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Characteristics, tissue-specific expression, and hormonal regulation of expression of tyrosine aminotransferase in the avian female reproductive tract

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ABSTRACT

Tyrosine aminotransferase (*TAT*) catalyzes the transamination of tyrosine to p-hydroxyphenylpyruvate. Accumulation of tyrosine in the body due to a genetic mutation in the *TAT* gene causes tyrosomia type II in humans. The *TAT* gene is regarded as a model for studying steroid-inducible factors regulating a variety of biological functions of *TAT*. However, little is known of the effects of estrogen on the expression of the *TAT* gene in chickens. Therefore, in the present study, we identified expression of the avian *TAT* gene in various organs. The results showed the *TAT* was detected predominantly in the liver and reproductive organs including testis, oviduct, and ovary. Specifically, *TAT* mRNA was expressed abundantly in the glandular and luminal epithelia of the oviducts in response to endogenous and exogenous estrogens which also induce dramatic morphological changes in the oviduct of chickens. In addition, target microRNAs of *TAT* (*miR-1460*, *miR-1626-3p*, *miR-1690-5p*, and *miR-7442-3p*) were found to modulate expression of the *TAT* gene. Especially, *miR-1690-5p* influenced *TAT gene* transcription by binding directly to its 3′-UTR region. Moreover, the expression of *TAT* was abundant in glandular epithelia of cancerous but not normal ovaries from laying hens. Taken together, our findings suggest that *TAT* plays an important role in the cytodifferentiation of oviducts in response to estrogen and in the progression of ovarian cancer in chickens.

1. Introduction

Estrogen is a major steroid hormone affecting the female reproductive system by regulating cell proliferation and differentiation, implantation, and pregnancy [1]. The activity of estrogen is controlled through its nuclear receptors (estrogen receptor α or β) which directly bind to a specific short DNA sequence within their promoter region of target genes called the estrogen response elements [2]. In the female reproductive organs of women, the oviduct is crucial for transport of oocytes and sperm, as well as being the site of fertilization of oocytes to initiate successful embryogenesis [3]. These processes are completely regulated by hormones, mostly estrogen. Chickens are regarded as one of the best animal models for research into function and signal transduction cascades induced by estrogen because of their exquisite responsiveness to estrogens. Especially, estrogen supports development of tubular glands in the oviduct of chickens for secretion of egg white proteins and induces cytodifferentiation of epithelial cells into goblet and ciliated cells [4]. For example, the chicken oviduct undergoes regression in response to decreases in circulating concentrations of endogenous estrogens during the induced molting period, then there is recrudescence of the oviduct and resumption of production of eggs in

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response to increasing concentrations of endogenous estrogens after molting [5]. In addition, the chick oviduct develops in response to the synthetic estrogen analog, diethylstilbestrol (DES) [6]. Moreover, estrogen stimulates formation of the egg shell by regulation of genes related to its calcification including secreted phosphoprotein 1 [7] and carbonic anhydrases [8].

Human tyrosine aminotransferase (*TAT*) is assigned to chromosome 16q22.1 and spans 10,244 bp composed of 12 exons and a long nucleotide sequence (2,757 bp). This gene encodes for a 454 amino acid protein with a molecular weight of 50,399 Da [9]. *TAT* is expressed in the liver, kidneys, and brain, but the *TAT* gene is abundantly expressed in the liver as an invaluable enzyme for converting tyrosine to p-hydroxyphenylpyruvate in a transamination reaction in response to a pyridoxal phosphate [10,11]. A deficiency in *TAT* gene expression and its enzymatic activity gives rise to tyrosinemia type II (also known as Richner-Hanhart syndrome), an autosomal recessive inheritance with keratitis, palmoplantar hyperkeratosis, mental retardation, and accompanying increases in concentrations of tyrosine in serum [12,13]. Glucocorticoids and glucagon stimulate *TAT* activity via the intracellular cyclic adenosine monophosphate (cAMP) pathway, whereas insulin suppresses the actions of *TAT* by modifying hormone response elements in the 5′-flaking region of the *TAT* gene [14,15]. However, little is known about expression of *TAT* in organs other than the liver.

