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Diethylstilbestrol regulates expression of avian apolipoprotein D during regression and recrudescence of the oviduct and epithelial-derived ovarian carcinogenesis

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ABSTRACT

Apolipoprotein D (APOD) is a glycoprotein which is widely expressed in mammalian tissues. It is structurally and functionally similar to the lipocalins which are multiple lipid-binding proteins that transport hydrophobic ligands and other small hydrophobic molecules, including cholesterol and several steroid hormones. Although multiple functions for APOD in various tissues have been reported, its expression, biological function, and hormonal regulation in the female reproductive system are not known. Thus, in this study, we focused on correlations between APOD and estrogen during development, differentiation, regression, and regeneration of the oviduct in chickens and in the development of ovarian carcinogenesis in laying hens. Results of the present study indicated that *APOD* messenger RNA (mRNA) expression increased ($P < 0.001$) in the luminal and glandular (GE) epithelia of the chicken oviduct in response to diethylstilbestrol (a nonsteroidal synthetic estrogen). In addition, the expression of APOD mRNA and protein decreased ($P < 0.001$) as the oviduct regressed during induced molting, and gradually increased ($P < 0.001$) with abundant expression in GE of the oviduct during recrudescence after molting. Furthermore, APOD mRNA and protein were predominantly localized in GE of cancerous, but not normal ovaries from laying hens. Collectively, results of the present study suggest that *APOD* is a novel estrogen-stimulated gene in the chicken oviduct which likely regulates growth, differentiation, and remodeling of the oviduct during oviposition cycles. Moreover, up-regulated expression of APOD in epithelial cell–derived ovarian cancerous tissue suggests that it could be a candidate biomarker for early detection and treatment of ovarian cancer in laying hens and in women.

1. Introduction

Apolipoproteins (APOs) are generally known to be associated with lipid to form lipoproteins in different proportions and to deliver the lipid to target tissues via the blood and lymphatic systems [1–3]. In addition, as enzyme cofactors and receptor ligands, they play important roles in various biological processes including regulation of metabolic pathways involving lipoproteins, cell migration, and brain development [4]. There are 7 types of APOs including apolipoproteins A (APOA), -B (APOB), -C (APOC), -D (APOD), -E (APOE), -H (APOH), and -J (APOJ) identified based on their various biological features, physiological and metabolic characteristics, and location of their genes on chromosomes [5]. Of these, APOD is a 29-kDa glycoprotein with a single glycosylated polypeptide containing 169 amino acids and found in various tissues such as spleen, brain, and testis in humans [1–3]. Interestingly, APOD is unusual because its amino acid sequence differs

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from that of other APOs, and it has a rather high degree of homology with the lipocalins superfamily of proteins which have a common β-barrel structure composed of an 8-stranded antiparallel β-sheet [6,7]. Thus, APOD is classified as a member of the lipocalin protein family which serves mainly as the transport system for small hydrophobic molecules including steroid hormones and retinoids. However, little is known about the exact physiological functions and hormonal control of expression of APOD.

