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Prenatal dexamethasone-induced programmed hypertension and renal programming

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ABSTRACT

Aims: Antenatal glucocorticoids can induce long-term effects on offspring health, including hypertension. Programmed hypertension has been observed in a prenatal dexamethasone (DEX) exposure model. However, how renal programming responds to prenatal DEX at different stages of development and the impact of DEX on programmed hypertension remain unclear. Therefore, we utilized RNA next-generation sequencing (NGS) to analyze the renal transcriptome in the offspring to examine whether key genes and pathways are responsible for DEX-induced renal programming and hypertension.
Main methods: Pregnant rats received intraperitoneal dexamethasone from gestational day 16 to 22. Prenatal DEX-induced programmed hypertension was examined in male offspring at 16 weeks of age.
Key findings: Prenatal DEX modified 431 renal transcripts from the nephrogenesis stage to adulthood in a constant manner. At the pre-hypertensive and established hypertension stages, we identified 11 and 13 differentially expressed genes related to blood pressure regulation, respectively. Among these genes, *Npr3*, *Ptgs2*, *Agt*, *Edn3*, *Ephx2*, *Agtr1b*, and *Gucy1a3* are associated with endothelium-derived hyperpolarizing and contractile factors (EDHF and EDCF). Genes in the arachidonic acid metabolism pathway may potentially be key genes contributing to programmed hypertension. In addition, DEX induced soluble epoxide hydrolase expression (*Ephx2* gene encoding protein).
Significance: Prenatal DEX elicits an imbalance between EDHFs and EDCFs that might lead to renal programming and hypertension. The arachidonic acid metabolism pathway is a common pathway contributing to programmed hypertension. Our results highlight candidate genes and pathways involved in renal programming as targets for therapeutic approaches to prevent programmed hypertension in children exposed to antenatal corticosteroids.
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1. Introduction

Hypertension is currently the most common medical problem, and it might originate from early life. Early-life insults can cause structural changes and functional adaptations resulting in a variety of adult diseases in later life, namely developmental programming [1]. One of the most commonly programmed phenotypes is programmed hypertension [2,3]. As the developing kidney is particularly vulnerable to environmental insults, renal programming is considered to be a key player in the developmental programming of hypertension [4].

Glucocorticoids are essential for the maturation of fetal organ systems and normal development. Although glucocorticoids are recommended in women at risk of preterm labor to accelerate fetal lung maturation [5], emerging evidence indicates that antenatal glucocorticoids may elicit fetal programming that leads to hypertension in adulthood [6–11].

In the kidneys, glucocorticoids might reduce the number of nephrons, elicit epigenetic regulation, induce oxidative stress, alter the renin–angiotensin system (RAS), and impair sodium handling, which can all lead to renal programming and hypertension [4,12,13]. However, none of the proposed mechanisms examined in various glucocorticoid-induced programming models were able to entirely determine the common underlying mechanism that drives the programmed hypertension process. To expand upon our previous findings, we utilized RNA next-generation sequencing (NGS) to analyze the renal transcriptome in the offspring and to capture candidate genes and pathways in transcriptional regulatory gene networks in the following developmental windows: 1 week (nephrogenesis stage) and 16 weeks (hypertensive stage) of age. First, nephrogenesis in rodents begins in late gestation and continues to postnatal weeks 1–2. Second, our previous reports indicated that programmed hypertension developed in 4-month-old adult male offspring exposed prenatally to DEX [9,10], Therefore, a

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clearer understanding of the similarities and differences in the regulated genes between different stages of development will aid in improving therapeutic strategies to explore primary and secondary programmed changes in response to prenatal DEX exposure.

2. Materials and methods

2.1. Animal study

All experiments were approved by Kaohsiung Chang Gung Memorial Hospital Institutional Animal Care and Use Committee. Female Sprague–Dawley (SD) rats were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). The animals were housed and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Male SD rats were caged with individual females until mating.

To construct a prenatal DEX exposure model, dexamethasone (0.1 mg/kg body weight) or vehicle was intraperitoneally administered to pregnant SD rats from gestational day 16 to 22 [9]. Because cardiovascular events occur at a higher rate and at an earlier age in males than in females [14], only male offspring were selected from each litter and used in all following studies. Half of the male offspring were killed at 1 week, and the other half were killed at 16 weeks ($n = 8$/group). Blood pressure was measured in conscious rats using an indirect tail-cuff method (BP-2000, Visitech Systems, Inc., Apex, NC, USA) as described previously [9,10]. Prior to the experiment, the rats were allowed to adapt to restraint and tail-cuff inflation for 1 week. At 16 weeks of age, the animals were placed in metabolic cages (Nalgene; Nalge Nunc International, Rochester, NY, USA) for 24-hour urine collection. Heparinized blood samples were collected. The kidneys were removed, frozen and stored at −80 °C until they were processed. Plasma (Cp) and urine creatinine (Cu) levels were analyzed using high-performance liquid chromatography (HPLC) as described previously [15]. The creatinine clearance (CCr) was calculated using the following equation: $CCr = (Cu / Cp) \times$ urine volume / body weight. Renal 14,15-dihydroxyeicosatrienoic acid (DHET) level, a vasoconstrictor synthesized from arachidonic acid, was measured using an ELISA kit (Detroit R&D Inc., Detroit, MI, USA) [15].

