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Toll-like receptor 3 and RIG-I-like receptor activation induces innate antiviral responses in mouse ovarian granulosa cells

Keqin Yan, Weiwei Zhu, Lili Yu, Nan Li, Xiaoyan Zhang, Peipei Liu, Qiaoyuan Chen, Yongmei Chen *, Daishu Han *

Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medicine, Peking Union Medical College, Beijing, PR China

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ABSTRACT

Viral infections of the ovary can cause pathological conditions. However, innate antiviral responses in the ovary are poorly understood. In this study, we demonstrate that Toll-like receptor 3 (TLR3), retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) are constitutively expressed in the mouse ovary and predominantly located in granulosa cells. Polyinosinic-polycytidylic acid [poly(I:C)], a common agonist of TLR3, MDA5 and RIG-I, induced innate antiviral responses in ovarian granulosa cells. Poly(I:C) up-regulated pro-inflammatory cytokines, including TNF-α and IL-6, and type I interferons (IFN-α/β). Moreover, poly(I:C) induced the expression of antiviral proteins, including 2′-5′-oligoadenylate synthetase, Mx GTPase 1 and IFN-stimulating gene 15, in granulosa cells. In contrast, P450 aromatase expression was inhibited by poly(I:C). The poly(I:C)-induced antiviral responses in TLR3 knockout ($TLR3^{-/-}$) ovarian granulosa cells were reduced, and completely abolished by blocking of MDA5/RIG-I signaling. Further, the poly(I:C)-induced cytokine expression in $TLR3^{-/-}$ cells was reduced by knockdown of MDA5 or RIG-I. Data suggest that TLR3, MDA5 and RIG-I cooperate in mediating innate antiviral responses in granulosa cells, which may contribute to the defense of the ovary against viral infections.

1. Introduction

Virus infections have been found in the ovaries of different species. Vaccinia viruses preferentially infect the ovary and impair ovarian functions (Zhao et al., 2011). Hepatitis B viruses can infect human oocytes and ovarian granulosa cells, which may represent one of the mechanisms underlying virus vertical transmission (Hu et al., 2011; Jin et al., 2011). The mechanisms underlying innate antiviral responses in the ovary remain poorly understood. The roles of pattern recognition receptors (PRRs) in mediating inflammatory responses in the ovary and regulating ovarian functions have been revealed (Sheldon and Bromfield, 2011). However, PRR-mediated innate antiviral responses in the ovary have yet to be investigated.

PRRs are broadly expressed in many cell types and recognize highly conserved components specific to microbes, termed pathogen-associated molecular patterns (PAMPs) (Kumar et al., 2011). Recognition of PAMPs by PRRs initiates innate immune responses and subsequently restructures adaptive immunity (Iwasaki and Medzhitov, 2010). Three families of PRRs in cells have been recognized thus far: Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and nucleotide-oligomerization domain-like receptors (NLRs). TLRs belong to a family of transmembrane proteins that include 13 members in mammals (Li et al., 2009). RLRs are a family of cytosolic viral sensors that include RIG-I, melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) (Yoneyama and Fujita, 2007). NLRs represent a large family of intracellular sensors that detect a broad spectrum of pathogens and stress signals (Martinon et al., 2009). Different PRRs may recognize the same PAMPs. For example, TLR3, MDA5 and RIG-I recognize viral double-strained RNA (dsRNA) and a synthetic dsRNA analog, polyinosinic-polycytidylic acid [poly(I:C)]. Activation of TLR3, MDA5 and RIG-I triggers antiviral responses by inducing the production of type I interferons (IFN-α/β) (Takeuchi and Akira, 2008; Thompson et al., 2011). IFN-α/β subsequently induce synthesis of antiviral proteins to inhibit viral replication within cells, and facilitate adaptive immunity against viruses (Samuel, 2001; Yoneyama et al., 2005). TLR3, MDA5 and RIG-I trigger different signaling pathways that converge at the activation of nuclear factor kappa B (NF-κB) and IFN regulatory factor 3 (IRF3), thereby inducing pro-inflammatory cytokines and IFN-α/β (Matsumoto et al., 2011; Takeuchi and Akira, 2008).

* Corresponding authors. Addresses: Department of Human Anatomy, Histology & Embryology, PUMC & CAMS, 5 Dong Dan San Tiao, Beijing 100005, PR China (Y. Chen), Department of Cell Biology, PUMC & CAMS, 5 Dong Dan San Tiao, Beijing 100005, PR China (D. Han). Tel.: +86 10 69156457; fax: +86 10 69156466.

E-mail addresses: chenym70@yahoo.com.cn (Y. Chen), dshan@ibms.pumc.edu.cn (D. Han).

