Indoleamine 2,3-dioxygenase and trophoblast invasion in caesarean scar pregnancy: implications for the aetiopathogenesis of placenta accreta spectrum

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Abstract

Immunohistochemical localisation of indoleamine 2,3-dioxygenase was studied in order to better understand the pathophysiology of placenta accreta spectrum. In the decidua staining for indoleamine 2,3-dioxygenase was found in the glandular epithelium with some additional positive cells. Extravillous cytotrophoblast invasion was present in the myometrium which was not covered by the decidual tissue whereas myometrial invasion of cytotrophoblasts was absent where this tissue lay deep to decidua. These results suggest that indoleamine 2,3-dioxygenase expression in the decidua may normally control trophoblast invasion and absence of its expression where decidua is absent may be involved in the pathogenesis of the over-invaded placenta.

Keywords: indoleamine 2,3-dioxygenase (IDO), trophoblast invasion, decidua, caesarean scar pregnancy, placenta accreta spectrum (PAS)

Abbreviations: IDO, indoleamine 2,3-dioxygenase; PAS, placenta accreta spectrum

1. Introduction

It is well recognised that a caesarean delivery is one of the predisposing factors for placental pathologies including placenta accreta spectrum (PAS) in subsequent pregnancies. Placental implantation at the site of a previous caesarean scar is an extremely serious complication of pregnancy in which trophoblasts invade into the myometrial layer, resulting in PAS. In normal implantation, trophoblast invasion into the maternal tissue is thought to be controlled by mechanisms in the decidual layer (Jauniaux et al., 2018). Factors responsible for regulating the extent of trophoblast invasion are poorly understood (Jauniaux and Burton, 2018). Immune cells, including macrophages and uterine natural killer cells, colonize the decidua and have been thought to be involved in the control of trophoblast invasion (von Rango et al., 2001).

The enzyme indoleamine 2,3-dioxygenase (IDO), widely expressed in a variety of tissues of mammals, catalyses the oxidative cleavage of the essential amino acid L-tryptophan (Yamazaki et al., 1985). One tissue with particularly high activity is the human placenta (Yamazaki et al., 1985). In vitro experiments demonstrated that IDO expressed by macrophages have the potential to actively induce apoptosis in extravillos cytotrophoblast cells by IDO mediated tryptophan depletion (Reister et al., 2001). They suggested tryptophan depletion by IDO expressed in decidual macrophages in vivo also

may be involved in inducing apoptosis and controlling trophoblast invasion (Reister et al., 2001).

In this study we redefine in vivo IDO localisation using a rare early pregnancy sample from a woman at 9 weeks of gestation who underwent hysterectomy for caesarean scar pregnancy. Caesarean scar pregnancy may be a model for studying pathophysiology/pathoaetiology of PAS. Hysterectomised specimen of caesarean scar pregnancy can preserve the detailed structure of abnormal trophoblast invasion and thus we believe that examination of caesarean scar pregnancy specimen may provide an important information of PAS pathophysiology/pathoaetiology. Here, we demonstrated some characteristics of caesarean scar pregnancy, which may be of use in further clarifying PAS mechanism. However, we do not know if caesarean scar pregnancy represents all PAS and the present data was based on a single case. Although further study is needed, this case thus sheds light on the aetiopathogenesis of PAS.

2. Material and methods

2.1. Tissue collection and processing

The specimen was collected from an early human pregnancy that included the entire uterus removed for medical reasons with 9 weeks of gestation in situ. The patient was diagnosed with caesarean scar pregnancy and chose surgical treatment with hysterectomy. She consented to have her surgical specimen used for research, and the uterus and the pregnancy tissues were removed intact. Freshly dissected tissues were fixed in neutral buffered formalin, embedded in paraffin and four micrometres sections were cut onto adhesive slides. Collection and study were approved by the Ethics Review Committee of Graduate School of Biomedical Sciences, Hiroshima University (reference number; Hi-222, date of approval; 14 October 2018).

