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Expression and Function of CTNNB1 in the Development of Avian Reproductive System

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ABSTRACT

Beta-catenin (*CTNNB1*, catenin (cadherin-associated protein), beta 1) is involved in various biological processes, including embryogenesis, tumorigenesis, angiogenesis and progression of metastasis. CTNNB1, as a multifunctional and oncogenic protein, has important roles in adhesion between Sertoli cells through an N-cadherin-dependent manner and in various cancer types through its over-activation. In addition, CTNNB1 can interact with estrogen/estrogen receptor alpha complex, which regulates the transcription of WNT (wingless-type MMTV integration site family)/CTNNB1 target genes. Recently, we investigated the functional roles and expression pattern of CTNNB1 during the morphological changes of embryonic gonads of chickens and the estrogen-dependent regulation of CTNNB1 in oviduct development and potential functions as a biomarker of CTNNB1 in human epithelial ovarian cancer using the chicken as a biological research model. Therefore, in this review, we provide a new insight of potential role of CTNNB1 in the development of the female reproductive tract during early embryogenesis and ovarian carcinogenesis of laying hen models.

(Key words : CTNNB1, Estrogen, Oviduct, Ovary, Cancer)

INTRODUCTION

The chicken animal model has provided insights into molecular mechanisms that are related with embryogenesis, hormone actions and ovarian cancer. The sex-determination related genes, including *AMH, SOX9* and *SF1*, are expressed in a similar manner in the gonads of both mammals and chickens. In addition, both species show a conserved genetic mechanism underlying sex differentiation. The chicken oviduct is a valuable tool for studying steroid hormone action because of the immediate-early response to estrogen. Furthermore, the chicken is the only animal that spontaneously develops ovarian carcinomas on the surface of the ovaries like humans.

EMBRYONIC GONAD DEVELOPMENT

Mammalian Gonad Differentiation

A wide variety of morphology and cell biology is described in vertebrate species. Most mammals use an *XX*:*XY* sex chromosome system; the male has a Y chromosome-linked *SRY* gene (Sex-determining Region Y) that acts within indifferent gonads to initiate testis differentiation and development while the female is homogametic (XX). The early mammalian gonads as bipotential gonads are composed of somatic cells and primordial germ cells (PGCs), which are capable of forming either testes or ovaries (Tilmann and Capel, 2002).

In the XY gonad, the specific expression of SRY triggers the testis differentiation pathway between embryonic day (E) 10.5 and 12.0. The early fetal testes at E13.5 are close to the mesonephros containing the Wolffian duct. Sertoli cells and peritubular myoid (PM) cells enclose a cluster of germ cells to form the testis cords, and the steroidogenic Leydig cells are located in the interstitum between the testis cords. Pre-Sertoli cells are differentiated by SOX9 which is upregulated by Sry and maintained in an autocrine and paracrine manner (Smith *et al*., 1999). PM cells originating from the mesonephros produces α-smooth muscle actin, which supports structurally the formation of the testis cords to bind with the Sertoli cells (Capel *et al*., 1999). Leydig

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cells arise from the mesonephros and produce androgen for fetal masculinization. In addition, vascular endothelial cells that contribute to the organization of testis-specific vasculature originate in the mesonephros and migrate into the XY gonads (Brennan *et al*., 2002).

In the female gonads, the absence of Sry initiates the development of ovaries from the cortex of the indifferent gonads. One of the early steps of ovarian differentiation is the germ cells enter meiosis at E12.5～13.5 but then becomes arrested in pachytene I at E18.5. The histological features are blocked, but the ovaries are already differentiated on a molecular level. R-spondin1 (Rspo1) as a key female-determining factor is up-regulated in the somatic cells of XX gonads at E11.5. Rspo1 also regulates the expression of Wnt4 in developing gonads which promotes the ovarian fate and suppresses testis development by stabilizing Ctnnb1. Foxl2, as a potential female-sex-determining gene, is also expressed at E12.5 and required for differentiation and maintenance of the ovaries by the Rspo1/Wnt4/Ctnnb1 signaling pathway in an independent manner (Nef and Vassalli, 2009)

Chicken Sex Differentiation

Sex is determined by a specific chromosome such as the sex chromosome. In avians and mammals, the sex chromosome of both groups evolved from a different pair of autosomes that resulted in different chromosome systems; the mammalian is an XX/XY sex chromosome system and the avian are a ZZ/ZW sex chromosome system. In the case of chickens, the Z-linked gene initiates sex determination, and one pair of Z chromosomes is the male sex (Smith and Sinclair, 2004).

