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The expression of aminoacyl-tRNA-synthetase-interacting multifunctional protein-1 (*Aimp1*) is regulated by estrogen in the mouse uterus

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ABSTRACT

Aimp1 is known as a multifunctional cytokine in various cellular events. Recent study showed Aimp1 is localized in glandular epithelial, endothelial, and stromal cells in functionalis and basalis layers of the endometrium. However, the regulatory mechanism of Aimp1 in the uterus remains unknown. In the present study, we found that *Aimp1* is expressed in the mouse uterus. *Aimp1* transcripts were decreased at diestrus stage. However, the level of Aimp1 protein was significantly increased in the luminal epithelium in the uterine endometrium at estrus stage during the estrous cycle. We found that treatment of estrogen increased the expression of *Aimp1* in the uterus in ovarectomized mice. We identified one estrogen receptor binding element (ERE) on mouse *Aimp1* promoter. The activity of *Aimp1* promoter was increased with estrogen treatment. Our findings indicate that Aimp1 might act as an important regulator to remodel the uterine endometrium and its expression might be regulated by estrogen during the estrous cycle. This will give us better understanding of the dynamic change of uterine remodeling during the estrous cycle.

1. Introduction

Aimp1 (ARS-interacting multifunctional protein) is one of the components of aminoacyl-tRNA synthetase (ARS) complex (Quevillon et al., 1997). Aimp1 acts as a cytokine in various sites such as monocytes, endothelial cells, dendritic cells, pancreatic α cells, and fibroblast (reviewed in Lee et al., 2008; Park et al., 2010). Therefore, Aimp1 is involved in diverse physiological processes including proinflammatory process (Ko et al., 2001; Kwon et al., 2010), adhesion (Park et al., 2002a), fibroblast proliferation (Park et al., 2005), glucose homeostasis (Park et al., 2006), angiogenesis (Park et al., 2002b), tumor suppression (Han et al., 2010) and wound repair (Park et al., 2005). In addition, Aimp1 is known as a precursor of EMAP II (endothelial monocyte-activating polypeptide II). EMAP II is released from the complex after cleavage of Aimp1 (Shalak et al., 2001). Recent study showed that EMAP II is present in endometirum of human uterus (Battersby et al., 2002). EMAP II is localized in glandular epithelial, endothelial, and stromal cells in functionalis and basalis layers of the endometrium (Battersby et al., 2002). However, the physiological function and regulation of Aimp1 in the uterus remains unknown. This study aimed to investigate the regulation of *Aimp1* expression in mouse uterus during the estrous cycle.

2. Materials and methods

2.1. Animal breeding and experiments

All mice experiments were performed on adult (7-week-old) ICR mice, provided by Orient Bio Company (Seongnam, Korea). Mice were housed under temperature and light controlled conditions with the lights on for 12 h daily and fed *ad libitum*. Care of mice, experimental and surgical procedures complied with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Agricultural Animal Care and Use Committee of CHA University.

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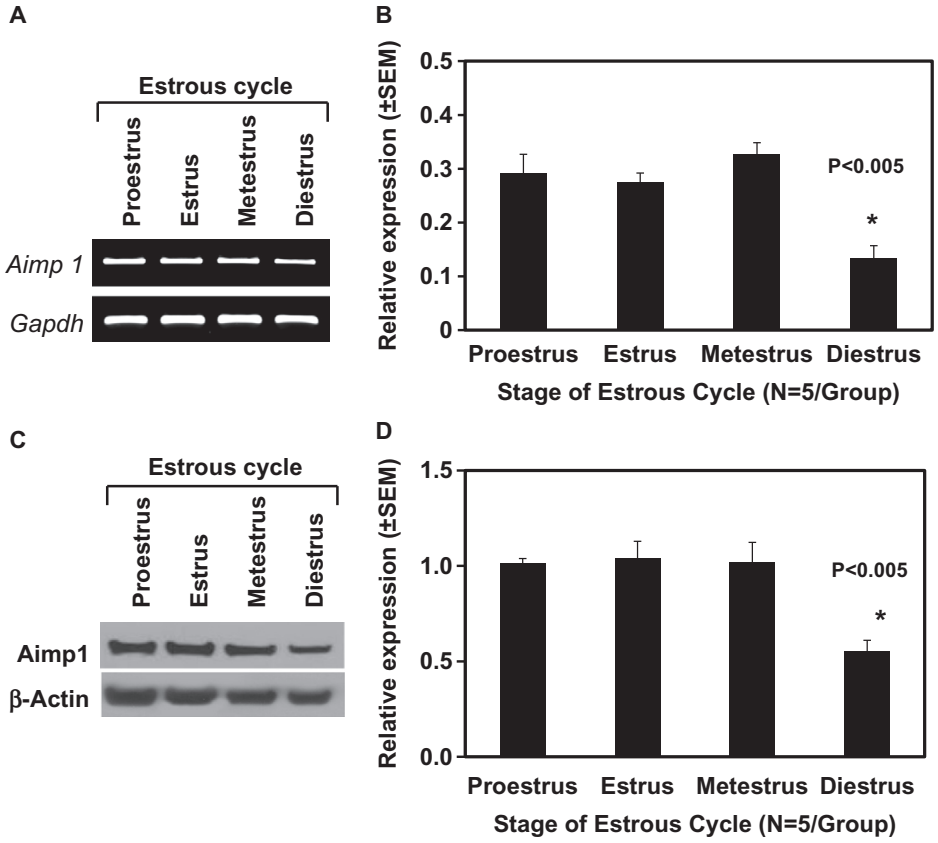

