Placenta 81 (2019) 1–8

Contents lists available at ScienceDirect

Placenta

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

Forkhead box P3 is selectively expressed in human trophoblasts and decreased in recurrent pregnancy loss

Xiaohui Hu[a], Yan Wang[b], Gil Mor[a,c], Aihua Liao[a,*]

[a] Institute of Reproductive Health, Center for Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, PR China
[b] Department of Obstetrics and Gynecology, Maternal and Child Health Hospital of Hubei Province, Wuhan, PR China
[c] C.S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, MI, USA

ARTICLE INFO

Keywords:
Foxp3
Trophoblasts
Invasion
Pregnancy
Placenta

ABSTRACT

Introduction: Forkhead box P3 (Foxp3) is necessary for induction of the immunosuppressive functions in regulatory T cells. Recent data indicate that some non-lymphoid cells, such as certain tumor cells, also express Foxp3, which participates in tumorigenesis and is related to the invasive and proliferative behavior of the tumor. However, the expression of Foxp3 in trophoblasts has not been studied previously.

Methods: Localization of Foxp3 in the villi and decidua in the first trimester was determined using immunohistochemistry. Foxp3 expression was detected by flow cytometry, and the expression levels in the first trimester placenta were compared with those in the term placenta. Additionally, the Foxp3 expression in trophoblasts in recurrent pregnancy loss (RPL) was analyzed by real-time polymerase chain reaction (RT-PCR), immunohistochemistry and Western blot.

Results: Foxp3 is expressed by trophoblasts in early pregnancy in the villi and decidua. Foxp3 is expressed in the nuclei of both trophoblast cell columns and cytotrophoblasts. However, low Foxp3 expression was observed in syncytiotrophoblasts. Foxp3 was also expressed by the extravillous trophoblasts in early pregnancy in decidua. The Foxp3 expression in trophoblasts in the term placenta was significantly decreased compared with that in the normal early pregnancy. Moreover, decreased levels of Foxp3 mRNA and protein were observed in women with RPL.

Discussion: The selective expression of Foxp3 in human trophoblasts suggest that Foxp3 expression may be associated with the proliferation and invasion behavior of trophoblasts. The decreased Foxp3 expression in the trophoblasts in RPL may contribute to better understanding of the pathological characteristics of RPL.

1. Introduction

Forkhead box protein 3 (Foxp3) is a nuclear transcription factor which is necessary for the induction of the immunosuppressive functions in regulatory T (Treg) cells [1].

It is well known that Treg cells play an important role in regulation of immune responses to self-antigens, allergens, and commensal microbiota as well as immune responses to infectious agents and tumors [1]. Transcriptional factor Foxp3 has been considered restricted to Treg cells and some populations of activated T cells [2]. However, recent studies indicate that Foxp3 is also expressed in some non-lymphoid cells including normal epithelial cells and cancer cells of multiple lineages and of various tissue origins [3,4]. It should be noted that the biological functions of Foxp3 in the tumor cells and their significance remain a matter of debate because of the contradictory research results. An association between increased Foxp3 expression levels in the tumor cells and poor prognosis has been reported [5–7]. However, Foxp3 was demonstrated to be a tumor suppressor gene acting as a transcriptional repressor of the breast cancer oncogenes including the S-phase kinase protein 2 (Skp2) [8] and human epidermal growth factor receptor 2 (HER2) [9]. The role of Foxp3 in tumorigenesis is conflicting and both tumor-promoting and tumor-suppressive functions were described; however, the studies suggest that the expression of Foxp3 in the tumor cells plays a significant role in tumorigenesis.

The formation of a tumor is a pathological process characterized by uncontrolled growth of malignant cells. However, placenta development is a highly modulated physiologic process that involves growth, proliferation and invasion of the trophoblasts. Tumorigenesis and placentation are different although they share similar processes including cell growth and invasion, neovascularization, and immunotolerance.

---

* Corresponding author. Institute of Reproductive Health, Center for Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, No. 13 Hangkong Rd, Wuhan, 430030, PR China.
E-mail address: aihua_liao@sina.com (A. Liao).

https://doi.org/10.1016/j.placenta.2019.04.003
Received 19 January 2019; Received in revised form 21 March 2019; Accepted 9 April 2019
0143-4004/

Trophoblasts participate in the early formation and development of placenta including implantation in the uterine wall under extremely harsh conditions, such as extreme hypoxia (~1–2% oxygen), lack of a vasculature supply, and potential immune attacks from the maternal immune system. Similar to the trophoblasts, the tumor cells can invade the normal tissue by establishing their own vasculature and defending against the host immune response. Both cell types are able to successfully survive and grow [10–12].

Considering the role of Foxp3 expression in the tumor cells and in tumorigenesis, it is tempting to speculate that Foxp3 may play a similar role in trophoblasts. To the best of our knowledge, the expression of Foxp3 by trophoblasts at the human maternal-fetal interface has not been studied previously. Therefore, we investigated the localization and expression of Foxp3 and its potential function to evaluate a possible role of Foxp3 expressed by trophoblasts in human pregnancy.

2. Material and methods

2.1. Human tissues

The collection and use of the samples was approved by Huazhong University of Science and Technology Clinical Trial Ethics Committee [IEC (S035)]. Every participant signed a written informed consent form. First-trimester (between 6 and 9 weeks of gestation) placentae were obtained from voluntary pregnancy termination (terminated for non-medical reasons) and recurrent miscarriage (diagnosed as recurrent spontaneous abortion excluding the cases resulting from endocrine, anatomic, and genetic abnormalities, infection, etc.); the third-trimester placentae (between 37 and 41 weeks of gestation) were obtained from the natural delivery. A total of 64 women with NP (elective termination or natural delivery) and 24 women with RPL who had two or more miscarriages. All sample information and application are presented in Tables I and II (supplementary material).

2.2. RNA extraction and RT-PCR analysis

Total RNA was isolated from the frozen tissues of normal preganncy and recurrent pregnancy loss by TRIzol extraction (Invitrogen, Carlsbad, CA, USA). Quantified RNA (1 μg) was reverse transcribed to cDNA with Prime Script RT master mix (Takara Bio, Shiga, Japan). Relative mRNA levels of Foxp3 and β-actin determined by quantitative RT-PCR (qRT-PCR) with SYBR Premix Ex TaqII (Takara Bio). For PCR analysis, the following primers were used (5′-3′): β-actin forward, CTACCTCATGAAGATCCTCACCGA; β-actin reverse, TTCTCCTTAATGT CACGCACGATT; Foxp3 forward, ACCTGGAAGAACGCCATC; and Foxp3 reverse, TGTTCGTCCATCCTCCTTTC.