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Tissue-resident macrophages are believed to be important innate immune cells that may construct the first line of defense against microbial infections. Some macrophages are located in the stroma of the normal ovary and regulate ovarian functions through the secretion of inflammatory cytokines (Takaya et al., 1997; Wu et al., 2004). The intra-follicular environment where numerous granulosa cells encompass an oocyte is devoid of macrophages in mice (Petrovska et al., 1996). These observations lead researchers to question whether or not the granulosa cells within a follicle possess innate immune capabilities to protect the oocyte against invading pathogens (Sheldon and Bromfield, 2011). Several recent studies have shown the expression and function of TLRs in the ovary. TLR1-9 are expressed in human normal ovary and ovarian cancer (Zhou et al., 2009). Human granulosa cells from in vitro fertilization express TLR4 (Serke et al., 2009). TLR4-initiated inflammatory responses in bovine granulosa cells perturb oestradiol synthesis (Herath et al., 2007). In mouse, the expression and function of TLR2 and TLR4 in cumulus cells have been investigated (Shimada et al., 2006, 2008). A previous study demonstrated that TLRs regulate ovulation in response to both exogenous and endogenous stimuli in mice (Liu et al., 2008). TLR signaling pathways have been recently analyzed in human granulosa cell lines, bovine and hen primary granulosa cells (Bromfield and Sheldon, 2011; Price et al., 2012; Woods et al., 2009).

Most studies on TLR-mediated innate immune responses in ovarian granulosa cells focus on TLR2 and TLR4. With the exception of a few studies on the expression of TLRs that recognize viruses in granulosa cells (Price et al., 2012; Zhou et al., 2009), the antiviral roles of PRRs in ovarian cells have yet to be exclusively investigated. The current study aims to evaluate the expression and function of the viral sensors in mouse ovary. We provide evidence that mouse ovarian granulosa cells constitutively express TLR3, RIG-I and MDA5, all of which participate in mediating innate antiviral responses in granulosa cells.

2. Materials and methods

2.1. Animals

C57BL/6J mice were obtained from the Laboratory Animal Center of Peking Union Medical College (Beijing, China). TLR3 knockout mice (B6/129S1-Tlr3tm1Flv/J) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). The mice were bred under specific pathogen-free conditions with a natural day/night cycle, fed freely with food and water, and handled in accordance with the Guidelines for the Care and Use of Laboratory Animals established by the Chinese Council on Animal Care.

2.2. Major reagents and antibodies

Poly(I:C) (tlrl-pic), BX795 (tlrl-bx7), and BAY11-7082 (tlrl-b82) were purchased from InvivoGen (San Diego, CA, USA). The antibodies used in this study are listed in Table 1. Small interfering RNA (siRNA) targeting mouse RIG-I (sc-61481), MDA5 (sc-61011), and control siRNA (sc-37007) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.3. Isolation and culture of granulosa cells

Mural ovarian granulosa cells were isolated based on previously described procedures with modifications (Vanderhyden et al.,

Table 1
Antibodies used in this study.

Vendor	Antibody	Host species	Catalog number	Use	Working dilution	Conjugation
Sigma–Aldrich, St. Louis, MO, USA	Anti-β-actin	Mouse	A5316	WB	1:4000	
Abcam, Cambridge, MA, USA	Anti-F4/80	Rat	ab6640	IF	1:500	
	Anti-ISG15	Rabbit	ab131119	WB	1:500	
	Anti-OAS1	Rabbit	ab86343	WB	1:1000	
	Anti-RIG-I	Rabbit	ab45428	WB	1:1000	
				IF	1:200	
				IHC	1:200	
	Anti-TLR3	Mouse	ab13915	WB	1:500	
				IF	1:200	
				IHC	1:200	
	Anti-MDA5	Rabbit	ab69983	WB	1:1000	
				IF	1:500	
				IHC	1:500	
Santa Cruz Biotechnology, Santa Cruz, CA, USA	Anti-FSHR	Goat	sc-7798	IF	1:200	
	Anti-PKR	Rabbit	sc-708	WB	1:500	
	Anti-MX1	Rabbit	sc-50509	WB	1:200	
	Anti-IRF3	Rabbit	sc-9082	WB	1:1000	
				IF	1:200	
Cell Signaling, Beverly, MA						
	Anti-p65	Rabbit	4764	WB	1:1000	
				IF	1:200	
	Anti-p-p65	Rabbit	3031	WB	1:1000	
	Anti-p-IRF3	Rabbit	4947	WB	1:1000	
Zhongshan Biotechnology Co., Beijing, China	Rat IgG	Goat	ZF-0315	IF	1:200	FITC
	Rabbit IgG	Goat	ZF-0311	IF	1:200	FITC
	Mouse IgG	Goat	ZF-0312	IF	1:200	FITC
	Goat IgG	Rabbit	ZF-0314	IF	1:200	FITC
	Mouse IgG	Goat	ZB-2305	WB	1:4000	HRP
				IHC	1:200	
	Rabbit IgG	Goat	ZB-2301	WB	1:4000	HRP
				IHC	1:200	

WB, Western blot; IHC, immunohistochemistry; IF, immunofluorescence staining; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase.