the fate of the cells constitutive of each zone through the expression of Ki-67 and cleaved Caspase-3. Thirty eight normal human adrenals (16 females, 22 males, ranging in age from 22 to 81 years old with a median age of 52 years old) were obtained from brain-dead organ donors (kindly provided by the Organ Transplant Clinics, University Hospital of Rouen). As early as 22 years old, we found that the histological ZG (h-ZG) does not correspond to the functional ZG (f-ZG) expressing CYP11B2. Moreover, the h-ZG CYP11B2- cells were CYP11B1+ showing that these cells ascribed to the h-ZG are in fact cortisol producing cells. The progressive replacement of CYP11B2+ cells by CYP11B1+ cells in the h-ZG might demonstrate the role of the extracellular matrix in the morphological maintenance of the adrenal cortex. Our analysis also showed that steroidogenic cells were either CYP11B1 or CYP11B2 positive. By immunofluorescence, we observed in many cases isolated or clusters of CYP11B2+ cells located deeply in the h-ZF and sometimes in the vicinity of the central vein. We were able to show that those cells were probably issued from CYP11B2+ cell clusters located in h-ZG which migrated centripetally. Ki-67 immunoreactivity was highly variable and observed throughout the entire cortex. We also found a positive correlation between the steroidogenic and endothelial cells proliferation. It is interesting to note that some Ki-67+ cells located in the h-ZG were CYP11B1+. Cortical cells positive for cleaved Caspase-3 were extremely rare but detected in all zones when present. These findings challenge the classic view of lineage conversion of differentiated ZG cells and show a new pathway where the CYP11B2+ cells migrate without changing their phenotype.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

The Impact of Cannabinoid Exposure on Glucocorticoid Receptor Signaling in Neural Stem Cells

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Preterm birth-birth before 37 weeks of pregnancy-can cause many short- and long-term complications in newborns, including respiratory distress syndrome (RDS). RDS results from incomplete lung development and a surfactant deficiency, and it is a major factor of pre-term mortality. Synthetic glucocorticoids (sGCs) such as Betamethasone or Dexamethasone (Beta, Dex) are administered prenatally to women at risk of pre-term birth to prevent preterm complications. While sGCs are known to improve outcome, they also cause alterations in brain development and neural stem cell biology that are associated with long-term neurological defects.

One common recreational drug used during pregnancy is cannabis. Some of the active components of cannabis include cannabinoids, which interact with the endocannabinoid receptor pathway in cells. Cannabinoids have been shown to induce proliferation and differentiation of embryonic neural stem cells (NSCs). We hypothesized that maternal cannabis use activates cannabinoid signaling pathways and leads to changes in glucocorticoid signaling in the developing brain. The purpose of this study was to determine whether cannabis use leads to a better or worse neurological outcome for children born pre-term and treated with sGCs for RDS.

Neural stem cell neurospheres (NSCs) were isolated from the cerebral cortex of mice and treated with Vehicle (ethanol), Dex, cannabinoid receptor agonist WIN-55,212-2 (Win), or a combination WinDex. The transcriptional profile induced by exposure to Vehicle, Dex, and WinDex RNA were analyzed using microarray analyses examining the complete expressed genome. Gene Chip profiles indicated that both glucocorticoids and cannabinoids induce distinct transcriptional responses in E14.5 NSCs. The genes involved in proliferation-including *S100a11, Jun,* and *Bex2-* were repressed by Dex whereas WinDex rescued some of these expression profiles. Some genes encoding microRNA that inhibit our top target coding genes implicated in proliferation showed a greater induction by Dex compared to WinDex.

Quantitative Polymerase Chain Reaction (qPCR) was performed to validate our genes of interest, including *Adm*, which has been shown to induce neural stem cell proliferation and differentiation. The biological impact of Winn on Dex-induced changes in NSC function were examined by in-vitro proliferation and differentiation studies using antibodies to Tuj1 (neurons), GFAP (glia), and CNPase (immature oligodendrocytes). The experiments indicate that Dex increased neuronal and oligodendrocyte differentiation, while WinDex appeared to reverse this phenotype in neurons.

These studies suggest that cannabis use during pregnancy may limit the biological impact sGCs for preterm birth and lead to distinct cellular responses.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Unravelling the Genetic Basis of ACTH-Mediated Aldosterone Hypersecretion in Hypertensive Patients Without Primary Aldosteronism