The undifferentiated bi-potential gonads are adjacent to the ventral surface of mesonephric kidneys and show up at E3.5. The first morphological differentiation of fetal gonads appear between E5.5 and 6.5 via the expression of the Z-linked gene, DMRT1 (Doublesex and mab-3-related Transcription factor 1). In early testicular development, DMRT1 contributes to the masculinization of the ZZ gonads, activates SOX9 (SRY (Sex determining region Y)-box 9), which triggers Sertoli cell proliferation and differentiation, and leads to the thickening of medullary cords. AMH (anti-Mullerian hormone) is produced in pre-Sertoli cells and inhibits mullerian ducts regression as in mammalians (Oreal *et al*., 1998).

Interestingly, the chicken female left gonad can differentiate into an ovary. The early stage of the left gonad undergoes morphological changes between E6.5 to E8.5. The medullar cords become vacuolated to form the lacunae during ovary formation, while the cortex begins to thicken by proliferation of somatic and germ cells. Germ cells that migrate to the cortex of the gonadal ridge undergo meiotic prophase and prepare for folliculogenesis. Molecular signaling such as the CTNNB1 pathway and FOXL2 may apply to chickens in the same manner as mammalians; however, AMH is expressed in the female gonad due to the regression of the right duct (Chue and Smith, 2011).

Avian Models

The chicken gonad is an appealing comparative model to understand the molecular mechanisms underlying the formation of the testes and ovaries. The embryonic gonads develop in a similar way in mammals and avian, involving conserved cell types and developmental processes that are generally shared (Chue and Smith, 2011; Lovell-Badge *et al*., 2002; Sekido and Lovell-Badge, 2007; Smith, 2007). This implies that the underlying genetic pathways leading to testis or ovary formation are also conserved. Indeed, the chicken shares with other vertebrates several key genes involved in gonadal sex differentiation, such as DMRT1, SF1, SOX9 and AMH, as outlined below (Smith and Sinclair, 2004). The chicken, therefore, provides a practical model to study embryonic gonad development (DeFalco and Capel, 2009; Morrish and Sinclair, 2002).

ESTROGEN ACTION IN THE FEMALE REPRODUCTIVE SYSTEM

Molecular Mechanisms of Estrogen

Estrogen is the essential female sex hormone in reproductive processes including the following: development of the ovulatory follicle, stimulating the midcycle preovulatory surge of gonadotropins, altering the consistency of cervical mucus to facilitate sperm transport and preparing the endometrial lining of the uterus for implantation. Synthesis of estrogen starts in the theca cells by conversion of cholesterol to androgen. The second enzymatic step is the aromatization of androgen into estrogen in granulose cells (Hewitt *et al*., 2005; Hillier *et al*., 1994). Estrogen has a multitude biological effects that are mediated through two estrogen receptor molecules, estrogen receptor alpha (ESR1) and beta (ESR2). These receptors consist of mainly two functional domains including a DNA binding domain and ligand-binding domain that are highly conserved domains (about 97 and 60%, respectively). The N-terminal domain of estrogen receptor has a low degree of homology at 18% and has activation function-1 (AF-1) which acts independent of the ligand (Hall *et al*., 2001; Hewitt *et al*., 2005).

The mechanism of estrogen action involves four pathways. First, in the classical ligand-dependent pathway, the ligand binds to the estrogen receptors (ESRs) inducing the binding to estrogen response elements (ERE) located within the regulatory region of the target genes, which regulates the expression of target genes (Rosenfeld and Glass, 2001). Second, in the ligand-independent pathway, peptide growth factors such as epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) interact with the N-terminal AF-1 domain that stimulate the expression of ESR target genes (Smith, 1998). Third, in ERE-independent genomic action, the estrogen-ESR complex binds to DNA-bound transcription factors such as Fos and Jun, interacting with alternative response element (AP-1) which regulates gene expression. Finally, in nongenomic signaling, ESR and plasma membranes involved in interactions with adaptor protein are linked and activate the MAPK signaling pathway (Simoncini *et al*., 2000).