Fig. 1. *Aimp1* expression in uteri at each stage of estrous cycle. (A) Relative expression of *Aimp1* in the uterus at different stages during the estrous cycle by RT-PCR. Total mRNAs from uteri at each stage of estrous cycle (proestrus, estrus, metestrus, diestrus) were isolated and also amplified with specific primers for *Aimp1*. *Gapdh* was used for internal control. Each group included five mice (n = 5). (B) Quantitation of the relative expression of *Aimp1* in the uteri by semi-quantitative RT-PCR. The ANOVA analysis was used to calculate *P* values. $^{*}p < 0.005$. (C) Relative protein expression of Aimp1 in the uterus at different stages during the estrous cycle by western blot analysis. Total protein extract from uteri at each stage of estrous cycle (proestrus, estrus, metestrus, diestrus) was used for western blot analysis using Aimp1 antibody (Aimp1). The β-actin antibody (β-actin) was used for loading control. (D) Quantitation of the relative expression of Aimp1 in the uteri by ChemiDoc. The ANOVA analysis was used to calculate *P* values. $^{*}p < 0.005$.

2.2. Determination of the estrous cycle and uterus sampling

Stages of the mouse estrous cycle were distinguished by morphology of epithelial cell of vaginal smear assay (Fig. 2). Sterile PBS (phosphate buffered saline) was gently flushed into the mouse vagina using pipettes. The lavage was smeared on the glass slides, stained with Giemsa stain (Sigma-Aldrich, USA). The stains were examined with microscopy and then the stages of the estrous cycles were determined by cytological features as described by Mettus and Rane (2003). Proestrus stage showed equal number of both leukocytes and nucleated epithelial cells (Fig. 2A). Estrus stage showed 75% nucleated epithelial cell and 25% non-nucleated epithelial cells (Fig. 2B). Metestrus stage showed increased number of leukocytes with epithelial cells (Fig. 2C). Diestrus stage showed that leukocytes were predominant cells (Fig. 2D).

Uteri at each stage of the estrous cycle were collected from adult female ICR mice with regular estrous cycles. Tissue was frozen in liquid nitrogen and stored at –80 °C (for RNA extraction and protein extraction), or fixed in 10% formalin solution and embedded in paraffin block for histological and immunostaining analysis.

2.3. Histology and immunostaining

Uteri collected from mice were fixed in 10% formalin for histology and immunostaining. After embedding in paraffin, paraffin sections (5 μm thick) were cut and put on Superfrost Plus Stain slides (Fisher Scientific, USA). Deparaffinization was performed as follows: wash slides three times for 5 minutes (min) each in xylene (Biosesang, Korea) using glass jars and then wash two times for 5 min in 100% ethanol (Merck, Germany), two times for 5 min in 95% ethanol, one time for 5 min in 70% and 50% ethanol, followed by washing slides two times for 5 min in distilled water. For hematoxylin staining, deparaffinized slides were stained in 250 ml of Harris hematoxylin solution (Cat. No. HHS128, Sigma-Aldrich) for 5 min and washed in running tap water for 5 min. The slides were rehydrated with series of ethanol and xylene followed by covering with coverslip with Permount Mounting Medium (Fisher Scientific). For immunofluorescent assay, deparaffinized slides were placed into slide rack and put in the container with antigen retrieval buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). The containers were placed in the microwave and heated with full power for 5 min. After boiling, the slides were reheated with 50% power for 5 min. The reheated slides were allowed to cool at room temperature for 15 min and then washed three times for 5 min in distilled water. Using the ImmEdge pen (Vector Labs, USA), a hydrophobic barrier was drawn around each tissue section and then slides were washed three times for 5 min in PBS buffer. The slides were put into a container with a permeabilization buffer (0.2% Triton X-100 in PBS) for 45 min at room temperature and then washed in PBS. The slides were tapped off excess PBS onto paper towel, and put in a humidified chamber followed by adding some blocking buffer (PBS with 10% BSA). The humidified chamber was closed off and incubated for 1 h at room temperature. The blocked slides were washed three times for 5 min in PBS. The rabbit polyclonal antibody against Aimp1 was