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Introduction: Primary aldosteronism (PA), a condition characterized by autonomous aldosterone hypersecretion, constitutes the most common cause of secondary hypertension. PA includes both sporadic and familial forms, inherited in an autosomal dominant manner. Recent evidence suggests a higher prevalence of aldosterone excess among hypertensive patients than previously thought, while chronic stress-related ACTH-mediated aldosterone hypersecretion has also been implicated in the pathogenesis of essential hypertension. **Objective:** To determine whether genetic variations of aldosterone regulating genes could be implicated in the ACTH-mediated aldosterone hypersecretion in hypertensive patients without PA. **Methods:** Twenty-one hypertensive patients without PA, who exhibited ACTH-mediated aldosterone hypersecretion, underwent Whole Exome Sequencing (WES) on Novaseq 6000 platform (Illumina). As hyper-responders were defined patients whose aldosterone (ALD) and aldosterone-to-renin ratio (ARR) response to ultra-low ACTH stimulation test was above the 97.5th percentile values of controls. The cutoff levels for ALD and ARR were 1300 pmol/L and 77 pmol/mIU, respectively. Variant calling was performed according to GATK best practices and VCF files were filtered for variants in 25 genes associated with PA. To identify new susceptibility genes for PA, VCF files were also intersected for variants in ion channels encoding genes involved in pathways responsible for PA. The analysis was restricted to rare variants with gnomAD frequency < 1%. Qualifying variants and pathogenicity were established by employing *in silico* tools. Copy Number Variant analysis was performed using ExomeDepth algorithm. **Results:** Eight out of twenty-one patients were heterozygous for rare variants in genes associated with PA, while two patients carried potentially damaging variants in genes encoding ion channels. Specifically, one patient was heterozygous for p.V259M in *KCNK5* and one patient was heterozygous for the novel variant p.V221M in *KCNK9*. Two additional patients carried a predicted pathogenic variant p.R492W in *SLC26A2*, a gene that has been associated with PA through GWAS. Germline variants in calcium channel genes were also detected in three patients: p.V249I in *CACNA1H*, p.R462Q in *CACNA1D* and p.L1801M in *CACNA1I*, while one patient carried an ultra-rare variant (p.R26L) in A*TP13A3*. Finally, in two patients we identified rare, likely pathogenic variants in two new susceptibility genes for PA: *KCNK16* (p.P255H) and *CACNA2D3* (p.V55I). **Conclusion:** These findings support the notion that mutations in aldosterone synthesis/secretion regulating genes may sensitize zone glomerulosa cells to ACTH stimulation, leading to aldosterone hypersecretion under conditions of stress. We also report two novel candidate susceptibility genes for PA, *KCNK16* and *CACNA2D3*, and one novel variant in *KCNK9*.

Adrenal

ADRENAL - BASIC AND TRANSLATIONAL ASPECTS

Wnt2b Is Essential for Adrenocortical Progenitor Cell Fate and Zona Glomerulosa Identity in Vivo

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Dysregulation of normal adrenal structure and function contributes to a spectrum of diseases from hypoplasia to cancer. Peripheral adrenocortical progenitor cells in the zona glomerulosa (zG) centripetally migrate and differentiate to replenish steroidogenic cells of the zG and the inner cortex over time. Both the fate of progenitor cells and aldosterone production by steroidogenic cells in the zG are regulated by Wnt/β-catenin signaling, but the cell-specific effects of individual WNT ligands in the adrenal cortex are not fully understood. To further characterize Wnt signaling components crucial for progenitor cell fate and zG identity, we analyzed mouse adrenals using single molecule *in situ* hybridization, which revealed the previously unknown expression of *Wnt2b* exclusively in the adrenal capsule. *Wnt2b* is co-expressed in the capsule with the Wnt signaling potentiator *Rspo3*, the loss of which causes zG depletion and reduced adrenal size in mice. Therefore, we hypothesized that capsular WNT2B activates Wnt signaling in the underlying zG to maintain the undifferentiated state of progenitor cells. To define the role of WNT2B in these processes, we first generated whole body *Wnt2*b knockout (KO) mice, which exhibit complete zG loss, as defined by known markers of zG identity (β-catenin and DAB2). To more fully determine the mechanism by which *Wnt2b* deletion results in zG loss, we crossed *Wnt2b*-floxed and capsule-specific *Gli1-CreERT2* mice to generate a *Wnt2b* conditional knockout (cKO) model and study the effects of *Wnt2b* loss on the zG during homeostasis of the adult adrenal cortex. Gli1-CreERT2 activation by tamoxifen in 6-week-old mice significantly decreased *Wnt2b* expression and resulted in a lower adrenal-to-body weight ratio in *Wnt2b* cKOs compared to controls four weeks later. Adrenocortical proliferation (Ki67) was also significantly decreased in *Wnt2b* cKO mice, suggesting that WNT2B may promote progenitor cell self-renewal. To characterize the consequences of WNT2B loss on canonical Wnt signaling, we assessed activation of β-catenin, the primary Wnt signaling effector. High β-catenin activity in the zG observed in wild-type mice was disrupted in *Wnt2b* cKO mice, together with markedly reduced expression of adrenocortical Wnt target genes *Axin2* and *Wnt4*. In addition, *Wnt2b* loss resulted in downregulation of steroidogenic genes *Cyp11b2* and *Hsd3b6*. Together, these data reveal that capsule-derived WNT2B is required for zG differentiation and maintenance, potentially through activating adrenocortical Wnt/β-catenin signaling and downstream target gene expression involved in both progenitor cell fate and steroid-producing cell function. Studies to more fully elucidate the dynamic effects of WNT2B on the adrenal zG are ongoing as they have important implications for adrenal homeostasis and disease, including both primary adrenal failure and neoplasia.