Regulation of CTNNB1 through Estrogen

CTNNB1 is a multifunctional protein found in the membrane, cytosol and nucleus. In the plasma membrane, CTNNB1 is an essential component of cell-adhesion complexes maintaining epithelial cell layers and barriers. This complex is stabilized by phosphorylation of CTNNB1 at serine/threonine; however, phosphorylated CTNNB1 at tyrosine can result in its dissociation from the adherin complex and transfer to the cytoplasm. The other function of CTNNB1 in the cytoplasm is regulating the expression of target genes by acting as a transcription factor. The binding of Wnt induces the inactivation of GSK3β and promotes binding to the TCF/LEF transcription factors, which activate the transcription of various target genes (MacDonald *et al*., 2009).

The over-expression of CTNNB1 induces uterine endometrial carcinomas as the most common cancer in the female reproductive tract. The accumulation of high levels of CTNNB1 in the cytoplasm originates from the mutation of specific proteins including APC and AXIN and the specific mutation of the GSK-3β phosphorylation site in the N-terminal of CTNNB1 in exon 3. In addition, estrogen regulates Wnt/CTNNB1 signaling in the uterus. Wnt/CTNNB1 signaling regulates the proliferation of uterine epithelial cells in an estrogen-dependent manner (Hou *et al*., 2004). Furthermore, estrogen not only directly modulates the transcriptional activation of Wnt ligands including Wnt4, Wnt5A, and Wnt7A but also regulates the expression of the Wnt/CTNNB1 target gene through EZH2 (the polycomb group protein enhancer of zets homolog2) in the ERE-independent pathway in endometrial cancer (Wang *et al*., 2010).

Effects of Estrogen in the Oviducts of Avian Models

Diethylstilbestrol (DES) is a widely used synthetic non-steroidal estrogen analog to study the biological roles of estrogen in the development of the chicken oviducts (Seo *et al*., 2009; Song *et al*., 2011). DES treatment by subcutaneous pellet implantation over two weeks triggers massive proliferation of reproductive organs and increases the expression of oviduct-related genes such as SERPINB 3 (Lim *et al*., 2012), SERPINB 11 (Lim *et al*., 2011b), A2M (Lim *et al*., 2011a), AHCYL1 (Jeong *et al*., 2012) and PTN (Lee *et al*., 2012).

Immature chicken oviducts undergo dynamic histological, ultrastructural and biochemical differentiation in response to estrogen. The morphological changes first appear in immature epithelial cells of the magnum in chicken oviducts. The differentiated cells are divided into three distinct types: tubular gland cells (synthesis of ovalbumin protein), goblet cells (synthesis of avidin) and ciliated cells (function in motility) (Kohler *et al*., 1968). During the cytodifferentiation of immature oviducts by estrogen, the weight and length increase, and the production of major egg white proteins, including ovalbumin, conalbumin and lysozyme, is also up-regulated (Palmiter *et al*., 1978).

Finally, estrogen has tissue-protective effects and prevents cell death through the regulation of apoptotic genes and bone morphogenetic proteins-7 (BMP-7) and caspases in the chicken oviduct. While the expression of BMP-7 is suppressed to prevent apoptosis during treatment with estrogen, estrogen withdrawal stimulated BMP-7 expression to induce apoptosis. Caspases 1 and 2 as an initiator caspase of apoptosis signaling are regulated by estrogen in the chicken oviduct (Anish *et al*., 2008; Monroe *et al*., 2000). Therefore, the chicken oviduct is an excellent model system to investigate the biological function and signaling pathway for estrogen because of the extensive and sensitive response to the minimal amount of steroid hormones in immature chicks (Dougherty and Sanders, 2005).