2.2. Next-generation sequencing and analysis

Kidney samples were used for RNA next-generation sequencing (RNA-Seq) (Welgene Biotech Co., Ltd., Taipei, Taiwan). Purified RNA was quantified and analyzed using a Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA) with an RNA 6000 LabChip kit (Agilent Technologies). Library construction was performed using the Solexa platform (Illumina, San Diego, CA, USA). The sequence was directly determined by sequencing-by-synthesis technology using the TruSeq SBS Kit (Illumina). Raw sequences were obtained using the Illumina GA Pipeline software CASAVA v1.8, which was expected to generate 30 million reads per sample. Gene expression was quantified as reads per kilobase of exon per million mapped reads (RPKM). The cuffdiff tool from the cufflinks package was run to calculate expression changes and associated q values (P values adjusted for false discovery rate) for each gene between control and DEX rats. Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed using NIH DAVID Bioinformatics Resources 6.7 to identify candidate genes and pathways [16].

2.3. Quantitative real-time polymerase chain reaction (PCR) analysis

RNA was extracted using procedures as previously described [9–11]. Two-step quantitative real-time PCR (qPCR) was conducted using Quantitect SYBR Green PCR Reagents (Qiagen, Valencia, CA) on an iCycler iQ Multi-color Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Six genes, *Ephx2*, *Hpgds*, *Ptgds*, *Ptgs1* (encodes for cyclooxygenase-1, COX-1), *Ptgs2* (encodes for COX-2), and *Cyp4a2*, which belong to arachidonic acid metabolism pathways, were analyzed. R18S was used as a reference. Primers were designed as described previously [15]. All PCR reactions were run in duplicate. For the relative quantification of gene expression, the comparative threshold cycle (C_T) method was employed. The average C_T for the unknown was subtracted from that of internal control to obtain ΔC_T. $\Delta\Delta C_T$ was obtained by subtracting the average control ΔC_T value from the average experimental ΔC_T. The fold increase was established by calculating $2^{-\Delta\Delta CT}$ for experimental vs. reference samples.

2.4. Western blot

Western blot analysis was performed according to previously reported methods, although nuclear extracts from the rat kidneys were used. A rabbit anti-rat soluble epoxide hydrolase (sEH) antibody was used as a 1:1000 dilution (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Bands of interest were developed (SuperSignal West Pico reagent, Pierce, Rockford, IL, USA) and quantified by densitometry as integrated optical density (IOD). Ponceau S red (PonS) staining was used to correct for any variations in total protein loading and for an internal standard. The protein level was shown as IOD/PonS.

2.5. Statistics

Data are shown as mean ± S.E.M. Statistical analysis was performed using an unpaired *t*-test. A P-value < 0.05 was considered to be statistically significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) 15.0 statistics software (SPSS Inc., Chicago, IL, USA).

3. Results

Litter sizes were not significantly affected by DEX exposure (pups per litter: control = 13 ± 2.7; HF = 11.8 ± 2.5). Male pup mortality rate and body weight (BW) did not differ between the 2 groups, and there were no differences in kidney weight at 1 and 16 weeks of age (Table 1). However, kidney weight-to-BW ratio, heart weight, and heart weight-to-BW ratio were higher in the DEX group than in the control group at 1 week of age. The systolic blood pressure (BP), diastolic BP, and mean arterial BP were significantly higher in the DEX group than those in the control group at 16 weeks of age. Renal function, as represented by creatinine clearance (CCr), did not differ between the DEX group and the control group at 16 weeks of age (Table 1).

Because renal programming events may manifest as a low nephron endowment in diet-induced programming models [17], and because we previously observed that DEX can impair nephrogenesis [9], we analyzed a panel of genes that has previously been reported to be relevant to kidney development [18,19]. As shown in Table 2, *Fgf10*, *Wt1*, *Gfra1*, *Eya1*, *Gata3*, *Kirrel*, *Etv4*, *Lhx1*, *Bmp2*, and *Cdh6* were found to be modified above the chosen threshold in the kidneys of 1-week-old offspring. Among them, only *Gfra1* and *Cdh6* were persistently upregulated at 16 weeks of age. *Pod1*, *Wnt9b*, *Lif*, *Fzd4*, *Osr1*, *Notch2*, and *Notch4* were identified as being above the chosen threshold at 16 weeks of age.

Next, epigenetic regulation has been proposed to contribute to glucocorticoid-induced developmental programming and hypertension [12,20]. Therefore, we examined five groups of epigenetic regulators [21], including histone deacetylases, histone methyl- and acetyl-transferases, bromodomain-containing proteins, DNA methyltransferases, and chromodomain-containing proteins that recognize methylated histones. We identified four genes above the chosen threshold, namely *Hdac4*, *Hdac7*, *Chd2*, and *Chd5* (Table 3).

In differential gene expression analysis, 2090 differentially-expressed genes (DEGs; 1150 up- and 940 down-regulated genes in DEX vs. control) in the kidneys of 1-week-old offspring met the selection criteria of (1) at least a 2-fold difference between groups and (2)

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