2.3. Western blot analysis

Frozen villi from normal pregnancy and recurrent pregnancy loss were ground and lysed for 1 h on ice by incubation in the lysis buffer containing protease inhibitors (1:100) (Thermo Fisher Scientific, MA, USA). Cell lysate was centrifuged for 15 min at 15,000 g. Protein was quantified and 40 μg was loaded on and separated through 10% Tris–glycine gels. The protein was transferred to the polyvinylidene difluoride membranes. The membranes were incubated in the blocking solution (5% nonfat milk in 20 mM Tris–HCl, 150 mM NaCl, and 0.1% Tween-20) (TBS-T) followed by the incubation with the antibodies (Foxp3 rabbit mAb at 1:1000 dilution) (Abcam, MA, USA) at 4 °C overnight. The membranes were washed in TBS-T and incubated with a peroxidase -conjugated secondary antibody (diluted 1:10000) for 2 h at room temperature. Antibody detection was performed by enhanced chemiluminescence reaction.

2.4. Immunohistochemistry (IHC)

The placental tissue was fixed in 4% paraformaldehyde, embedded in paraffin and sectioned at a thickness of 5 μm. The sections were deparaffinized in xylene and rehydrated in graded alcohol solutions. After antigen retrieval, the slides were treated with 3% hydrogen peroxide for 15 min to suppress endogenous peroxidase activity and blocked with 5% Bovine Serum Albumin (BSA) (Thermo Fisher Scientific, MA, USA) for 30 min, as previously described [13]. Samples were incubated with a Foxp3 antibody (rabbit monoclonal; diluted 1:100) (Abcam, MA, USA) at 4 °C overnight followed by incubation with a secondary antibody (Abcam, MA, USA) (diluted 1:200) at room temperature for 2 h. Images were acquired and analyzed with a microscope (Leica AF CTR6500HS).

2.5. Isolation of trophoblasts from the early pregnancy villi

Villus tissue (between 6 and 8 weeks of gestation) was washed in cold phosphate-buffered saline (PBS) to clarify the solution and to remove excess blood. Then, the tissue was digested with 2.5% trypsin (Gibco, Grand Island, NY, USA) for 10 min at 37 °C. After the digestion, pre-warmed fetal bovine serum was added to stop the trypsinization process. Then adding culture media to the tissue until the final volume is between 30 ml and 40 ml depending on the amount of tissue. Vortexing the mixture for 20 s and then allow the tissue to sediment. After sedimenting, the supernatant will be centrifuged to collect pellet. The pellet digested cells were carefully layered over the lymphocyte separation medium (GE Healthcare Life Sciences, Marlborough, MA, USA) and centrifuged at 800 g for 30 min with the centrifuge brake turned off. The white band of the cells was collected with a transfer pipet and placed into a conical tube.

2.6. Preparation of placental cells

Placental tissue was sliced and digested in RPMI 1640 medium (Sigma, Oakville, ON, Canada) supplemented with collagenase type IV (1.0 mg/ml) (Gibco) for 1 h at 37 °C with gentle agitation. The suspension was filtered through an 80-mm mesh strainer into a fresh medium.

2.7. Flow cytometry analysis

The cell surface of isolated trophoblasts or placental cells was stained with an appropriate fluorescently labeled conjugated mAb for 20 min away from light. Cells were washed three times with cold PBS. Then, the cells were fixed and labeled according to the manufacturer's protocol for intracellular Foxp3 and CK7 staining. For intracellular staining, the cells were fixed and permeabilized using a Fix/Perm kit (eBioscience, San Diego, CA, USA). The antibodies used in this study included FITC-conjugated mAbs against CK7 (Merck, Temecula, CA, USA), PE-conjugated mAbs against HLA-G and Foxp3 (BioLegend, San Diego, CA, USA), APC-conjugated mAbs against CD45 (BioLegend), and PE-CY7-conjugated mAbs against Foxp3 (eBioscience). Flow cytometry was performed using a FACScalibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed with the CellQuest program (Becton Dickinson, Franklin Lakes, NJ).

2.8. Statistical analysis

GraphPad Prism software version 6.0 (GraphPad, San Diego, CA, USA) was used for data analysis. Initial normality tests were used to determine if all data sets fit to the normal Gaussian distribution. Statistical analysis was performed using Mann-Whitney U test. Values of $P < 0.05$ were considered statistically significant. Data are presented as the mean ± SEM.

