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nAChRs-ERK1/2-Egr-1 signaling participates in the developmental toxicity of nicotine by epigenetically down-regulating placental 11β-HSD2

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ABSTRACT

Impaired placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity which inactivates maternal glucocorticoids is associated with poor fetal growth and a higher risk of chronic diseases in adulthood. This study aimed to elucidate the epigenetically regulatory mechanism of nicotine on placental 11β-HSD2 expression. Pregnant Wistar rats were administered 1.0 mg/kg nicotine subcutaneously twice a day from gestational day 9 to 20. The results showed that prenatal nicotine exposure increased corticosterone levels in the placenta and fetal serum, disrupted placental morphology and endocrine function, and reduced fetal bodyweight. Meanwhile, histone modification abnormalities (decreased acetylation and increased di-methylation of histone 3 Lysine 9) on the *HSD11B2* promoter and lower-expression of 11β-HSD2 were observed. Furthermore, the expression of nicotinic acetylcholine receptor (nAChR) α4/β2, the phosphorylation of extracellular regulated kinase 1/2 (ERK1/2) and Ets-like protein-1 (Elk-1), and the expression of early growth response-1 (Egr-1) were increased in the nicotine groups. In human BeWo cells, nicotine decreased 11β-HSD2 expression, increased nAChRα9 expression, and activated ERK1/2/Elk-1/Egr-1 signaling in the concentration (0.1–10 μM)-dependent manner. Antagonism of nAChRs, inhibition of ERK1/2 and Egr-1 knockdown by siRNA were able to block/abrogate the effects of nicotine on histone modification and expression of 11β-HSD2. Taken together, nicotine can impair placental structure and function, and induce fetal developmental toxicity. The underlying mechanism involves histone modifications and down-regulation of 11β-HSD2 through nAChRs/ERK1/2/Elk-1/Egr-1 signaling, which increases active glucocorticoids levels in the placenta and fetus, and eventually inhibits the fetal development.

1. Introduction

Maternal smoking during pregnancy is a serious public health problem. Epidemiological studies have estimated that the prevalence of active smoking among pregnant women is as high as 25% worldwide and that approximately 50% of non-smoking pregnant women are exposed to passive smoking (Pereira et al., 2017). Nicotine, a toxic alkaloid, is a major psychotropic drug that is one of the 4800 chemicals in cigarettes and mediates some of the deleterious effects of smoking (Machaalani et al., 2014). Epidemiological and clinical studies have shown that active or passive tobacco smoke exposure significantly reduces placental weight and fetal birthweight (Pereira et al., 2017), leading to increased risk of diseases such as obesity (Chaiton and Holloway, 2016) and type 2 diabetes (Montgomery and Ekbom, 2002) in adulthood for the offspring. Studies in rodents have shown that prenatal nicotine exposure (PNE) affects placental amino acid transport and vascular function (Bainbridge and Smith, 2006), reduces the bodyweight of fetal rats (Chen et al., 2007), results in an increased risk of cardiovascular disease in adulthood for the offspring (Lim and Sobey, 2011). We previously confirmed that PNE decreases the bodyweight of

Abbreviations: 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; PNE, prenatal nicotine exposure; nAChR, nicotinic acetylcholine receptor; ERK1/2, extracellularly regulated kinase 1/2; Elk-1, Ets-like protein 1; Egr-1, early growth response-1; HPA, hypothalamic-pituitary-adrenal; H3K9ac, histone 3 Lysine 9 acetylation; H3K9me2, histone 3 Lysine 9 di-methylation

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fetal rats and increases susceptibility to multiple adult chronic diseases (e.g. non-alcoholic fatty liver disease, glomerulosclerosis and osteoarthritis) in adult offspring rats (Chen et al., 2007; Liu et al., 2012; Ao et al., 2015; Xu et al., 2015; Tie et al., 2016).

Numerous studies on the relationship between adverse environmental exposures during pregnancy, low birth weight and risk of chronic diseases in adulthood have led to the hypothesis that the risk of developing adult disease originates from fetal development in early life. Glucocorticoids (GCs, cortisol in human, corticosterone in rodents) are the main effectors of the hypothalamic-pituitary-adrenal (HPA) axis. They are involved in the growth and maturation of various organ systems and are essential for the normal development of the fetus. A number of studies have shown that high intrauterine levels of GCs induced by maternal stress can cause fetal HPA axis dysfunction, leading to low birth weight; and increased susceptibility to chronic diseases in later life through a programming effect (Moisiadis and Matthews, 2014a, 2014b). In addition, epidemiological studies have shown that changes in placental characteristics are associated with a variety of adult diseases, laying the foundation for the "placental origin of chronic disease" concept (Burton et al., 2016). More and more studies have demonstrated the programming power of the placenta (Sferruzzi-Perri and Camm, 2016), whereby placental impairment will indirectly result in permanent structural and functional changes in the fetus, leading to a variety of adult diseases in the offspring.

