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Prenatal retinoic acid up-regulates pulmonary gene expression of COUP-TFII, FOG2, and GATA4 in pulmonary hypoplasia

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Abstract

Purpose: Retinoids play an important role in lung development. Recently, prenatal treatment with retinoic acid (RA) has been reported to stimulate alveologenesis in hypoplastic lungs in the nitrofen model of congenital diaphragmatic hernia (CDH). Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) is a transcription factor in the steroid/thyroid hormone receptor superfamily, and targeted ablation of COUP-TFII causes CDH and associated lung hypoplasia in mice. Friend of GATA 2 (FOG2) is a zinc finger-containing protein that modulates the transcriptional activity of GATA proteins. GATA4 is a member of a family of DNA-binding proteins, which is found in the promoter regions of many genes. The *COUP-TFII*, *FOG2*, and *GATA4* genes, regulated by the retinoid signaling pathway, are located on chromosomes 15q26, 8q23, and 8p23.1 respectively, regions reported to be deleted in individuals with CDH. The aim of this study was to examine the pulmonary gene expression of COUP-TFII, FOG2, and GATA4 in the nitrofen model of CDH.

Materials and Methods: Pregnant rats were exposed to either olive oil or 100 mg nitrofen on day 9 of gestation (D9). 5 mg/kg of RA was given intraperitoneally on days D18, D19, and D20. The fetuses were recovered by caesarean section on D21, and the diaphragm was carefully examined for the presence of a hernia under a microscope. Left lungs were obtained from CDH fetuses and controls and divided into four groups: control (n = 9), control + RA (n = 9), CDH (n = 9), and CDH + RA (n = 9). The relative mRNA expression levels of COUP-TFII, FOG2, and GATA4 were analyzed in each lung by real-time reverse transcriptase–polymerase chain reaction from cDNA generated by mRNA from pulmonary total RNA.

Results: The relative mRNA expression levels of COUP-TFII, FOG2, and GATA4 were significantly increased in CDH + RA lungs compared to control, control + RA, and CDH ($P < .05$).

Conclusions: Up-regulation of pulmonary gene expression of COUP-TFII, FOG2, and GATA4 after prenatal treatment with retinoic acid in the nitrofen model of CDH suggests that RA may have a therapeutic potential in modulating lung growth. Furthermore, these results support the concept that these proteins work together to regulate downstream target genes that play an important role in the development of lung.

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Despite remarkable progress in postnatal treatment, the mortality and morbidity in patients with congenital diaphragmatic hernia (CDH) remain high [1,2]. Pulmonary hypoplasia, characterized by immaturity and small lung size, is considered to be one of the principle contributors to the high morbidity and mortality in infants with CDH [1,3]. Although our understanding of the pathogenesis of CDH still remains unclear, there is increasing evidence to suggest that the retinoids play a crucial role in its pathogenesis [4]. Nitrofen-induced CDH model has been widely used as an experimental model and has provided important insights into the pathogenesis of this developmental anomaly [5-8]. Recent studies have suggested that the retinoid signaling pathway (RSP) is inhibited in the nitrofen-induced hypoplastic lung [9-12]. However, the exact mechanism by which nitrofen acts in the RSP remains incompletely understood.

Retinoids are the family of molecules derived from vitamin A and are essential for normal development of various organs including the lungs and diaphragm during embryogenesis [13]. Retinoic acid (RA) is the active metabolite of vitamin A that is essential in each of the lung developmental stages [14]. Recent work from our laboratory has shown that retinoic acid rescues lung hypoplasia in nitrofen-induced hypoplastic fetal rat lung explants [9]. We also reported that lung retinol storage is decreased and gene expression of most downstream components of the retinoid signaling pathway was increased in the nitrofen model of CDH [15]. Based on these findings, we studied the effect of retinoic acid in lung growth during late gestation in the nitrofen model of CDH and showed that retinoic acid promoted alveologenesis by increasing the number and reducing the size of alveoli, therefore obtaining an increased gas exchange surface area [5]. Our result demonstrated that prenatal treatment with retinoic acid stimulates alveologenesis in hypoplastic lungs in the nitrofen model of CDH.

Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), is a member of the steroid/thyroid hormone receptor superfamily and encodes a transfactor regulated by the RSP [16,17]. The COUP-TFII is expressed in the developing lung, the foregut mesenchyme, the developing posthepatic mesenchymal plate, and the septum transversum, all components that are important for the formation of the diaphragm [18]. Homozygous tissue-specific ablation of COUP-TFII in mice has been shown to cause left-sided posterolateral CDH similar to Bochdalek-type CDH seen most commonly in humans [18]. The *COUP-TFII* gene is located on chromosome 15q26. Recently, Kantarci and Donahoe [19] reported that deletion of COUP-TFII gene is strongly associated with CDH. FOG2, friend of GATA 2, is a multi-zinc finger transcriptional corepressors that bind specifically to members of the GATA family of transcription factors [20]. It has been reported that FOG2 is required for normal diaphragm and lung development in mice and humans [21]. The *FOG2* gene is located on chromosome 8q23 in a region commonly deleted in individuals with CDH. It has also been shown that FOG2 interacts physically with COUP-TFII, which in turn, modulates the transcriptional activity of GATA4, GATA5, and GATA6 [18,22]. GATA4 is a RA-inducible GATA-binding transcription factor which is found in the promoter regions of many genes [23]. GATA4 is also known to functionally interact with FOG2, and they are coexpressed in mesenchymal cells of developing lung in mice [23,24]. GATA4 is located on chromosome 8p23.1, a region recurrently deleted in individuals with CDH. It has been shown that heterozygous mutation of mice demonstrates diaphragmatic hernia and primary lung defects [25]. Although the precise mechanism of these genes in RSP during lung development is not clearly understood, it is possible to speculate that COUP-TFII, FOG2, and GATA4 are affected together in RSP by nitrofen administration and therefore disrupting regulation of downstream target genes that play a key role in the lung development, causing hypoplastic lung in nitrofen CDH model.

Based on our previous work of therapeutic potential of RA in stimulating alveologenesis in hypoplastic lung, we designed this study to investigate the hypothesis that the pulmonary gene expression of COUP-TFII, FOG2, and GATA4 are up-regulated after prenatal treatment with RA in the nitrofen CDH model.

1. Materials and methods

1.1. Animals and drugs

Adults Sprague-Dawley rats were mated and the presence of spermatozoids in the vaginal smear was verified and was considered as gestational day 0. Pregnant female rats were then randomly divided into two groups. Animals in the experimental group received intragastrically 100 mg of Nitrofen (Wako Chemicals, Osaka, Japan) dissolved in 1 mL of olive oil on day 9.5 of gestation, whereas those in the control group received only vehicle. On gestational day 18, the rats were randomly injected intraperitoneally with all *trans*-retinoic acid 5 mg/kg in cottonseed oil (Sigma, St Louis, Mo) or with diluents. The injections were repeated on days 19 and 20, and then the rats were sedated with isofluorane and killed by cervical dislocation on the day 21. The fetuses were recovered by caesarean section and the diaphragm was carefully examined for the presence of a hernia under a microscope (Leica S8 APO). We analyzed the left lungs of fetuses from four treatment groups: control (n = 9), control + RA (n = 9), CDH (n = 9), and CDH + RA (n = 9). The control group consisted of fetuses that only received olive oil, and the control fetuses that exposed to RA were defined as the control + RA group. Fetuses with CDH were defined as the CDH group and exposed to RA were defined as the CDH + RA group. In order to obtain representative numbers, the fetuses in each group came from at least four different dams. The Department of Health and Children approved all the animal experiments (ref. B100/4022) under