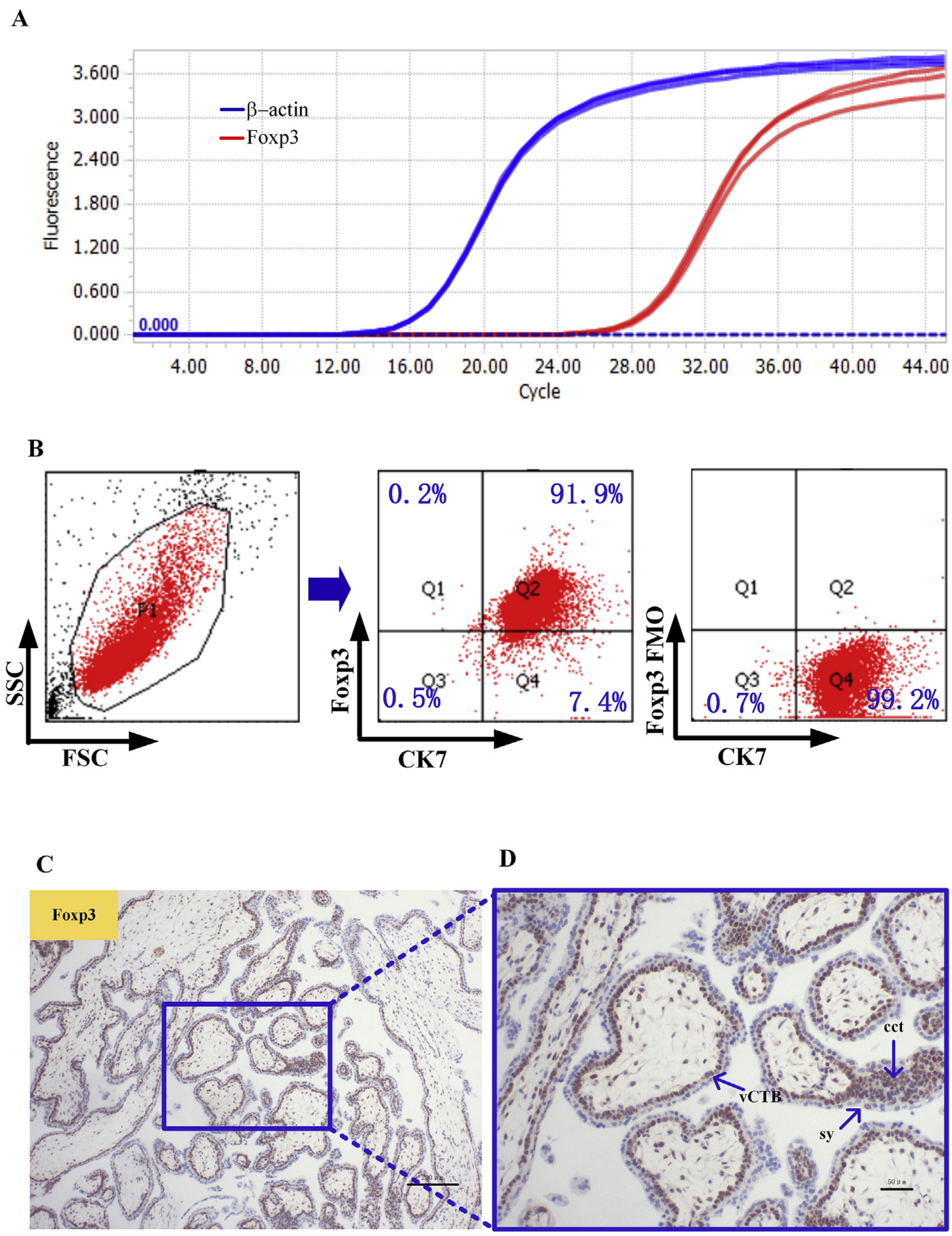

Fig. 1. Gene and protein expression of Foxp3 in the first trimester villi. RNA extracted from the villi was subjected to quantitative real-time RT-PCR using primers specific for Foxp3 and β-actin. In (A), fluorescence intensity was plotted versus cycle number. (B) Representative flow cytometry analysis of freshly isolated first trimester villus trophoblasts stained with mAbs to CK7 and Foxp3. Dot plots for CK7-FITC and Foxp3-PE staining of the trophoblasts. In (C), the Foxp3 protein was analyzed by immunohistochemical staining of the normal first trimester tissues from voluntary pregnancy termination using rabbit *anti*-Foxp3 monoclonal antibody. In (D), a higher magnification of the zone delimited by the frame in C is shown. Note that while cytotrophoblasts and trophoblast cell columns are strongly positive for Foxp3, most syncytiotrophoblasts remain negative as shown by the arrow. vCTB, villous cytotrophoblast; sy, syncytiotrophoblast; CCT, cell column trophoblast. Magnification: × 40 in C; × 100 in D.

3. Results

3.1. Foxp3 expression in trophoblasts in the early pregnancy villus tissue

We investigated the *Foxp3* gene expression in the villus tissue using RT-PCR. *Foxp3* mRNA was detected in the early pregnancy villi (Fig. 1A). Treg cells are absent in the floating villi and thus, we hypothesized that all detected Foxp3 mRNA comes from trophoblasts [14]. To confirm that trophoblasts express Foxp3, we isolated trophoblasts from the early pregnancy floating villi. Cells prepared from human first-trimester floating villi were directly stained for cytokeratin-7 (CK7, the trophoblast marker expressed by extravillous trophoblasts and villous trophoblasts) [15] and Foxp3 expression was determined by flow cytometry (FCM). FCM analysis demonstrated that 99% of the cells isolated from the floating villi were CK7-positive (Fig. 1B). Nearly 90% of the CK7-positive cells were Foxp3-positive. Thus, these data confirmed that Foxp3 is expressed in trophoblasts in early pregnancy.

To investigate the localization of Foxp3 in the villi, Foxp3 expression was analyzed by immunohistochemistry. Floating placental villi are composed of an inner layer of the cytotrophoblasts and an outer layer of the syncytiotrophoblasts that are continuously generated by the asymmetrical cell division, differentiation and fusion of the inner layer cytotrophoblasts within the developing syncytium. As shown in Fig. 1C and D, immunohistochemical analysis revealed significant Foxp3 expression in the villi in early pregnancy. Foxp3 was mainly expressed in the nuclei of both trophoblast cell columns and cytotrophoblasts while most syncytiotrophoblasts remain negative. However, it is somewhat of a puzzle that some positive staining is present in the villous connective tissue.

3.2. Foxp3 expression in trophoblasts in the decidua in early pregnancy

Villi connected to the decidua give rise to the proliferative cell columns which generate differentiated extravillous trophoblasts (EVT). These EVTs gradually replace the vascular epithelial cells thus remodeling the spiral artery into the larger conduits to deliver the low-pressure, high blood flow to the growing fetus. We detected a large amount of fusiform-shaped Foxp3-positive trophoblasts present in the decidua in early pregnancy. These Foxp3-expressing trophoblasts were replacing the endothelial cells of the spiral artery (Fig. 2C–E) and vein

