Acta Histochemica 117 (2015) 297–304

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Expression and regulation of scavenger receptor class B type 1 in the rat ovary and uterus during the estrous cycle

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ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form 11 March 2015
Accepted 11 March 2015

Keywords:
SR-B1
Ovary
Uterus
Estrous cycle
E_2
P_4

ABSTRACT

Scavenger receptor class B type 1 (SR-B1) preferentially mediates the selective uptake of high density lipoprotein–cholesterol ester and the delivery of cholesterol for steroidogenesis. Although multiple analyses have investigated the function of SR-B1 in the liver, adrenal and ovary, its expression in rat ovary and uterus during the estrous cycle is lacking. In the present study, real-time PCR, western blot and immunohistochemistry (IHC) were used to investigate SR-B1 expression in the rat ovary and uterus during the estrous cycle. The results demonstrated that ovarian SR-B1 expression was in a stage-dependent manner, continuously increased from proestrus and kept elevated during metoestrus, while uterine SR-B1 expression decreased from proestrus to diestrus. To determine whether ovarian and uterine SR-B1 expression were affected by sex steroid hormones, immature rats were treated with 17 β-estradiol (E_2), progesterone (P_4), or their antagonists from postnatal days 24–26. Results showed that the levels of *SR-B1* mRNA and protein were significantly up-regulated by E_2 in both the ovary and uterus. IHC results showed that SR-B1 was primarily localized in the oocytes, theca internal cells (T-I) of follicles, interstitial cells (IC) as well as corpus luteum (CL), but not granulosa cells (GC) in the ovary during the estrous cycle. Uterine SR-B1 was highly expressed in the endometrial luminal epithelial cells (LEC) and glandular epithelial cells (GEC) as well as in the circular muscle (CM) cells, and weak staining in stromal cells (SC) through estrous cycle. Taken together, SR-B1 expression in the ovary and uterus across the estrous cycle demonstrate that SR-B1 may be involved in uterine function, follicular development as well as luteal function.

Introduction

Cholesterol is an essential component of mammalian cell membranes and acts as a precursor of bile acids and steroid hormones, which are necessary and essential for proper development (Woollett, 2001). High density lipoprotein (HDL) is a major cholesterol-carrying lipoprotein. Steroidogenic cells can obtain much of the cholesterol from circulating HDL, which serves as substrate for steroid hormone synthesis. The selective uptake of HDL-derived cholesteryl ester (CE) occurs in a variety of human and other mammalian cell types (Pittman et al., 1987).

Scavenger receptor class B type 1 (SR-B1), a member of the class B scavenger receptor family, has been demonstrated as the first physiologically relevant HDL receptor and a mediator of selective cholesterol uptake *in vivo* and *in vitro* (Acton et al., 1996). SR-B1 has been observed notably expressed in tissues, such as the liver, and a strong cholesterol demand for steroidogenesis (adrenals, ovaries and testes) (Kozarsky et al., 1997; Temel et al., 1997). SR-B1 can specifically bind to HDL to facilitate cellular transport of cholesterol, regulation of vasculature and plays an important anti-atherosclerotic role (Berrougui and Khalil, 2009; Papale et al., 2010). Alteration in SR-B1 expression profoundly influences diverse physiological functions including female fertility and development of red blood cell and coronary heart diseases (Ueda et al., 2000).

SR-B1 plays a major role during different stages of reproduction and fetal development by providing substrate cholesterol for steroid hormone synthesis. SR-B1 has been implicated in protection of female fertility, and homozygous SR-BI KO females have abnormal HDLs, ovulate dysfunctional oocytes, and are infertile (Miettinen et al., 2001). A recent study shown that SR-B1 expression in extra embryonic tissues was involved in the maternal-fetal transport of cholesterol and/or other lipids with a role during neural tube

Abbreviations: SR-B1, scavenger receptor class B type 1; IHC, immunohistochemistry; E_2, β-estradiol; P_4, progesterone; T-I, theca internal cells; IC, interstitial cells; CL, corpus luteum; GC, granulosa cells; LEC, luminal epithelial cells; GEC, glandular epithelial cells; CM, circular muscle; SC, stromal cells; HDL, high density lipoprotein; CE, cholesteryl ester; hCG, human chorionic gonadotropin; ERs, estrogens receptors; PRs, progesterone receptors.

