Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Expression patterns of claudin-5 and its related signals during luteal regression in pseudopregnant rats: The enhanced effect of additional PGF treatment

Lina Qi, Jingle Jiang, Pengjin Jin, Meiqian Kuang, Quanwei Wei, Fangxiong Shi, Dagan Mao*

College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, PR China

ARTICLE INFO

Keywords:
Corpus luteum
PGF
P_4
Claudin-5
STAT3
Rats

ABSTRACT

To study the expression patterns of claudin-5 and its related signals during luteal regression in rats, a sequential PMSG/hCG treatment paradigm was used to obtain a single, well-defined generation of corpus luteum (CL). A total of 35 rats were treated with one PGF or two PGF at an interval of 24 h from day 7 of pseudopregnancy to induce CL regression. Serum and ovaries were collected at 0, 2, 4, 8 or 24 h after one PGF injection (1 PGF), 2 or 24 h after two PGF injections (2 PGF). The serum progesterone level was detected by RIA; the ovarian expression of claudin-5, the phosphorylations of STAT3 (*p*-STAT3), Akt (*p*-Akt), ERK1/2 (*p*-ERK) and p38 MAPK (*p*-p38) were detected by western blot, real-time PCR and IHC. Results showed that serum progesterone (P_4) decreased after PGF treatment. *Claudin-5* mRNA decreased at 4 h and 8 h after 1 PGF and 2 h after 2 PGF, and claudin-5 protein decreased at 4 h after 1 PGF. *p*-STAT3 increased at 4 h after 1 PGF and 2 h after 2 PGF. *p*-ERK increased at 2 h after 2 PGF. The level of *p*-Akt decreased at 4 h after 1 PGF. PGF treatment did not alter the phosphorylation of p38 MAPK at any time points in this study. IHC results revealed that claudin-5 was expressed in the nuclei and cytoplasm of steroidogenic cells and in the vessels, while PGF induced-*p*-STAT3 was expressed uniformly in the cytoplasm of luteal steroidogenic cells. In conclusion, PGF treatment decreased the expression of claudin-5 and the additional PGF treatment enhanced the decrease in *claudin-5* mRNA expression and the increases in ERK1/2 and STAT3 phosphorylation in the corpus luteum of pseudopregnant rats, which will contribute new information to the further study of molecular mechanism of luteal regression.

1. Introduction

The corpus luteum (CL) is a temporary endocrine gland formed by the ovulated follicle. The lifespan of the rodent CL can be divided into four stages, including CL during the estrous cycle, pseudopregnancy, pregnancy and lactation. The main function of the CL is to synthesize and secrete progesterone (P_4), a kind of steroid hormone playing an important role in the regulation of estrous cycle and maintenance of pregnancy. If the oocyte is not fertilized, the CL will regress and the next estrous cycle begins. It is widely accepted that CL regression or luteolysis includes functional regression characterized by a marked decrease in P_4 secretion and structural regression characterized by apoptosis of endothelial and steroidogenic cells (Davis and Rueda, 2002).

$PGF_{2\alpha}$, a major luteolytic factor in mammals, caused the reduction of serum progesterone level in cows, ewes and rats (Mccracken et al., 1999; Fiedler et al., 1999; Mccracken et al., 1970). $PGF_{2\alpha}$ activated phospholipase C and led to increase the intracellular Ca^{2+}, PKC and MAPK (Atli et al., 2012), which reduced the stability of the key enzymes in progesterone biosynthesis or activated the progesterone catabolism (Currie et al., 1992; Knauthe et al., 1996; Stocco et al., 2000). Moreover, a study by Wiltbank et al. showed that treatment with two PGF at an interval of 24 h in the Ovsynch procedure enhanced luteal regression in cows with different ranges of circulating P_4 level (Wiltbank et al., 2015), and 4 × PGF decreased P_4 concentrations at all treatment time points (Atli et al., 2012).