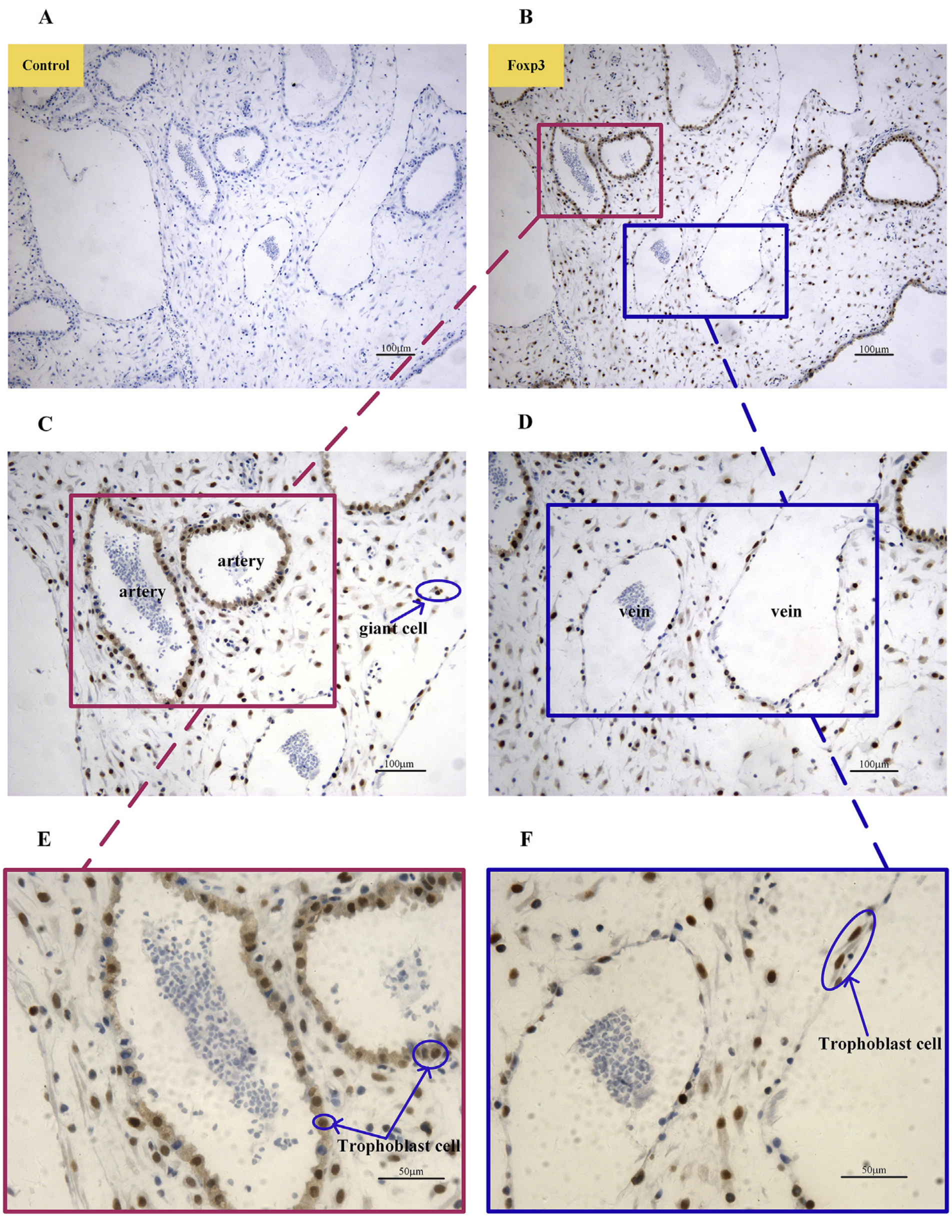

Fig. 2. Foxp3-expressing trophoblasts invading decidua and vessels. Sections from the first trimester decidua were analyzed by immunohistochemistry with control rabbit Abs (A) and rabbit polyclonal *anti*-Foxp3 Abs (B, C, D, E, F). In (C) and (D), a higher magnification of the zone delimited by the red frame and blue frame in B is shown, respectively; In (E) and (F) are higher magnification captures of regions from (C) and (D), respectively. The Foxp3-expressing trophoblast cells (brown) are replacing the endothelial cells of the spiral artery and vein. Foxp3 is also expressed in the multinuclear giant cells as shown by the arrow. Magnification: × 100 in A and B; × 200 in C and D; and × 400 in E and F.

(Fig. 2D–F). Foxp3 is also detected in the giant cells which are the end stage of invasive differentiation of interstitial cytotrophoblasts (Fig. 2C) [16].

3.3. *Decreased Foxp3 expression in trophoblasts in the third trimester*

To explore the possible clinical significance of the Foxp3 expression in trophoblasts, we analyzed the samples of placentae of the first trimester (between 6 and 9 weeks of gestation) and third trimester (between 37 and 41 weeks of gestation) donors.

Expression of CK7 (the trophoblast marker), human leukocyte antigen-G (HLA-G, the EVT maker), CD45 (the common leukocyte antigen marker) and Foxp3 was simultaneously examined by flow cytometry. $CD45^-CK7^+$ cells were defined as villus trophoblasts and $CD45^-HLA\text{-}G^+$ cells were defined as EVT [17]. Flow cytometric analysis revealed significantly lower Foxp3 expression in $CD45^-CK7^+$ trophoblasts from the third trimester compared with that in the cells from the first trimester ($P < 0.01$, Fig. 3). Moreover, a slight decrease in Foxp3 expression in $CD45^-HLA\text{-}G^+$ cells, albeit not statistically significant, was observed in the third trimester ($P > 0.05$, Fig. 4).

3.4. *Foxp3 expression in trophoblasts is impaired in the recurrent pregnancy loss*

To further investigate the potential role of Foxp3 in placental development and successful pregnancy, we analyzed the Foxp3 expression in placentae from the normal pregnant subjects and the patients who suffered from recurrent pregnancy loss (RPL). As shown in Fig. 5A, the levels of Foxp3 mRNA in the RPL patients were lower than that in the normal pregnant women ($P < 0.05$). Furthermore, reduced Foxp3 protein levels were found in the RPL villi (Fig. 5B and C). Consistently, immunohistochemical staining showed lower Foxp3 expression in the cytotrophoblasts and the cell columns in RPL (Fig. 5D and E). These data suggest that the reduced expression levels of Foxp3 in trophoblasts may be associated with abnormal early pregnancy.

4. Discussion

In the current study, we demonstrated that the transcription factor Foxp3 is expressed in human villi and decidua. The profile of Foxp3 expression in trophoblasts is schematically represented in Fig. 6. The

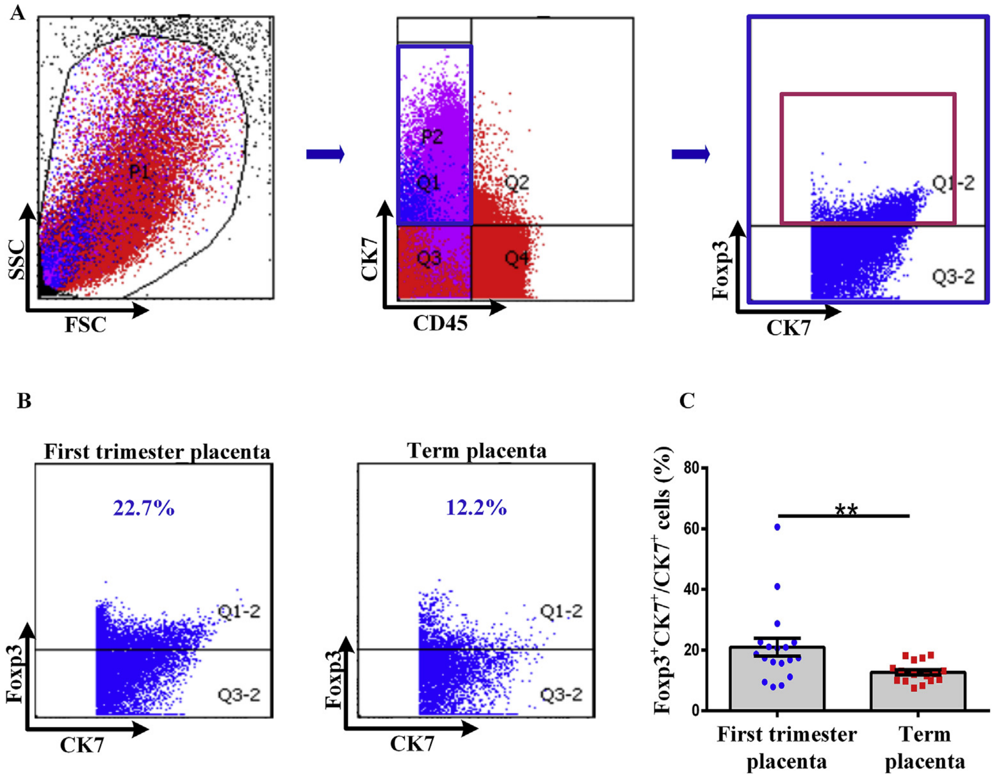

Fig. 3. The Foxp3 expression in the CK7^{+}CD45^{-} trophoblasts from the first trimester and third trimester placenta. Representative flow cytometry analysis of isolated placental cells from the first trimester (n = 18) and term placentae (n = 18) stained with mAbs to CD45, CK7 and Foxp3. (A) A live gate (P1) was set in the forward-side scatter plot and the representative density plots showing the analysis of Foxp3 expression in CD45^{-}CK7^{+} trophoblasts (P2) were delineated. (B) The percentages of Foxp3^{+}CK7^{+} trophoblasts from the first trimester placenta were compared with that from the term placenta. (C) Summary of the data showing the frequency of Foxp3 expression in the CD45^{-}CK7^{+} trophoblasts. Data are presented as the mean ± SEM. $^{**}P < 0.01$.