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http://dx.doi.org/10.1016/j.acthis.2015.03.007
0065-1281/

closure and fetal growth (Santander et al., 2013). Previous research had demonstrated that SR-B1 was expressed in theca internal cells (T-I) at all stages of follicular development and in corpus luteum cells (CL) of rat ovaries (Johnson et al., 1998; Mizutani et al., 1997). In addition, it could mediate the recognition of apoptotic granulosa cells by the surrounding thecal cells and therefore might play a role in the remodeling of atretic follicles to subordinate interstitial cells (Svensson et al., 1999). *SR-B1* gene knockout (KO) mice were accompanied by abnormal structure, composition, and abundance of lipoproteins, which altered the development of female gametes and caused a significant proportion of ovulated oocytes to die soon after ovulation, resulting in infertility (Trigatti et al., 1999). More recently, Jimenez reported that *SR-B1* KO mice had 50% lower serum progesterone levels than normal wild-type mice (Jimenez et al., 2010), and markedly lower progesterone secretion was associated with SR-B1 protein deficiency in human granulosa cells (Kolmakova et al., 2010).

In animal models, *SR-B1* appears to be regulated by the action of pituitary hormones (such as LH), or human chorionic gonadotropin (hCG) that stimulate steroidogenesis, suggesting its important role for steroid hormone production in supplying precursor cholesterol (Li et al., 1998). When treated with E_2 *in vivo*, there was an increase in expression of *SR-B1* in the rat adrenal gland and corpus luteum, but a decrease in the liver (Landschulz et al., 1996). Therefore the up-regulation in expression of *SR-B1* by key steroid hormones makes provision for extra supplies of precursor cholesterol to tissues for synthesizing additional steroid hormones.

Sex steroid hormones such as progesterone, testosterone and estradiol are recognized as regulators of cell proliferation and differentiation, and are essential for reproduction (Bhangoo et al., 2006). Estrogens also act as fundamental regulator of the metabolic signaling molecule system and coordinating various functions of genes, cells and organs (Rettberg et al., 2014). Its action occurs through estrogens receptors (ERs) that are conserved and widely distributed in the reproductive organs (ovary, uterus and testis) and mamma, as well as brain (Fixemer et al., 2003; Sakaguchi et al., 2005). Contrary to the results obtained in hepatic cells, high estrogen treatment could increase SR-B1 expression and selective lipid uptake in adrenal and ovarian luteal cells (Azhar et al., 2002; Landschulz et al., 1996; Stangl et al., 2002). The biological effects of progesterone (P_4) are mediated through progesterone receptors (PRs) to regulate growth and differentiation in the human body (Bagchi et al., 1992). It is well accepted that progesterone responsiveness in the endometrium is mediated by the coordinated actions of PR-A and PR-B (Graham and Clarke, 1997).

Although multiple analyses have investigated the function of SR-B1 in the rat liver, adrenal and ovary, its expression in rat ovary and uterus especially during the estrous cycle and its regulation are not well understood. In the present study, the expression of SR-B1 in rat ovary and uterus during the normal estrous cycle was firstly analyzed. To determine whether ovarian and uterine SR-B1 expressions are influenced by the sex steroid hormones, we treated the immature rats with E_2 and P_4 to investigate the ovarian and uterine SR-B1 expression. The present study could act as an attempt to understand the role of SR-B1 in female development and reproduction.