2.2. Immunohistochemistry

Sections were heated at 60℃ for 15 minutes and then standard deparaffinisation with xylene was performed. Antigen retrieval was carried out using an electric kettle at 98℃ for 40 minutes in 0.2 M citrate buffer at pH6.0. Inactivation of endogenous peroxidase activity was performed in 0.3% H_2O_2/methanol at room temperature for 20 minutes. Sections were blocked with horse serum (for mouse

antibodies) or donkey serum (for rabbit antibodies) at room temperature for 30 minutes. Primary antibodies were diluted as IDO1 (#86630: CST; 1/4000), HLA-G (ab7759: abcam; 1/3000) and Rabbit-isotype control (#3900: CST; depending on Abs concentration). Sections were incubated with primary antibodies overnight at 4℃. Slides were washed three times in PBS and exposed to biotinylated antibodies at room temperature for 30 minutes, then to the streptavidin-HRP antibody at room temperature for an additional 30 minutes (VECTORSTAIN Elite ABC HRP kit: PK-6102, PK-6101: VECTOR Laboratories, Burlingame, CA, USA or Streptavidin-Biotin Complex Peroxidase kit: 30462-30: nakalai tesque, Kyoto, Japan). Colour development utilised DAB (Peroxidase Stain DAB kit: 25985-50: nakalai tesque, Kyoto, Japan) and routine haematoxylin staining was performed at room temperature for 5 minutes.

3. Results and discussion

Serial sections were stained with antibodies to IDO and HLA-G with rabbit immunoglobulin as the primary antibody control. Figure 1A shows the section of an implantation site at the previous caesarean scar. This section includes the placental-decidual interface as well as myometrial tissue where in some areas decidual tissue was disrupted between placental villous tissue and myometrium (i.e. at the site of caesarean scar pregnancy). IDO immunoreactivity was seen to be strongly expressed on the glandular epithelium in the decidua. Some IDO positive cells were also seen in the decidual stroma which are probably macrophages (Figure1B, i). In the myometrium IDO immunoreactivity could not be observed. HLA-G positive extravillous cytotrophoblast cells were observed in the decidua (Figure1B, i) and in the myometrium where decidual tissue is absent (Figure1B, iii). In contrast HLA-G positive extravillous cytotrophoblast cells could not be found in the myometrium covered with decidual tissue (Figure1B, i).

During implantation cytotrophoblast cells invade across the maternal decidua as interstitial extravillous cytotrophoblast. In early pregnancy there is a particularly dense infiltrate of activated T cells and natural killer cells in the decidua having a different phenotype from those in peripheral blood (King et al., 1998). It has been

speculated that at the invasion front the invading trophoblast may be recognised by these leukocytes which are responsible for preventing the over invasion of foetal cells, possibly by induction of apoptosis (von Rango et al., 2001). It is therefore possible to speculate that in caesarean scar pregnancy the absence of activated T cells and natural killer cells because of lack of decidual tissue may be the reason for the extensive pathological invasion of the myometrium that usually results in an over-invaded placenta.

IDO is a widely distributed enzyme that catabolises the essential amino acid tryptophan and is primarily induced by interferon-γ, but other pro-inflammatory stimulants are also effective (Taylor and Feng, 1991). It has been suggested that IDO-mediated tryptophan depletion by macrophages induces apoptosis of extravillous trophoblast cells in the decidua, thereby limiting over invasion into the myometrium (Reister et al., 2001). In the present study we mapped IDO expression using a rare pregnancy sample which we obtained from hysterectomy for caesarean scar pregnancy in situ. Obvious expression of IDO was seen on the glandular epithelium and on CD68 positive (data not shown) macrophages in the decidua. HLA-G positive extravillous cytotrophoblast cells were observed only within the decidua and they did not invade the myometrium. However extravillous cytotrophoblast cells obviously invaded the

myometrium at a site of caesarean scar where decidual tissue was not present. From these results it is possible to suggest that IDO expressed in the decidua may be involved in controlling trophoblast invasion.

Central regulatory cytokines of IDO expression such as IFN-γ and TNF-α have been shown to be secreted by activated T cells and natural killer cells in the decidua of early human pregnancy (King et al., 1998). The local concentration of tryptophan which is controlled by the extent of IDO expression may thus control the differentiation and function of extravillous cytotrophoblast. This is obviously of relevance to the possible regulatory mechanisms of extravillous cytotrophoblast invasion at the site of implantation. In this context the absence of IDO expression at the implantation site due to the absence of decidual tissue (caesarean scar pregnancy) may be responsible for the abnormal trophoblast invasion in this pathological situation. We feel that the data we describe here allows us to add IDO to the list of mechanisms causing abnormal placental implantation.