The chicken oviduct is a well-known tissue model to investigate cellular growth and differentiation that is dependent of hormonal regulation during various biological processes such as oviduct development, egg formation, oviposition, and molting [8,9]. As a primary sex hormone of females, estrogen plays pivotal roles in these processes in the female chickens. For instance, estrogen stimulates proliferation and differentiation of oviduct cells including tubular glands, goblet cells, and ciliated cells during the period of oviduct development [9–11], and estrogens also induce synthesis of egg-white proteins such as ovalbumin, ovomucoid, conalbumin, and lysozyme in the tubular glandular cells of the magnum of the oviduct [12]. In addition, estrogen triggers complete remodeling of the reproductive tract to enhance the subsequent rate of egg production after normal or induced regression and recrudescence phases of the molting period [13]. On the other hand, the laying hen is a well-established animal model for research in epithelial-derived ovarian cancer (EOC) which may arise from genetic mutations and damage of the ovarian surface epithelium of laying hens and women [14,15]. Indeed, estrogen is a well-known risk factor for gynecological carcinogenesis in women [16] and expression of *estrogen receptor alpha* messenger RNA (mRNA) is significantly greater in EOC of laying hens [17]. However, little is known about relationships between APOD and estrogen in the female reproductive organs. Our preliminary gene profiling data revealed that expression of *APOD* mRNA decreased with decreases in circulating concentrations of estrogen in plasma of laying hens as the ovary and oviduct regressed during induced molting and increased with increases in circulating concentrations of estrogen with recrudescence of the ovary and oviduct after diet-induced molting in laying hens [13]. Therefore, this study was designed to test the hypothesis that APOD plays important roles in the growth, development, differentiation, and regression of the oviduct and ovary of chickens in response to estrogen. Indeed, in the present study, we used diethylstilbestrol, a nonsteroidal synthetic estrogen that binds strongly to estrogen receptors. Diethylstilbestrol effects are similar to natural estrogens with respect to its effects on the developmental pattern of the oviduct of neonatal chicks oviduct including differentiation of tubular glands and ciliated cells, and expression of egg-white proteins in the magnum of the oviduct [18,19]. Finally, the experimental goals of this study were to (1) investigate whether APOD mRNA and protein expression are regulated by estrogen during oviduct development and differentiation; (2) determine the expression pattern for APOD during regression and regeneration of the oviduct induced by molting; and (3) compare APOD expression in normal and cancerous ovaries of hens. Results of the present study indicate that *APOD* is a novel estrogen-stimulated gene expressed during development and differentiation of the oviducts of postnatal chicks and involved in remodeling and recrudescence of the chicken oviduct during the peri-molting periods. Furthermore, APOD is a candidate gene associated with development of EOC in laying hens.

2. Materials and methods

2.1. Experimental animals and animal care

The experimental use of chickens for this study was approved by the Animal Care and Use Committee of Korea University. All chickens were exposed to a light regimen of 15-h light and 9-h dark with ad libitum access to feed and water and subjected to standard poultry husbandry guidelines.

2.2. Tissue samples

2.2.1. Study 1

Female chicks were identified by polymerase chain reaction (PCR) analysis using W chromosome–specific primer sets. Treatment of chicks with diethylstilbestrol and recovery of the oviduct ($n = 5$) were conducted as reported previously [19]. Briefly, a 15-mg diethylstilbestrol pellet was implanted subcutaneously in the abdominal region of 1-week-old female chicks for 10 d. The diethylstilbestrol pellet was removed for 10 d, and then, a 30-mg dose of diethylstilbestrol was administered for 10 additional days. Five 37-day-old chicks in each group were euthanized using 60% to 70% carbon dioxide. The collected samples were either frozen or fixed in 4% paraformaldehyde for further analyses. Paraffin-embedded tissues were sectioned at 5 μm.

2.2.2. Study 2

Molting of laying hens was induced as described previously by adding 20,000-ppm zinc to the diet to effectively reduce feed intake and induces molting [20,21]. Briefly, molting was induced by feeding hens in the zinc-fed group a diet containing high zinc (mixed 252 g zinc oxide per 10-kg feed to achieve a final concentration of 20,000 ppm of zinc). Laying hens in the molting group completely ceased egg production within 12 d after feeding the high-zinc diet. The 35 laying hens (47 wk) were divided into 2 larger groups, including molting-progressing or postmolting-progressing group, and kept in individual cages. The molting group was divided into 3 subgroups based on the number of days of feeding the high-zinc diet (normal feeding group, 6 d and 12 d after onset of zinc feeding). The recrudescence (postmolting) group was divided into 4 subgroups based on the number of normal feeding days after complete cessation of egg laying and initiation of feeding a normal commercial diet: 20, 25, 30, or 35 d after onset of zinc feeding or 8, 13, 18, or 23 normal feeding days after cessation of egg production and removal from the high-zinc diet. Hens ($n = 5$ per time point) in each subgroup (0, 6, 12, 20, 25, 30, and 35 d after onset of zinc feeding) were euthanized using 60% to 70% carbon dioxide.