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Differential expression and anti-oxidant function of glutathione peroxidase 3 in mouse uterus during decidualization

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ABSTRACT

Glutathione peroxidase 3 (GPX3) is an important member of antioxidant enzymes for reducing reactive oxygen species and maintaining the oxygen balance. *Gpx3* mRNA is strongly expressed in decidual cells from days 5 to 8 of pregnancy. After pregnant mice are treated with GPX inhibitor for 3 days, pregnancy rate is significantly reduced. Progesterone stimulates *Gpx3* expression through PR/HIF1α in mouse endometrial stromal cells. In the decidua, the high level of GPX3 expression is closely associated with the reduction of hydrogen peroxide (H_2O_2). Based on our data, GPX3 may play a major role in reducing H_2O_2 during decidualization.

1. Introduction

Early pregnancy in mammals is vulnerable to stressors [1]. Reactive oxygen species (ROS) is involved in the pathophysiology of infertility and assisted fertility [2,3]. ROS is deleterious by-products of aerobic metabolism, including superoxide ($O_2^{\cdot-}/HO_2^{\cdot}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^{\cdot}$) [4]. In a healthy body, ROS and antioxidants remain in a balance. Oxidative stress is a disturbance in the pro-oxidant–antioxidant balance [5]. It is estimated that up to 2% of the oxygen consumed by mitochondria is partially reduced to form $O_2^{\cdot-}$, which is subsequently converted to H_2O_2 [6].

In the presence of high H_2O_2 concentration, catalase is most effective for metabolizing H_2O_2. However, the glutathione system plays a critical role in the presence of low concentrations of either H_2O_2 or other peroxides [7]. The key enzyme in the redox cycle responsible for the reduction of H_2O_2 is GPX. The mammalian glutathione peroxidase family consists of 8 classes (i.e., *Gpx1–Gpx8*) [8]. Glutathione peroxidase 3 (GPX3) belongs to the selenocysteine-containing GPX family and is a main antioxidant enzyme in plasma for scavenging ROS derived from normal metabolism or oxidative insult [9]. GPX3 attenuates oxidant stress by reducing H_2O_2 and organic hydroperoxides to their corresponding alcohols [10].

GPX3 is one of the key enzymes in the cellular defense against oxidative stress [11]. *GPX3* expression is down-regulated both in rat and human endometrial adenocarcinoma, regardless of tumor grade or histopathological subtype [11]. *GPX3* is one of the up-regulated genes expressed in receptive phase of human endometrium, which could represent a useful prognostic tool for selecting IVF patients [12]. Compared to natural cycles, *GPX3* expression shows a delay in controlled ovarian stimulation (COS) cycles [13]. Based on our preliminary analysis, *Gpx3* is highly expressed in mouse decidua from days 5 to 8 of pregnancy, suggesting that *Gpx3* may play a role during decidualization. This study was to examine the expression, regulation and function of *Gpx3* during decidualization.

2. Materials and methods

2.1. Animal treatments

Mature mice (CD1 strain) were maintained in a controlled environment (14 h light and 10 h dark cycle). All animal procedures

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were approved by the Institutional Animal Care and Use Committee of South China Agricultural University. Uteri under pseudopregnancy, delayed implantation and activation, steroid hormonal treatments and deciduoma were collected as described previously [14]. Day 1 is the day of vaginal plug.

2.2. In situ hybridization

Total RNAs from the mouse uterus on day 8 of pregnancy were reverse transcribed and amplified with primers 5′-AGAAAGGAGATGTGAACG and 5′-TAGAATGACTGGGAATGTG (367 bp, NM_001083929). The amplified PCR fragment was recovered from the agarose gel and cloned into pGEM-T plasmid. The cloned fragment of *Gpx3* was further verified by sequencing. The preparation of digoxigenin-labeled cRNA probes and in situ hybridization of *Gpx3* mRNA in mouse uteri was performed as described previously [15].

2.3. Isolation of mouse endometrial stromal cells and in vitro decidualization

Mouse endometrial stromal cells were isolated from day 4 pregnant uteri as previously described [16]. The cell pellets were resuspended in DMEM/F-12 (Sigma) with 2% heat-inactivated FBS (Gibco). Cells were plated onto 35-mm culture dishes at the concentration of 1×10^6 cells/well. After an initial culture for 1 h, the medium was changed to remove free floating cells. In vitro decidualization of endometrial stromal cells was performed as previously described [17].

2.4. Isolation of human endometrial stromal cells and in vitro decidualization

Human endometrial samples were collected from normally cycling women undergoing hysterectomy or endometrial biopsy with written informed consent. All human procedures were approved by the Institutional Committee on the Use of Human Subjects in Medical Research of Bailu Hospital (Xiamen, China). Human endometrial stromal cells were isolated as described previously [18]. In vitro decidualization of human endometrial stromal cells was performed as previously described [19].

2.5. Transfection of Gpx3 siRNA

Transfections of *Gpx3* small interfering RNA (siRNA) were performed according to Lipofectamine 2000 protocol (Invitrogen).

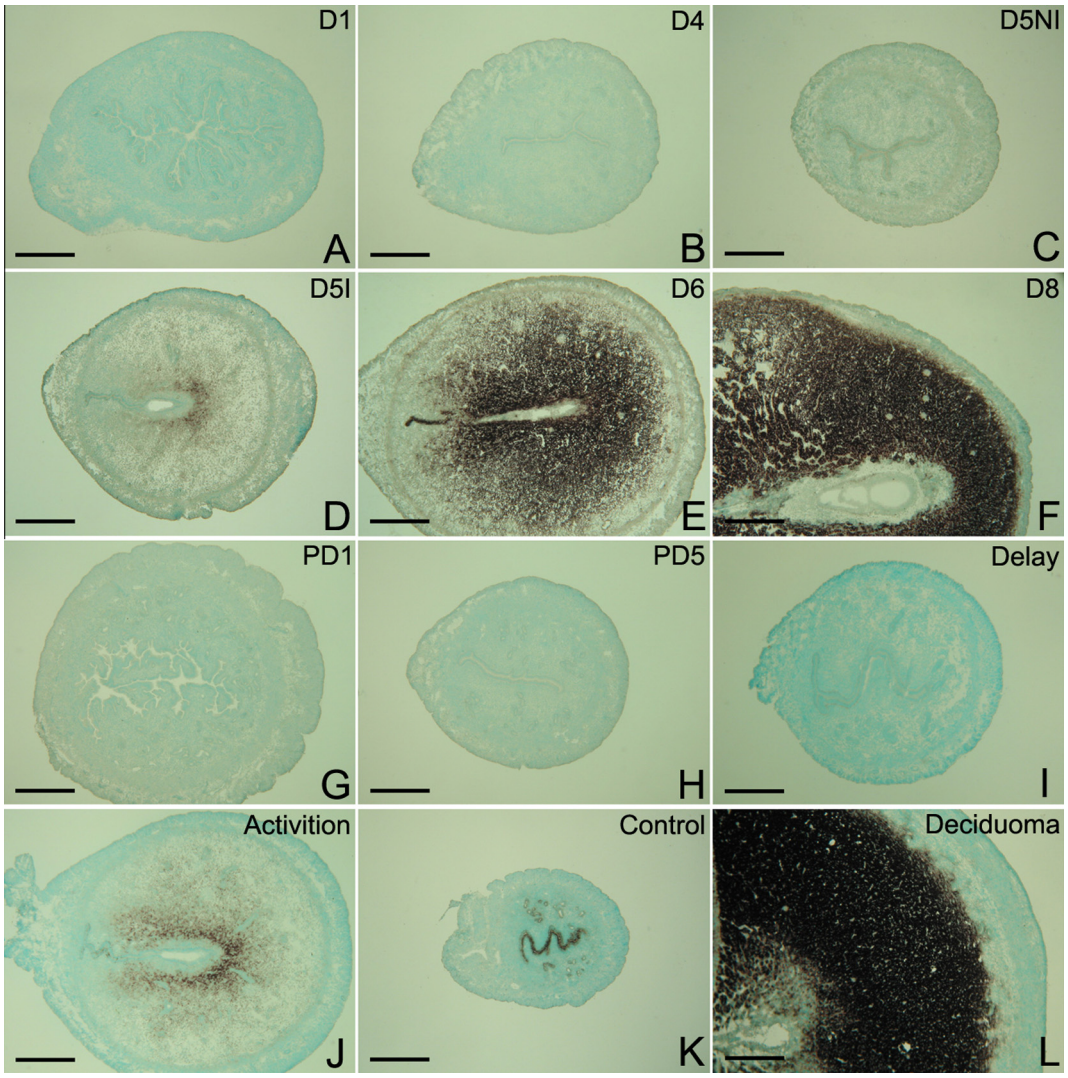

Fig. 1. In situ hybridization of *Gpx3* mRNA expression in mouse uteri. (A) Day 1 of pregnancy; (B) Day 4 of pregnancy; (C) Inter-implantation site on day 5 of pregnancy (D5NI); (D) Implantation site on day 5 of pregnancy (D5I); (E) Day 6 of pregnancy; (F) Day 8 of pregnancy; (G) Day 1 of pseudopregnancy (PD1); (H) Day 5 of pseudopregnancy (PD5); (I) Delayed implantation; (J) Activation of delayed implantation by estrogen; (K) Uninjected uterine horn for control (Control); (L) Uterine horn under artificial decidualization (Deciduoma). Bar = 80 μm.