villus is composed of various types of trophoblasts with specialized functions. The cytotrophoblast cells, which are located in the inner layer of the villi, proliferate and cover the mesenchyme and fetal vessels that form the placental villous tree. The syncytiotrophoblasts covering the surface of the floating villus are continuously generated by the fusion of the inner layer cytotrophoblasts. These syncytiotrophoblasts secrete numerous hormones, such as human chorionic gonadotropin, into maternal circulation and this secretion is required for maintenance and immunological adaptation of pregnancy. At the tip of villus, the cytotrophoblasts proliferate to form the cell columns which give rise to various invasive EVTs. These EVTs do not proliferate but invade the uterine wall and the uterine spiral arteries thus remodeling the placental vasculature to enable sufficient placental perfusion [18,19]. Abnormalities of EVT invasion, remodeling and cytotrophoblast gene

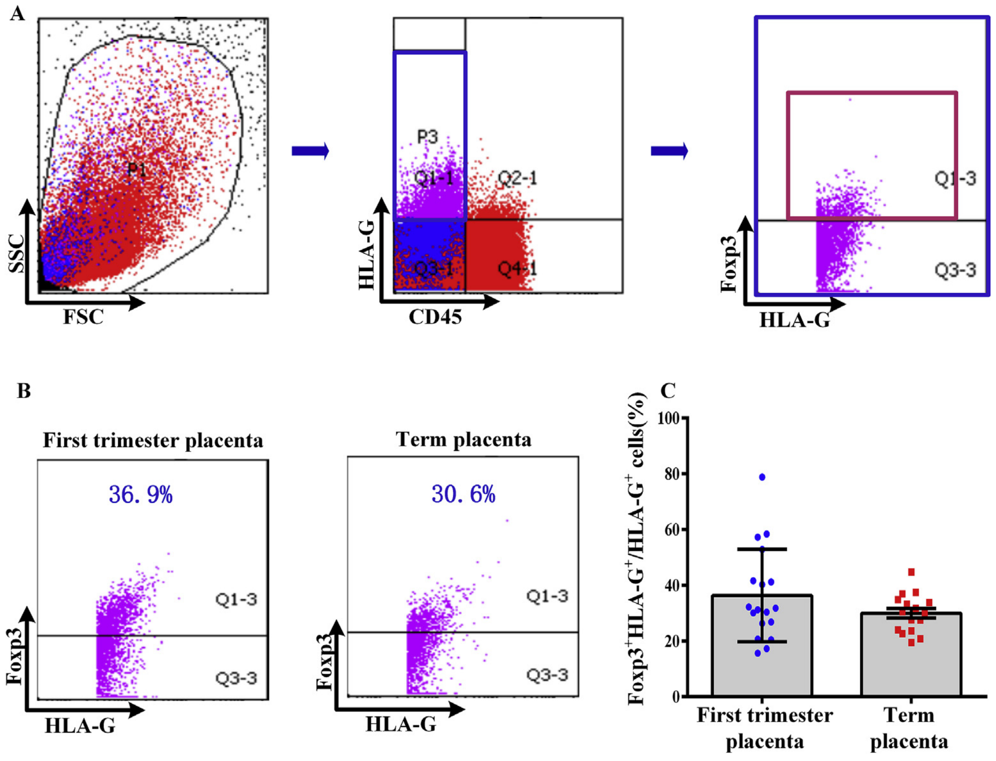

Fig. 4. The Foxp3 expression in the HLA-G^{+}CD45^{-} trophoblasts from the first trimester and third trimester placentae. Representative flow cytometry analysis of isolated placenta cells from the first trimester placenta (n = 18) and term placenta (n = 18) stained with mAbs to CD45, HLA-G and Foxp3. (A) A live gate (P1) was set in the forward-side scatter plot and the representative density plots showing the analysis of Foxp3 expression in CD45^{-}HLA-G^{+} trophoblasts (P3) were delineated. (B) The percentages of Foxp3^{+}HLA-G^{+} trophoblasts from the first trimester placenta and that from the term placenta were compared. (C) Summary of the data showing the frequency of Foxp3 expression in the CD45^{-}HLA-G^{+} extravillous trophoblasts. Data are presented as the mean ± SEM.