Cell-cell and cell-matrix adhesion are basic requirements for maintaining the stability of tissue structures (Garcia et al., 2017). The

Abbreviations: CL, corpus luteum; ECL, enhanced chemoluminescence; mTORC2, mammalian target of rapamycin complex 2; MAPK, mitogen-activated proteinkinases; PGF, prostaglandin$F_{2\alpha}$ analogue; $PGF_{2\alpha}$, prostaglandin$F_{2\alpha}$; P_4, progesterone; *p*-ERK, phosphorylated ERK1/2; *p*-p38, phosphorylated p38 MAPK; p38, p38 MAPK; *p*-STAT3, phosphorylated STAT3; *p*-Akt, phosphorylated Akt; PKC, protein kinase C; P450scc, cytochrome P450scc; PDK1, phosphoinositide-dependent kinase 1; STAR, steroidogenic acute regulatory protein; STAT3, signal transducer and activator of transcription; TJs, tight junctions; 3β-HSD, 3 beta-steroid dehydrogenase

* Corresponding author at: Laboratory of Animal Reproduction, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China.
E-mail address: maodagan@njau.edu.cn (D. Mao).

https://doi.org/10.1016/j.acthis.2018.02.001
Received 22 November 2017; Received in revised form 29 January 2018; Accepted 6 February 2018

Table 1
Antibodies used for western blot (WB) analysis and immunohistochemistry (IHC).

Antibodies	Catalogue no.	Supplier	Dilution	
			WB	IHC
Primary antibodies				
Rabbit anti-claudin-5	abs130067a	Absin Bioscience Inc	1:1000	1:100
Mouse anti-STAT3	cs9139	Cell Signaling Technology	1:1000	–
Rabbit anti-*p*-STAT3	cs9145	Cell Signaling Technology	1:2000	1:100
Mouse anti-α-Tubulin	ABM0010	Zoonbio Biotechnology	1:5000	–
Rabbit anti-*p*-ERK1/2	cs9101	Cell Signaling Technology	1:1000	–
Mouse anti-ERK1/2	AM2189b	Abgent (Suzhou, China)	1:1000	–
Rabbit anti-*p*-p38	cs9216	Cell Signaling Technology	1:1000	
Rabbit anti-p38	AM065	Beyotime Institute of Biotechnology (Nantong, China)	1:1000	–
Rabbit anti-*p*-Akt (Thr 308)	cs13038	Cell Signaling Technology	1:1000	–
Mouse anti-Akt	cs2926	Cell Signaling Technology	1:1000	–
Secondary antibodies				
Goat anti-rabbit IgG–HRP	ZB-2301	ZSGB-BIO	1:3000	–
Goat anti-mouse IgG–HRP	ZB-2305	ZSGB-BIO	1:5000	–

adhesion structures consist of 4 types of intercellular junctions: desmosomes, adherens junctions, gap junctions and tight junctions (TJs) (Khan and Asif, 2015), and TJs are characterized by fusion of the adjacent cell membranes which are very important for the integrity and function of epithelial barrier (Farquhar and Palade, 1963; Jia et al., 2014). As one of integral membrane proteins of TJs (Mineta et al., 2011; Turksen and Troy, 2004), claudin-5 has been reported to be an endothelial specific tight junction protein, and in the ovary the claudin-5 is exclusively localized to the vasculature (Rodewald et al., 2007). However, whether claudin-5 expression can be altered during PGF-induced luteal regression is not yet known.

Several studies have showed that the expression of claudins could be regulated by STAT3 activation (García-Hernández et al., 2015; Sonoki et al., 2017). STAT3 can be activated by various cytokines and involved in different cellular responses (Yang and Zhang, 2016), and the phosphorylation of STAT3 at Tyr705 induced dimerization, nuclear translocation and DNA binding (Wakahara et al., 2012). Previous study showed that PGF treatment increased the luteal STAT3 expression in pregnant rats (Curlewis et al., 2002). Therefore, we hypothesized that activation of STAT3 might be correlated with the alternated expression of claudin-5 during PGF-induced luteal regression in pseudopregnant rats.