EPITHELIAL-DERIVED OVARIAN CANCER

General Characteristics

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer-related deaths, and approximately 15,000 to 22,000 women are diagnosed with this cancer disease in the United States. Over 70% of the patients diagnosed with EOC are at an advanced stage of EOC due to a poor detection system for the early stage of

EOC (Bovicelli *et al*., 2011; Jemal *et al*., 2011). Therefore, to resolve these problems, molecular approaches to discovery biomarkers for the early diagnosis of EOC are necessary.

Ovarian cancer originates from three different ovarian cell types. Germ cell tumors arise most frequently in the second and third decade of life and account for 3~5% of all ovarian cancers. Sex-cord-stromal tumors arise from the ovarian connective tissue and account for 7% of all ovarian malignancies. Epithelial ovarian cancers generally develop after age 40 and include approximately 90% of all malignant ovarian tumors (Chen *et al*., 2003). The histological subtypes of epithelial ovarian cancer can be divided into four types which are Fallopian tube (serous), endometrium (endometrioid), mucin-secreting endocervical glands (mucinous) and glycogen-filled vaginal rests (clear cell) (Auersperg, 2011; Auersperg *et al*., 2001).

The reasons for the occurrence of ovarian cancer are 15% familial and 85% sporadic. Generally, the origin of ovarian cancer has been thought to be from ovarian surface epithelial cells, but recently, fallopian tubes (oviducts) have also been shown to be a candidate for the origin of tumors (Tahira *et al*., 2010). Molecular alterations that are commonly observed in ovarian cancer are mutations in BRCA causing a defect in DNA repair in membrane ruptures (Sowter and Ashworth, 2005).

Classifications

Four types of EOC are classified on the basis of morphology and histology which are serous, endometrioid, mucinous and clear cells. (Auersperg, 2011; Auersperg *et al*., 2001). Serous carcinoma, accounting for over half of all EOCs, has various characteristics, including multiple cysts, solid areas, glands and parts of papillae. Endometrioid carcinoma accounts for approximately between 10 and 20 % of all EOCs and arises from the alteration of genetic molecules such as oncogenes, tumor suppressor gene, phosphatase and tensin homolog (PTEN) and other DNA repair-related genes. Several features are observed in this type of tumor such as glands, solid masses and fibrous consistency. In the mucinous carcinomas, morphological properties have been found as follows: papillae and soli areas, mucin-riched cytoplasm and large areas of necrosis and hemorrhage. Finally, clear cell carcinoma makes up 10% of all EOCs based on the development of tubular, papillary solid and hobnail cells containing apical nuclei (Barua *et al*., 2009).

Avian Models for Epithelial-Derived Ovarian Cancer

To investigate the specific molecular targets responsible for the therapeutic effect of EOCs, the laying hen is an optimal animal model for the discovery of novel biomarkers. The following advantages of this model provide reasonable evidence for the applicability of this animal to research human EOCs. First, the cause of chicken ovarian cancer is incessant ovulation which is the same reason in human ovarian cancer (Johnson and Giles, 2013). Second, chicken ovarian cancer spontaneously develops from ovarian surface epithelium at a high rate, just like in women (Vanderhyden *et al*., 2003). Chicken ovarian cancer usually spontaneously develops after chickens stop their ovulation to produce eggs when they are over 2 years old. Third, the histological classification and stage of chicken ovarian cancer are remarkably consistent with that of women: histological subtypes of ovarian carcinoma are serous, endometrioid, mucinous and clear cell carcinomas, and the stage is divided by the International Federation of Gynecologists (FIGO) staging system (Barua *et al*., 2009; Heintz *et al*., 2006). Finally, biomarkers for detecting the early-stages of human EOCs, including CA125, EGFR and ERBB-2, are detected in very similar patterns in the cancerous ovaries of hens (Rodriguez-Burford *et al*., 2001).

CONCLUSION

In conclusion, this review shows the advantages of using a chicken as an animal model to research the molecular mechanisms of the reproductive system and ovarian carcinogenesis. The results of our lab show that the CTNNB1 is an essential modulator in both the male and female reproductive tracts. This review also shows not only the potential roles of CTNNB1 during male gonad development but also the availability of CTNNB1 as a biomarker in ovarian cancer, which provides a better understanding for further studies on fetal gonad development and reproductive systems at a molecular level.

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