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Pivotal roles for hormonally regulated expression of the HEP21 gene in the reproductive tract of chickens for oviduct development and in ovarian carcinogenesis

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ABSTRACT

Hen egg protein (HEP21) is a 21-kDa secreted protein and has a single copy of the Ly6/uPAR domain. Although HEP21 is expressed primarily in the chicken oviduct, its biological function(s) in the reproductive system of chickens is not known. Thus, in the present study, we investigated expression patterns of HEP21 with respect to hormonal regulation, oviduct development, changes in expression in laying hens undergoing induced molting, and in the development of ovarian carcinogenesis in laying hens. Results of present study indicated that *HEP21* messenger RNA (mRNA) expression increased ($P < 0.001$) in the chicken oviduct in response to estrogen. *In situ* hybridization analyses revealed expression of *HEP21* mRNA predominantly in glandular (GE) and luminal epithelia of the magnum of the chicken oviduct in response to estrogen. The expression of *HEP21* mRNA decreased ($P < 0.001$) as the oviduct regressed during induced molting and increased ($P < 0.001$) with recrudescence of the oviduct following molting. *HEP21* mRNA was most abundant in GE of the oviduct during recrudescence, but not during oviduct regression following induced molting. Moreover, we found abundant expression of *HEP21* in GE of cancerous ovaries, but not in normal ovaries of hens. Collectively, results of present study suggest that HEP21 is an estrogen-responsive gene in the oviduct of hens that likely regulates development of the chicken oviduct, and egg production and formation. Furthermore, there is increased expression of HEP21 in epithelial-derived ovarian cancer suggesting that HEP21 could be used for diagnosis and monitoring carcinogenesis in laying hens and in women.

1. Introduction

Egg white of hens is a functional material for food and pharmaceutical industries through the application of biological activities of separated major components including ovalbumin, ovotransferrin, ovomucoid, lysozyme, and ovomucin [1]. For this reason, most previous studies focused on structures, properties, and modifications with physicochemical conditions of those main egg white proteins whereas a large number of minor egg white proteins remain to be characterized. HEP21 is one component of egg white proteins that is synthesized in the chicken oviduct as a secreted 21-kDa protein [2]. It is predominantly expressed in the chicken oviduct, especially the magnum where the production of egg white proteins occurs. HEP21 is known as a new member of the uPAR/Ly6/CD59 superfamily, and topology of these domains is similar to that of snake venom neurotoxins [3,4]. However, research to define the biological functions of HEP21 in chickens is very limited.

As an oviparous animal, the chicken oviduct is a well-established organ for investigating reproductive biology including structural, cellular, and biochemical changes with respect to sex hormones during oviduct development, egg formation, and oviposition [5,6]. Estrogen, as a primary sex

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hormone of females, induces development and maintenance of cell proliferation and differentiation of cells of tubular glands, goblet cells, and ciliated cells in the chicken oviduct [6–8]. Estrogen stimulates epithelial cells of the tubular glands of the magnum of the chicken oviduct to synthesize and produce an abundance of critical egg-white proteins including conalbumin, lysozyme, ovalbumin, and ovomucoid [9]. Also, exposure of chicks to diethylstilbestrol (DES) affects the growth, development, and differentiation of the immature oviduct in chicks [10].

Concentrations of estrogens in serum of laying hens declines during regression of the chicken oviduct and increases during recrudescence of the oviduct in response to molting [11]. In hens, molting is a unique biological phenomenon associated with cyclic process to shed and replace feathers and to establish complete remodeling of the reproductive tract [12]. Naturally, laying hens experience an annual period of molting at the end of each laying cycle to enhance the subsequent rate of egg production as the hen ages, and there is a decrease in egg production. To restore a new effective egg laying cycle, the reproductive organs such as ovary and oviduct undergo regression and regeneration during molting [13]. In this way, artificial induction of molting is fully used as an intensive method to increase the number and quality of eggs produced through a revival in the laying cycle of hens in commercial poultry operations [14]. Moreover, with respect to enhancing animal welfare, feeding a diet containing high levels of zinc is a remarkable way for induction of molting in hens instead using an alternative method of starvation of laying hens to induce molting [15].

Recent studies have shown that ovarian cancer can arise from epithelium from the oviduct as oviduct-related genes are upregulated in epithelial-derived ovarian cancer (EOC) of laying hens [16]. Generation of EOC is associated with incessant ovulation that likely causes genetic mutation and damage of the ovarian surface epithelium of laying hens and women; therefore, laying hens are well known as the most suitable animal model for studies EOC [17,18]. However, little is known about HEP21 with respect to hormonal regulation in the developing chick oviduct or its expression in ovarian carcinogenesis of laying hens. Therefore, the objectives of this study were to: (1) investigate whether the expression of HEP21 is regulated via estrogen during oviduct development in chickens; (2) determine the distribution and localization of HEP21 in the oviduct during its regression and recrudescence associated with induced molting; and (3) compare HEP21 expression between normal and cancerous ovaries from hens. Results of the present study indicate that HEP21 is a novel estrogen-stimulated gene during oviduct development in chicks, and that the expression of HEP21 is mediated by regression, remodeling, and recrudescence of the chicken oviduct during the pre- and post-molting periods. In addition, HEP21 is a candidate gene in the development of ovarian carcinogenesis 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. White Leghorn 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 [19]. Treatment with DES and recovery of the oviduct ($n = 5$) were conducted as reported previously [20]. Briefly, a 15 mg DES pellet was implanted subcutaneously in the abdominal region of 1-week-old female chicks for 10 d. The DES pellet was removed for 10 d, and then a 30 mg dose of DES was administered for 10 additional days. Five 37 d 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 [21,22]. 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 post-molting-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 (post-molting) 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 the onset of zinc feeding) 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.

2.2.3. Study 3

A total of 136 laying hens (88 hens older than 36 mo of age and 48 hens older than 24 mo of age), which had completely stopped laying eggs, were euthanized for biopsy and cancerous ($n = 10$) ovaries were collected. As a control, normal ($n = 5$) ovaries were also collected from egg-laying hens. We examined tumor stage in 10 hens with cancerous ovaries based on characteristic features of chicken ovarian cancers [18].

2.3. RNA isolation

Total RNA was isolated from frozen tissues using Trizol reagent (Invitrogen, Carlsbad, CA) according to