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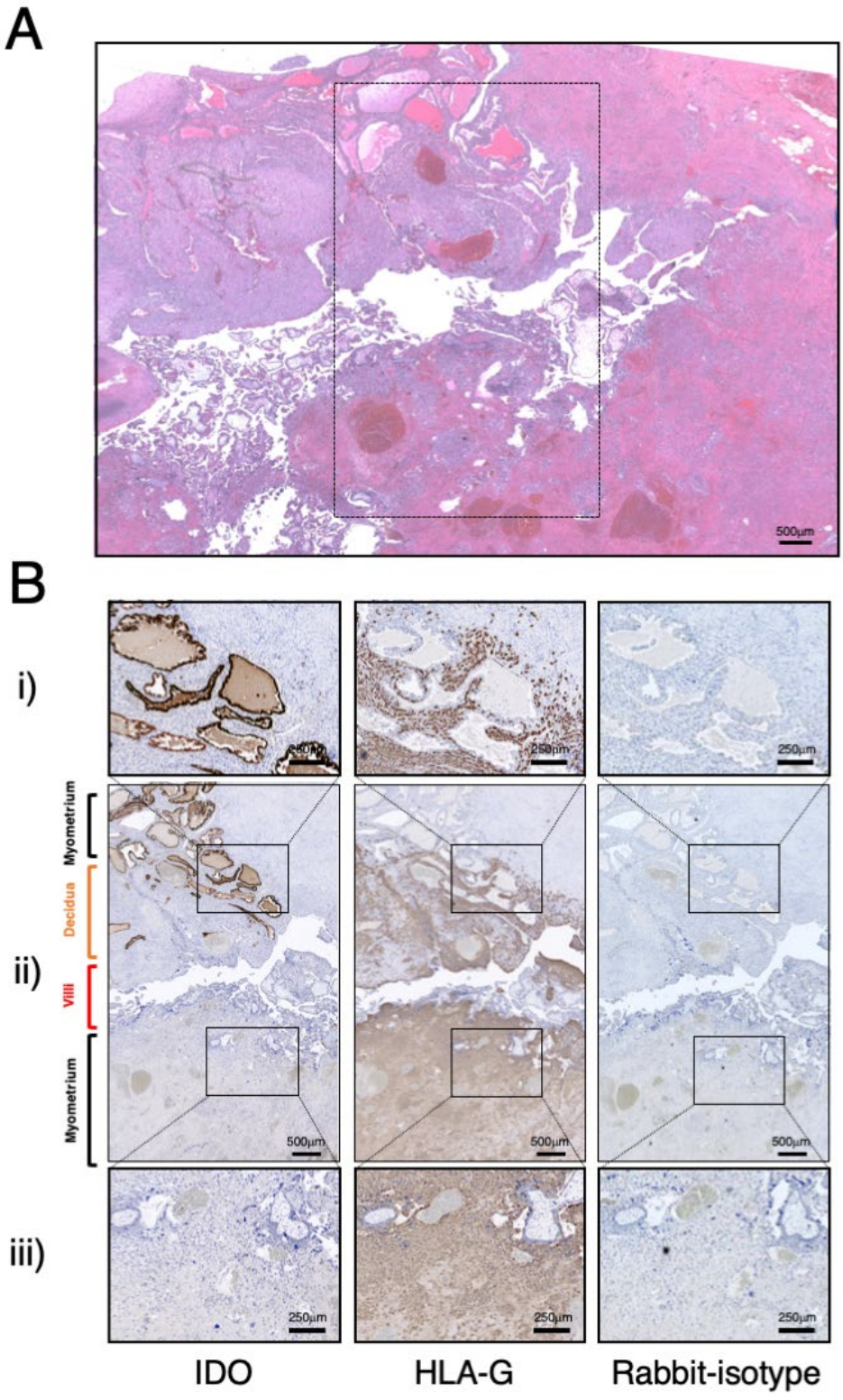
Figure legend

Figure 1. Immunohistochemical localisation of indoleamine 2,3-dioxygenase in human decidua.

Immunohistochemical analysis of a tissue sample from a woman at a nine weeks of gestation who underwent elective hysterectomy for caesarean scar pregnancy. (A) HE staining. Scale bars represent 500 micrometres, (B) Immunostaining for indoleamine 2,3-dioxygenase (IDO), HLA-G and rabbit-isotype control were performed. The panel i) shows area with decidual tissue. The panel ii) shows the same area of the specimens depicted in Figure 1A. The panel iii) shows areas of the myometrium containing a previous caesarean scar without decidual tissue. Scales for images are as indicated.


A
500μm
B
i)
ii)
iii)
Myometrium
Decidua
Villi
Myometrium
250μm
500μm
IDO
HLA-G
Rabbit-isotype