Based on a previous study using microarray analysis, the expression of avian TAT was detected in the chicken oviduct and its expression was associated with changes in concentrations of endogenous estrogens in plasma during diet-induced molting periods. Therefore, in the present study, we validated the temporal and cell-specific expression of the *TAT* gene using the chicken as an animal model for study of its regulation by endogenous and exogenous estrogens. The objectives of this study were to: (1) analyze the primary amino acid sequence of avian TAT as compared with other species; (2) demonstrate tissue- and cell-specific expression of *TAT* mRNA in organs of chickens; (3) investigate whether endogenous and exogenous estrogens regulates expression of *TAT* during regeneration and growth of the chicken oviduct; (4) determine target microRNAs for expression of *TAT* during estrogen-induced development of the chick oviduct; and (5) compare the expression of *TAT* between normal and cancerous ovaries from laying hens. Results of the present study provide valuable insights into the *TAT* gene with respect to development and regression of the female reproductive tract in response to changes in circulating concentrations of estrogen in chickens.

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 hours light and 9 hours 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

Hens (n = 5 per time point) in each subgroup including the molting group (normal feeding group, 6 d and 12 d after the onset of zinc feeding) and the recrudescence group (20, 25, 30, or 35 d after the onset of zinc feeding, and 8, 13, 18, or 23 d of normal feeding after cessation of egg production and removal from the high-zinc diet) were euthanized using 60% to 70% carbon dioxide before collecting the oviduct on each assigned day. Molting is the loss of feathers along with regression of the reproductive organs due to a decrease in circulating concentrations of estrogen, but it can also be induced by feeding a high-zinc diet as we reported previously [5].

2.2.2. Study 2

Fifteen female chicks were identified by PCR analysis using W chromosome-specific primer sets (F: 5′-CTA TGC CTA CCA CAT TCC TAT TTG C-3′ and R: 5′-AGC TGG ACT TCA GAC CAT CTT CT-3′). Treatment with (DES, a synthetic estrogen agonist) and recovery of the oviduct were conducted as described previously [16]. We implanted a 10-mg DES pellet into the abdominal region of 1-week-old female chicks to release the hormone for 10 days. The DES pellet was then removed from all chicks for 10 days, and then a 10-mg daily dose was administered for 10 additional days. Control chicks were raised normally without any treatment for 37 days after hatching. Five 37-day-old control chicks and five 37-day-old DES-treated chicks were euthanized using 60% to 70% carbon dioxide to provide samples of the oviduct which were cut into 5- to 7-mm pieces and frozen in liquid nitrogen. The remaining tissues were cut into 10- to 15-mm pieces and fixed in fresh 4% paraformaldehyde in phosphate buffered saline (PBS) (pH 7.4). After 24 hours, fixed tissues were changed to 70% ethanol for 24 hours and then dehydrated and embedded in Paraplast-Plus (Leica Microsystem, Wetzlar, Germany). Paraffin-embedded tissues were sectioned at 5 μm.

2.2.3. Study 3

A total 136 laying hens (88 older than 36 mo of age and 48 older than 24 mo of age), which had completely stopped laying eggs were euthanized for biopsy and collection of cancerous (n = 10) ovaries. As a control, normal (n = 5) ovaries were collected from hens that were laying eggs. We examined the stage of ovarian tumors in 10 hens using characteristic features of chicken ovarian cancer [17,18].

2.3. Sequence analysis

For pairwise comparisons and multiple sequence alignment, the amino acid sequences of TAT genes from each of several species (see Supplemental Table 1), were aligned using Geneious Pro, version 5.5, software (Biomatters Ltd, Auckland, New Zealand), with default penalties for gap, and the protein weight matrix of Blocks Substitution Matrix (BLOSUM) [19].