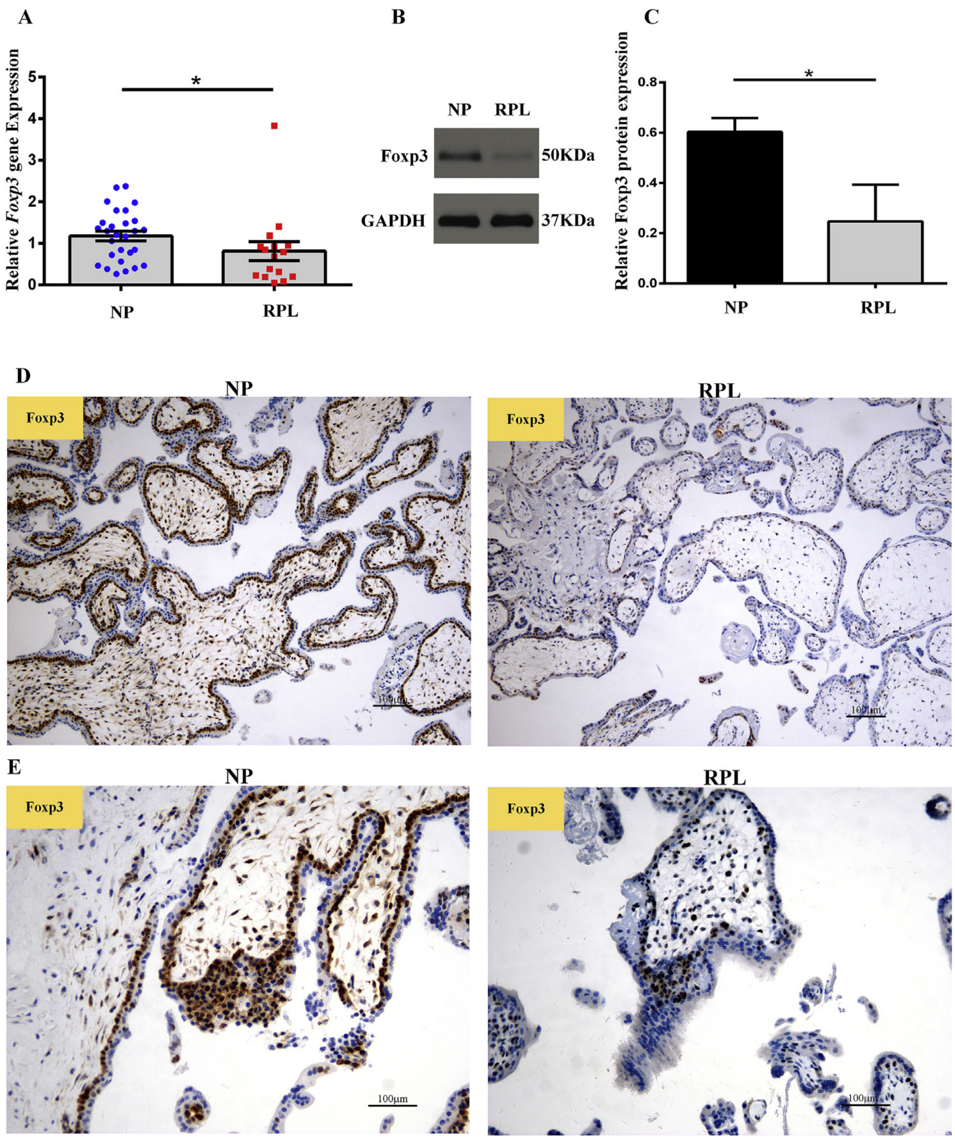

Fig. 5. Abnormal Foxp3 expression in trophoblasts from women with NP and RPL. Foxp3 gene and protein expression was significantly lower in the villi from patients who suffered from recurrent pregnancy loss (RPL) versus that in the normal pregnant (NP) subjects. (A) The mRNA levels of Foxp3 were decreased in the RPL group (n = 16) compared with that in the NP group (n = 28). β-Actin was used as an internal reference. Data are presented as the mean ± SEM. $^*P < 0.05$. (B, C) Foxp3 protein expression was detected by Western blot and GAPDH was used as a loading control. Data are presented as the mean ± SEM. $^*P < 0.05$. (D, E) Lower Foxp3 expression in the cytotrophoblasts and the cell columns in RPL compared with that in NP according to the data of immunohistochemistry. Magnification: × 100 in D; × 200 in E.

expression were observed in various pregnancy complications including RPL, preeclampsia and intrauterine growth restriction [20–22].

The data of immunohistochemistry revealed significant Foxp3 expression mainly in the nuclei of both trophoblast cell columns and cytotrophoblasts in the villi. Lower Foxp3 expression was observed in the syncytiotrophoblasts. Moreover, in the decidua in early pregnancy, Foxp3 is expressed by the fusiform-shaped interstitial EVTs, multinuclear giant cells and EVTs which are replacing the endothelial cells of the spiral artery and vein. In fact, Foxp3 expression has been reported in the mouse giant cells and the Rcho-1 cell line derived from rat placenta [23,24]. As mentioned above, columnar EVT detach from the cell columns, adhere to the extracellular matrix proteins, and migrate through decidua. The specific and selective Foxp3 expression in the cytotrophoblasts, the cell columns and the invasive EVTs suggested that Foxp3 expression may be associated with the proliferation and invasion behavior of trophoblasts.

Additionally, we found significantly lower Foxp3 expression in the $CD45^-CK7^+$ trophoblasts from the term placenta compared with that in the cells from the first trimester placenta. Current view is that proliferation and invasion of trophoblasts are very strong in the first trimester and are gradually decreasing as the gestation progresses [25]. Lower Foxp3 expression may partially explain the reduced proliferation and invasion of trophoblasts in the term placenta. Moreover, at the end of pregnancy there is a change in the immunological profile of the pregnant woman. The pro-inflammatory profile characterizes the third trimester, promoting delivery. Therefore, the decreased Foxp3 expression might promote the immunological profile shifting to pro-inflamatory at the end stage of pregnancy.

Zuo et al. [9] were the first to report that Foxp3 expressed by breast cancer cells is an important suppressor of human breast cancer; this finding initiated numerous studies about the association between Foxp3 expression and cancer prognosis. Studies of the Foxp3 expression in tumor cells indicate that Foxp3 plays an important role in proliferation, apoptosis and invasion of the tumor cells. Although the role of Foxp3 in tumorigenesis is conflicting and may involve tumor-promoting and tumor-suppressive functions, these studies led to the recognition that the expression of Foxp3 in the tumor cells is important in tumorigenesis. Considering the similarities in the proliferative, invasive, and immune tolerance mechanisms between the tumor cells and trophoblasts, Foxp3 expressed by trophoblasts may have a unique biological function in normal human pregnancy.

Shallow trophoblast invasion and incomplete spiral artery remodeling have been described in patients with RPL and preeclampsia. Our data of RT-PCR and Western blot analysis indicate that in RPL patients, Foxp3 expression is reduced in the villi indicating a possible clinical significance of Foxp3. Moreover, Foxp3 is expressed selectively