11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is encoded by the *HSD11B2* gene, it converts potent 11β-hydroxyglucocorticoids (cortisol, corticosterone) into their inactive 11-keto forms (cortisone, 11-dehydrocorticosterone); it is expressed in the cytotrophoblast and syncytiotrophoblast in placenta and provides a barrier between maternal and fetal GCs (Driver et al., 2001). Over-exposure of the fetus to maternal GCs due to impaired 11β-HSD2 function can lead to low birthweight, which is recognized as the main mechanism of increased risk for metabolic diseases in later life (Stroud et al., 2016). In addition, 11β-HSD2 is also important for the development and function of the placenta. It has been reported that placental dysfunction in humans is associated with reduced 11β-HSD2 activity (Kosicka et al., 2017), and placental transport of nutrients and placental vascularization are inhibited in 11β-HSD2 (−/−) mice (Wyrwoll et al., 2009). Our previous studies have reported that PNE causes over-exposure to maternal corticosterone in fetal rats by increasing corticosterone levels in maternal serum and inhibiting the expression of 11β-HSD2 in placenta (Chen et al., 2007); and that high corticosterone levels not only lead to HPA axis-associated neuroendocrine metabolic programming alterations in fetal rats (Liu et al., 2012; Xu et al. 2012); but also increase susceptibility to high-fat diet-induced metabolic syndrome and related metabolic diseases (such as nonalcoholic fatty liver) in adult offspring rats (Xu et al., 2013, 2015). However, the potential molecular mechanism of PNE-induced inhibition of 11β-HSD2 expression in placenta has not yet been elucidated.

Early growth response-1 (Egr-1) is a multifunctional transcription factor that is reported to be involved in complex stress responses (such as cigarette smoke) (Bhattacharyya et al., 2013). It has been reported that inflammation-induced Egr-1 can negatively regulate 11β-HSD2 expression in vascular smooth muscle cells (Tsugita et al., 2008). We hypothesized that Egr-1 is also involved in the inhibition of 11β-HSD2 expression by PNE. Thus, this study aimed to clarify the underlying molecular mechanism of the inhibitory effect of PNE on 11β-HSD2 expression in placenta in Wistar rats (in vivo) and human BeWo cells (in vitro). This study will provide important theoretical and experimental insights into the fetal/placental origin of adult diseases.

2. Materials and methods

2.1. Drugs and reagents

Nicotine (N3876-25ML) was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Corticosterone ELISA kits were purchased from Assaypro LLC (Saint Charles, MO, USA) and commercial kits for the placental lactogen (PL) radioimmunoassay were purchased from Beijing North Institute (B13PZB, Beijing, China). Vecuronium bromide (ab 120536, Abcam) and PD-184161 (ab 143,847, Abcam) were purchased from Abcam (Cambridge, UK). Egr-1 siRNA was purchased from GenePharma Co., Ltd. (Shanghai, China). Real-time quantitative polymerase chain reaction (RT-qPCR) kits were purchased from Takara Biotechnology Co., Ltd. (Dalian, Liaoning, China). Chromatin immunoprecipitation (ChIP) assay kits were purchased from Millipore Co., Ltd. (Billerica, MA, USA). The other reagents used for experiments were of analytical grade.

2.2. Animals and treatment

Specific pathogen-free (SPF) rats for experiments were obtained from the Experimental Center of Hubei Medical Scientific Academy (No. 2012–2014, Hubei, China). Animal experiments were conducted in the Center for Animal Experiment of Wuhan University (Wuhan, China). The Committee on the Ethics of Animal Experiments of the Wuhan University School of Medicine approved the protocol (permit No. 14016). All procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee.

Wistar rats raised in this study were bred as previously described (Ao et al., 2015). A sperm-positive vaginal smear served as confirmation of mating, and the day of the positive smear was set as gestational day (GD) 0. Individually caged pregnant rats were then randomly divided into a control group and a PNE group. The subsequent treatments were the same as in our previous research (Xu et al., 2013). Briefly, the PNE group was administered 1.0 mg/kg nicotine tartrate subcutaneously twice a day, whereas the control group received the same volume and frequency of saline administration from GD9 to GD20. The pregnant rats were anesthetized with isoflurane on GD20 and euthanized. There were 11 pregnant rats in each group and the litter size was 8–15. Maternal and fetal blood was collected by cutting the bilateral carotid arteries and collecting drops of blood. Serum samples were obtained from whole blood by centrifugation at 4000 rpm; and 4 °C for 15 min. The fetuses and placentas were quickly removed for weighing and then divided into four groups: female control, male control, female nicotine-exposed and male nicotine-exposed groups. Pooling of placental tissue was performed as previously described (Wu et al., 2015). All samples were transferred to liquid nitrogen immediately, followed by storage at −80 °C for subsequent hematoxylin and eosin (H&E) staining, total RNA extraction and total protein extraction.

2.3. Corticosterone and PL concentration measurement

Corticosterone concentrations in fetal serum (n = 11) and placenta (n = 11) were measured using ELISA kits according to the manufacturer's protocol. Maternal serum concentrations of PL were determined by radioimmunoassay, in accordance with the manufacturers' protocols.

2.4. H&E staining

H&E staining was performed using the routine method. Briefly, placenta tissue samples from different treatment groups were sequentially fixed in paraformaldehyde, dehydrated in ethanol, embedded in paraffin, and then cut into 5-μm sections. After deparaffinization and rehydration, 5-μm placenta slices were stained with hematoxylin and eosin. Images were photographed under 20× and 400× magnification using the Nikon Eclipse Ci (Nikon, Japan).