was expected to be of less benefit.

Overall, our therapeutic experiments confirmed these predictions: Long-term therapy led to significantly reduced tumor sizes for L + Ab and IL treated NCIH295R xenografts. Furthermore, in both specific treatment groups high rates of complete remissions were evident (L + Ab: complete remission (CR) 43%, partial remission (PR) 14%, stable disease (SD) 14%, progressive disease (PD): 29% ; IL: CR 29%, PR 0%, SD 14%, PD 57%; NaCl: CR 0%, PR 0%, SD 14%, PD 86%). Moreover, after a single therapeutic intervention SJ-ACC3 displayed trends towards anti-tumoral activity regarding endpoints as Ki67 positive cells, area of proliferating cells and P-Akt/Akt ratio for SJ-ACC3 (NaCl: 71.1±14.3%, L + Ab: 38.5±14.3%, IL: 45.8±7.2%; n.s.) while opposite trends were detectable for MUC-1(NaCl: 39.8±8.5%, L + Ab: 46.4±9.5%, IL: 73±3.7%; n.s.).

In summary, our experiments reveal strong therapeutic efficacy of this regimen against NCI-H295R, but suggest furthermore sub-group dependent differences in therapeutic outcome reflecting clinical observations. Thereby, implementation of this panel of tumor models might be helpful for clinical translation of novel regimens in the future.

P6 – Holger S. Willenberg (Rostock) *et al*.

11-deoxycortisol and other markers to define adrenal insufficiency in the overnight oral metyrapone test

Background: The oral overnight metyrapone test (OMT) serves to identify patients with central adrenal insufficiency (sAI) and its performance is comparable to the insulin tolerance test (ITT). By blocking 11-beta-hydroxylase activity and cortisol synthesis, metyrapone leads to an increase in corticotropin (ACTH) and 11-deoxycortisol (11S). However, measurements of 11S serum concentrations and test results are not easily available and this has hampered a wider distribution despite the disadvantages of the ITT. Therefore, we asked whether determination of the 11S precursor 17-hydroxyprogesterone (17-OHP) and its metabolite androstenedione can be utilized as equally good indicators of sAI in the OMT.

Methods: We studied 21 patients (mean age 63.8 yrs., 45.8 % women) who were evaluated for the presence of sAI on the basis of pituitary disease. Plasma ACTH concentrations, serum cortisol, 11S, 17-OHP and androstenedione were determined at baseline and at 8 a.m. in the morning after oral application of 2 gr metyrapone at 11 p.m.

Results: Of the patients, 12 had sAI and 9 had intact adrenal function as defined mainly by the outcome of the ITT (max. cortisol < 500 nmol/l for the diagnosis of sAI). The best parameter to distinguish sAI from normal adrenal function was 11S, followed by ACTH and 17-OHP with areas under the receiver-operating curves of 1.0, 0.97 (1 false positive) and 0.938 (3 false negatives), respectively. Best cut-off values were 9.0 µg/dL for 11S, 65 pg/mL for ACTH and 10.0 nmol/L for 17-OHP. The maximal ACTH concentration correlated significantly with 11S (Spearman-rho 0.893, $p<0.001$) and 17-OHP (Spearman-rho 0.883, $p<0.001$).

Conclusion: Our study shows that 11S and ACTH are good markers for the read-out of the OMT, while the latter is currently easier to assay than 11S. Whether 17-OHP really produces false negative test results should rather be evaluated against a long-term clinical judgement than against the comparison to other biochemical markers. Androstenedione performed inferior to the other markers for diagnosing sAI.

P7 – Abeer El Wakil (Dresden) *et al*.

Biological *in vivo* characterization of adrenal physiopathology in murine neuroblastoma models.

Background: Neuroblastoma is a highly heterogeneous disease, which arises from the developing sympathetic nervous system and is commonly found in the adrenal medulla. It is the most frequent malignant tumor in children accounting for 15% of childhood cancer mortality. A number of characteristic chromosomal aberrations are observed and activating mutations of Anaplastic Lymphoma Kinase (ALK) have been described. The expression pattern of ALK in vertebrates has been described in several studies, where ALK has been shown to be expressed in the CNS and the PNS, as well as in testis and ovary. Despite this, deletion of *ALK* in mice does not result in serious phenotypes. The precise physiological role of ALK in mammals is still unclear. This study is aimed at the molecular characterization of the role of ALK mediated signaling in adrenal pathophysiology as well as the assessment of its role in neuroblastoma biology.

Methods: The key aims of this study were threefold: (1) First, characterization of the impact of the loss (knockout) or gain (gain-of-function) of ALK activity on the cellular and/or the zonal identity of the mouse adrenal glands and investigation of its modulation by sex and age in both mouse models; (2) Second, identification of differentially expressed transcripts in the adrenal glands of both mouse models compared to wild type mice with respect to sex; and (3) Third, assessment of the relevance of the genetic pathways identified by these studies for the tumorigenicity of the adrenals.

Results: These preliminary studies using a mouse model of mutated *ALK* within the kinase domain which transforms the receptor into a constitutively active version, revealed a key role for this gene in regulating aldosterone secretion in the adrenal gland. The adrenals from these mice displayed intense aberrant *Cyp11b2* expression in the *zona fasciculata* as compared to normal expression in the adrenals of wild type (WT). This leads to hyperaldosteronism and consequent hypertension. Remarkably, this phenotype is not evident in *ALK*-deficient mice in which the kinase domain encoding exons have been removed. Moreover, mouse models of increased *ALK* signaling resulted in multifocal adrenal neoplasia as compared to wild type and/or *ALK*-deficient murine adrenals. Surprisingly, these neoplasias in the adrenals of ALK gain of function mice failed to develop adrenal hyperplasia or tumors over the time course of 18 months during the study.

Conclusion: Together, these data have shown the pivotal importance of ALK in the regulation of aldosterone production in the adrenal cortex *in vivo* and have revealed an unexpected effect of ALK upon the functional zonation of the adrenal cortex. The availability of these ALK mouse models provides a formidable tool for the assessment of the role of *ALK* in regulating aldosterone production and subsequent adrenocortical functional zonation as well as for deciphering its contribution to adrenal neoplasias. Moreover, these data have suggested that ALK is an adrenal oncogene, but is not fully oncogenic as additional genetic events are thought to be required for malignant progression.

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Herzlich, Ihr Holger Willenberg