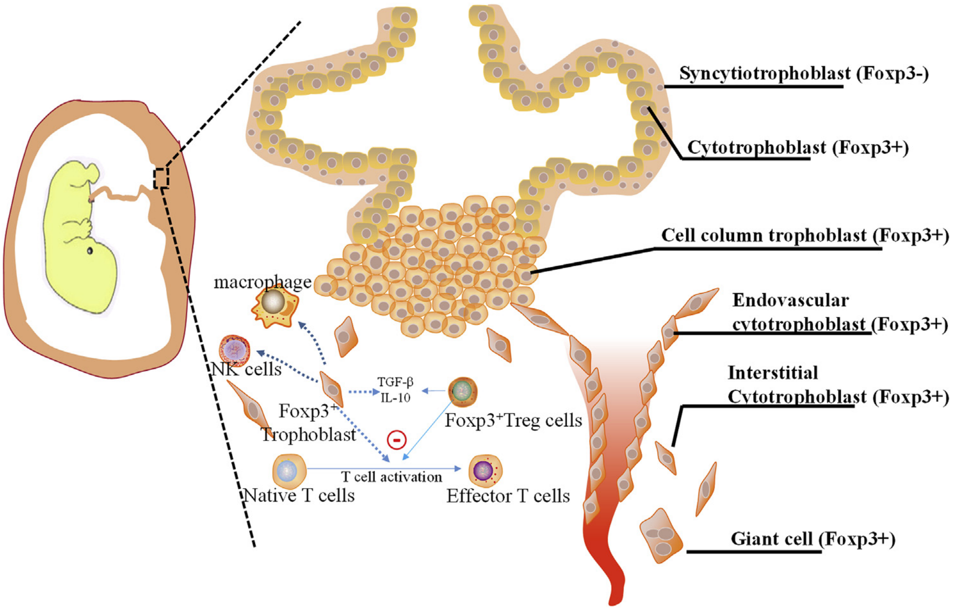

Fig. 6. Schematic diagram of Foxp3 expression in trophoblasts in the villi and decidua. The actively proliferating cytotrophoblasts form column cytotrophoblasts. Cytotrophoblasts at the distal portion of the column differentiate into various invasive extravillous trophoblast subtypes. Interstitial extravillous trophoblasts invade into the decidua and eventually differentiate into the placental bed giant cells at the final stage of the invasive differentiation pathway. Endovascular trophoblasts penetrate the uterine artery and vein and replace the vascular epithelial cells. Various immune cells, including NK cells, macrophages and T lymphocytes, communicate with the extravillous trophoblasts to promote the maternal-fetal tolerance. Foxp3 is expressed in the nuclei of cytotrophoblasts and proliferating cell columns. A large amount of fusiform-shaped Foxp3-expressing trophoblasts exist in the decidua. These Foxp3-expressing trophoblasts gradually replace the vascular epithelial cells. Foxp3-expressing trophoblasts may secrete immunosuppressive cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGF-β), similar to the Foxp3$^+$ regulatory T (Treg) cells, thus inhibiting the activation of naive T cells. Foxp3 expressed in trophoblasts may participate in the "education" of decidual immune cells. Dotted arrows are speculation requiring further experimental demonstration. Filled arrows are facts that have already been confirmed by other research group.

in the cytotrophoblasts and cell columns. These findings suggest that Foxp3 expression in trophoblasts may participate in trophoblast invasion and placental development. Besides, the abnormal Foxp3 expression might be associated with the dysregulated immune homeostasis in RPL patients. These issues need further investigation.

Foxp3 has been identified as a marker of CD4$^+$CD25$^+$ regulatory T (Treg) cells and is the key determinant of their immunomodulation functions. In normal pregnancy, Treg cells play the key role in the induction of tolerance to fetal antigens [26]. Considering the potent immunosuppressive function of Foxp3 in Treg cells, Foxp3 expressed in trophoblasts may have immunosuppressive effects similar to Treg cells and mimicking the functions of Treg cells may represent a novel mechanism of maternal-fetal tolerance. Mia Kim and colleagues have observed that the expression of Foxp3 in the colorectal cancer cells was significantly correlated with that of IL-10 and TGF-β, which are the key cytokines that help tumor cells to evade immune response [27]. Interestingly, IL-10 is also expressed in trophoblast cells that prevent maternal immunity attack [28,29]. However, it is not clear whether Foxp3 expression is directly attributed to the IL-10 secretion by trophoblasts. Moreover, a successful pregnancy involves a complex cross-talk between the fetal trophoblasts and maternal decidual immune cells that enable in utero development of the embryo and fetus. Trophoblasts act as a conductor that can educate the decidual immune cells that determine specialized composition and function of the maternal immune cells. Thus, the uterine environment is modulated to sustain a successful pregnancy [30]. It is not known whether the Foxp3 expression is associated with the "education" ability of trophoblasts. In fact, the proliferation of anti-CD3/anti-CD28-stimulated T cells can be strongly inhibited by the Foxp3-expressing pancreatic cancer cells [31]. Moreover, coculture of Foxp3-expressing melanoma cells with CD4$^+$CD25$^-$ T cells led to strong Foxp3-dependent inhibition of T-cell proliferation [32]. Therefore, Foxp3 expressed in trophoblasts may participate in the development and maintenance of maternal immune tolerance.

Further studies are needed to define the exact function of Foxp3 in normal and aberrant proliferation, invasion and differentiation of trophoblasts and to delineate the role of Foxp3 expressed in trophoblasts in the placental development and successful pregnancy. Moreover, Foxp3 cannot be considered a direct therapeutic target because of its crucial role in regulation of autoimmunity. Therefore, the molecules that regulate Foxp3 expression in trophoblasts rather than that in the Treg cells should be identified. The molecules regulating Foxp3 expression may provide a strategy for development of potential therapeutic targets other than Foxp3 itself.

In summary, we demonstrate for the first time that in addition to the Treg cells, Foxp3 is also expressed in human trophoblasts. Moreover, abnormal Foxp3 expression in trophoblasts may be associated with occurrence of RPL.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Dr. Zhihui Li and Dr. Yonghong Zhang from Tongji Medical College, Huazhong University of Science and Technology for their valuable comments on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.04.003.

Funding

This study was supported by research grants from the National Natural Science Foundation of China (No. 81871186) and National key R&D Program of China (2018YFC1003904).

References

[1] A.Y. Rudensky, Regulatory T cells and Foxp3, Immunol. Rev. 241 (1) (2011) 260–268.
[2] J.D. Fontenot, J.P. Rasmussen, L.M. Williams, J.L. Dooley, A.G. Farr, A.Y. Rudensky, Regulatory T cell lineage specification by the Forkhead transcription factor Foxp3, Immunity 22 (3) (2005) 329–341.
[3] G.Y. Chen, C. Chen, L. Wang, X. Chang, P. Zheng, Y. Liu, Cutting edge: broad expression of the FoxP3 locus in epithelial cells: a caution against early interpretation of fatal inflammatory diseases following in vivo depletion of FoxP3-expressing cells, J. Immunol. 180 (8) (2008) 5163–5166.

[4] F. Martin, S. Ladoire, G. Mignot, L. Apetoh, F. Ghiringhelli, Human FOXP3 and cancer, Oncogene 29 (29) (2010) 4121–4129.

[5] F.I.D. Dimitrakopoulos, H. Papadaki, A.G. Antonacopoulou, A. Kottorou, A.D. Gotsis, C. Scopa, H.P. Kalofonos, A. Mouzaki, Association of FOXP3 expression with non-small cell lung cancer, Anticancer Res. 31 (5) (2011) 1677–1683.

[6] A. Merlo, P. Casalini, M.L. Carcangiu, C. Malventano, T. Triulzi, S. Menard, E. Tagliabue, A. Balsari, FOXP3 expression and overall survival in breast cancer, J. Clin. Oncol. 27 (11) (2009) 1746–1752.

[7] M.E. Winerdal, P. Marits, M. Winerdal, M. Hasan, R. Rosenblatt, A. Tolf, K. Selling, A. Sherif, O. Winqvist, FOXP3 and survival in urinary bladder cancer, BJU Int. 108 (10) (2011) 1672–1678.