Materials and methods

Materials

Sesame oil, Estrogen receptor antagonist (ICI-182780), and Progesterone receptor antagonist (mifepristone, RU-486) were purchased from Santa Cruz company (USA). 17 β-estradiol (E_2) and Progesterone (P_4) were purchased from Sigma Company (USA).

Animals and treatment

Twenty mature (14 weeks old, 200–250 g) and 30 immature female Sprague-Dawley rats (21d, 40–50 g) were purchased from the Experimental Animal Center of Nanjing Agricultural University (Nanjing, China). Animals were kept in standard animal-house conditions with a 12-h light cycle and room temperature 23 °C, with free access to food and water. The mature rats were monitored for 14 days and vaginal smears were taken and examined twice daily. Ovaries and uteri from rats exhibiting at least two consecutive 4-day estrous cycles were collected on proestrus (P), estrus (E), metoestrus (M) and diestrus (D) phase.

To determine the effects of steroid hormones on SR-B1 expression, immature female rats ($n = 30$) were randomly divided into six groups, and each rat received subcutaneously (s.c.) injections of sesame oil 200 μL (C), P_4, E_2, E_2 *plus* P_4, E_2 *plus* ICI-182780 or P_4 *plus* RU486, respectively, according to earlier studies (Ahn et al., 2011; Gangula et al., 2001; Jung et al., 2011; Xiao et al., 2013). The P_4 (2 mg/kg BW), E_2 (20 μg/kg BW), ER antagonist ICI-182780 (500 μg/kg BW) and PR antagonist RU486 (400 μg/kg BW) were dissolved in 200 μL of sesame oil, respectively. And antagonists were injected 30 min prior to E_2 or P_4. Immature rats were treated daily for 3 days from postnatal day 24 and were sacrificed by cervical dislocation under ether 12 h after the final injection. One set of the ovaries and uteri were fixed in 4% paraformaldehyde for immunohistochemistry, and the other sets of tissue were stored at −80 °C for RNA and protein. All surgical procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Real-time PCR

Total RNA was isolated from the rat ovary and uterus with TRIzol reagent (Invitrogen, USA) and processed for quantitative real time-PCR as described (Gorrini et al., 2013). Total RNA (1 μg) was reverse transcribed into cDNA with PrimeScript reverse transcriptase reagent kit (Perfect real time; TaKaRa, Dalian, China). For real-time PCR, cDNA was amplified using a SYBR Premix Ex Taq kit (TaKaRa; Dalian, China) on StepOnePlus Real-Time PCR System. PCR reactions were carried out with DNA polymerase activation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s. Primer sequences were as follows: *SR-B1* (sense: 5′-TCTGAACCCTGACCCA-3′, anti-sense: 5′-CCCTTGACGGATTTGA-3′), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (sense: 5′-CAAGTTCAACGGCACAGTCAAG-3′, anti-sense: 5′-ACATACTCAGCACCAGCATCAC-3′). All samples were measured in triplicates, and the data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

The quantitive expressions of SR-B1 protein were assessed by Western blotting as described (Meng et al., 2014). Briefly, the frozen tissues were homogenized in a Dounce homogenizer (Wheaton, Millville, NJ210) using RIPA lysis buffer (Beyotime, Nantong, China) containing 10 mM phenylmethylsulfonyl fluoride (PMSF; Beyotime), followed by centrifugation at 12,000 g for 10 min at 4 °C. Protein concentration was qualified by bicinchoninic acid (BCA) protein assay kit (Beyotime).

For each sample, 40 μg of total protein per lane was separated by 10% SDS-PAGE (Bio-Rad) and then transferred to polyvinylidene fluoride (PVDF) transfer membranes (Millipore, Bedford, MA, USA) using standard techniques. The protein blots were incubated with anti-rabbit SR-B1 antibody (1:1500; Novus Biologicals, NB400-104) and β-Tubulin (1:10000; CMC Scientific Co., Ltd) overnight at 4 °C. After washing in TBST, the membranes were probed with