Recent study showed that Akt was immediately down-regulated accompanied with the activation of STAT3 in the corpus luteum after PGF treatment in cattle (Rovani et al., 2017). Moreover, ERK and p38 MAPKs have also been reported to regulate the expression of cell-cell junction proteins (Ramos, 2008), including occludin and Connexin 43 in Sertoli cells (Qiu et al., 2016) and the blood-epididymis barrier (Kim and Breton, 2016). Furthermore, the transcriptional activity of STAT3 can also be regulated by phosphorylation at Ser727 through the MAPK pathway (Sakaguchi et al., 2012). Therefore, the AKT and MAPK signaling might be associated with the expression of junction proteins in the corpus luteum.

Our current study aimed to investigate the expression patterns of claudin-5 and its related signals during the luteal regression in pseudopregnant rats. We tested the hypothesis that PGF-induced activation of STAT3 and MAPK signaling was associated with the decrease in claudin-5 expression, and the additional PGF treatment would enhance the alterations in the CL of pseudopregnant rats.

2. Materials and methods

2.1. Experimental animals

A total of 35 SD female rats aged 21 days were obtained from the Nanjing Qinglongshan Animal Breeding Grounds, China. Rats were housed under identical conditions and allowed free access to rat chow (Qinglongshan, Nanjing, China) and water *ad libitum*. All experimental procedures were approved by the Nanjing Agricultural University Institutional Animal Care and Use Committee.

2.2. Treatment

To obtain a single, well-defined generation of CL, rats aged 28 days were injected subcutaneously with 30 IU PMSG and 20 IU hCG (both from Ningbo Sansheng Pharmaceutical Co., Ltd., Zhejiang, China) 48 h later (Day 0) to induce pseudopregnancy. On Day 7 of pseudopregnancy, rats were randomly divided into 7 groups (n = 5) and injected with one PGF or two PGF (30 μg each time) at an interval of 24 h, and the control rats (0 h) were injected with 0.3 mL 0.9% saline. Rats were then sacrificed by cervical dislocation under ether at 0, 2, 4, 8 or 24 h after one PGF injection (1 PGF), 2 or 24 h after two PGF injections (2 PGF). Ovaries and blood were collected. The blood was centrifuged at 5000*g* for 10 min, and the supernatant was taken for P_4 measurement. For each rat, one ovary was stored at −80 °C for protein and RNA extraction; the other was fixed by immersing in 4% formalin for 48 h and then stored in 70% alcohol until embedded in paraffin.

2.3. Progesterone assay

The serum concentrations of progesterone were detected using commercial RIA kit (Beijing North Institute of Biological Technology, Beijing, China) by the Shanghai Xinfan Bio Techology Co., Ltd. The sensitivity for progesterone determinations was 0.2 ng/mL, the intra-assay coefficient of variation (CV) was < 10%, and the inter-assay coefficient of variation was < 15%.

2.4. Immunohistochemistry

The tissue slides for IHC were subjected to deparaffinization, hydration and blocking of peroxidase activity. After being washed with PBS, sections were incubated with 5% BSA at 37 °C for 2 h, and then incubated with specific primary antibodies (Table 1) at 4 °C overnight, while negative controls were performed by replacing the primary antibody with normal goat serum. Then sections were incubated with an SABC (Boster Biological Technology, Wuhan, China) and visualized with 0.05% DAB (3, 3′-diaminobenzidine tetrachloride, Solebo biotech Ltd) in PBS. Finally, the sections were counterstained with hematoxylin, dehydrated through alcohol, and cover slipped with VectaMount (Vector Laboratories, Burlingname, CA,USA). Images were acquired under a Nikon YS100 microscope (Nikon, Tokyo, Japan).