[8] T. Zuo, R. Liu, H. Zhang, X. Chang, Y. Liu, L. Wang, P. Zheng, Y. Liu, FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2, J. Clin. Investig. 117 (12) (2007) 3765–3773.

[9] T. Zuo, L. Wang, C. Morrison, X. Chang, H. Zhang, W. Li, Y. Liu, Y. Wang, X. Liu, M.W. Chan, FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene, Cell 129 (7) (2007) 1275–1286.

[10] K.D. Beaman, M.K. Jaiswal, G.K. Katara, A. Kulshreshta, S. Pamarthy, S. Ibrahim, J. Kwak-Kim, A. Gilman-Sachs, Pregnancy is a model for tumors, not transplantation, Am. J. Reprod. Immunol. 76 (1) (2016) 3–7.

[11] S.G. Holtan, D.J. Creedon, P. Haluska, S.N. Markovic, Cancer and pregnancy: parallels in growth, invasion, and immune modulation and implications for cancer therapeutic agents, Mayo Clin. Proc. 84 (11) (2009) 985–1000.

[12] H. Zhao, M. Ozen, R.J. Wong, D.K. Stevenson, Heme oxygenase-1 in pregnancy and cancer: similarities in cellular invasion, cytoprotection, angiogenesis, and immunomodulation, Front. Pharmacol. 5 (2015) 295.

[13] P. Tantbirojin, C.P. Crum, M.M. Parast, Pathophysiology of placenta creta: the role of decidua and extravillous trophoblast, Placenta 29 (2008) 639–645.

[14] Y.P. Wang, S. Zhao, Vascular Biology of the Placenta, first ed., Morgan & Claypool Life Sciences, San Rafael, 2010.

[15] L. Ji, J. Brkić, M. Liu, G. Fu, C. Peng, Y.L. Wang, Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia, Mol. Aspect. Med. 34 (5) (2013) 981–1023.

[16] J. Pollheimer, V. Fock, M. Knöfler, Review: the ADAM metalloproteinases–Novel regulators of trophoblast invasion? Placenta 35 (2014) S57–S63.

[17] T. Tilburgs, Â.C. Crespo, A. van der Zwan, B. Rybalov, T. Raj, B. Stranger, L. Gardner, A. Moffett, J.L. Strominger, Human HLA-G + extravillous trophoblasts: immune-activating cells that interact with decidual leukocytes, Proc. Nati. Acad. Sci. U S A. (2015) 201507977.

[18] S.K. Gupta, S.S. Malhotra, A. Malik, S. Verma, P. Chaudhary, Cell Signaling pathways involved during invasion and syncytialization of trophoblast cells, Am. J. Reprod. Immunol. 75 (3) (2016) 361–371.

[19] M. Knöfler, J. Pollheimer, Human placental trophoblast invasion and differentiation: a particular focus on Wnt signaling, Front. Genet. 4 (2013) 190.

[20] G. Fu, G. Ye, L. Nadeem, L. Ji, T. Manchanda, Y. Wang, Y. Zhao, J. Qiao, Y.L. Wang, S. Lye, MicroRNA-376c impairs transforming growth factor-β and nodal signaling to promote trophoblast cell proliferation and invasion, Hypertension 61 (4) (2013) 864–872.

[21] B. Huppertz, Placental origins of preeclampsia, Hypertension 51 (4) (2008) 970–975.

[22] M. Langbein, R. Strick, P.L. Strissel, N. Vogt, H. Parsch, M.W. Beckmann, R.L. Schild, Impaired cytotrophoblast cell-cell fusion is associated with reduced Syncytin and increased apoptosis in patients with placental dysfunction, Mol. Reprod. Dev. 75 (1) (2008) 175–183.

[23] L.R. Guerin, J.R. Prins, S.A. Robertson, Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? Hum. Reprod. Update 15 (5) (2009) 517–535.

[24] A.C. Zenclussen, K. Gerlof, M.L. Zenclussen, S. Ritschel, A. Zambon Bertoja, S. Fest, S. Hontsu, S. Ueha, K. Matsushima, J. Leber, H.D. Volk, Regulatory T cells induce a privileged tolerant microenvironment at the fetal-maternal interface, Eur. J. Immunol. 36 (1) (2006) 82–94.

[25] N. Ishihara, H. Matsuo, H. Murakoshi, J. Laoag-Fernandez, T. Samoto, T. Maruo, Changes in proliferative potential, apoptosis and bcl-2 protein expression in cytotrophoblasts and syncytiotrophoblast in human placenta over the course of pregnancy, Endocr. J. 47 (3) (2000) 317–327.

[26] A. Schumacher, A.C. Zenclussen, Regulatory T cells: regulators of life, Am. J. Reprod. Immunol. 72 (2) (2014) 158–170.

[27] M. Kim, T. Grimmig, M. Grimm, M. Lazariotou, E. Meier, A. Rosenwald, I. Tsaur, R. Blaheta, U. Heemann, C.T. Germer, Expression of Foxp3 in colorectal cancer but not in Treg cells correlates with disease progression in patients with colorectal cancer, PLoS One 8 (1) (2013) e53630.

[28] D.T. Rein, M. Breidenbach, B. Hönscheid, U. Friebe-Hoffmann, H. Engel, U.J. Göhring, L. Uekermann, C.M. Kurbacher, T. Schöndorf, Preeclamptic women are deficient of interleukin-10 as assessed by cytokine release of trophoblast cells in vitro, Cytokine 23 (4) (2003) 119–125.

[29] I. Roth, D.B. Corry, R.M. Locksley, J.S. Abrams, M.J. Litton, S.J. Fisher, Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10, J. Exp. Med. 184 (2) (1996) 539–548.

[30] M. PrabhuDas, E. Bonney, K. Caron, S. Dey, A. Erlebacher, A. Fazleabas, S. Fisher, T. Golos, M. Matzuk, J.M. McCune, Immune mechanisms at the maternal-fetal interface: perspectives and challenges, Nat. Immunol. 16 (4) (2015) 328–334.

[31] S. Hinz, L. Pagerols-Raluy, H.-H. Oberg, O. Ammerpohl, S. Grüssel, B. Sipos, R. Grützmann, C. Pilarsky, H. Ungefroren, H.D. Saeger, G. Klöppel, D. Kabelitz, H. Kalthoff, Foxp3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer, cancer, Res. 67 (17) (2007) 8344–8350.

[32] J. Niu, C. Jiang, C. Li, L. Liu, K. Li, Z. Jian, T. Gao, Foxp3 expression in melanoma cells as a possible mechanism of resistance to immune destruction, Cancer Immunol. Immunother. 60 (8) (2011) 1109–1118.