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Role of Platelets in Placentation

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1

2 Abstract

3

4 In human placenta, embryo-derived trophoblasts aggressively invade maternal spiral
5 arteries and transform the arteries to low-resistant large-caliber vessels. This process that
6 ensures adequate placental perfusion is called maternal vascular remodeling. Histological
7 examination showed deposition of maternal platelets in the trophoblast aggregates formed in
8 the spiral arteries. Several lines of evidence suggest that these platelets are activated.
9 Soluble factors released from the activated platelets, as whole, enhanced invasive capacity of
10 isolated trophoblasts in vitro. These findings suggest importance of non-hemostatic platelet
11 function in maternal vascular remodeling. In contrast, gene knockout studies suggest that
12 maternal platelet defects are compatible with successful pregnancy in mice. Moreover,
13 pregnant women with severe platelet defects usually fulfill uneventful pregnancy. Thus,
14 promotion of endovascular trophoblast infiltration by maternal platelets might not be the only
15 mechanism that regulates maternal vascular remodeling.

16 The maternal vascular remodeling is an essential component of human reproduction and
17 should be secured by several complementary mechanisms. Future studies should aim to
18 elucidate other mechanisms that could regulate endovascular trophoblast infiltration.

19

1 Introduction

2

3 In human placenta, cytotrophoblasts show two distinct patterns of differentiation (Figure 1).

4 In floating villi, cytotrophoblasts differentiate into syncytiotrophoblast and form the syncytial
5 layer, where exchange of gas and nutrients takes place. On the other hand, at
6 villus-anchoring sites, cytotrophoblasts differentiate into extravillous trophoblasts and form
7 the stratified structure called cell column. After losing proliferative activity and acquiring
8 invasive activity in the cell column ¹, extravillous trophoblasts begin to invade decidual tissue
9 (interstitial trophoblasts) or maternal blood vessels (endovascular trophoblasts).

10 Endovascular trophoblasts destroy the muscular linings and replace the endothelium of the
11 maternal spiral arteries, transforming them from small resistant vessels to flaccid large-caliber
12 vessels. This process is called maternal vascular remodeling. The maternal vascular
13 remodeling ensures adequate placental perfusion and contributes to establishment of
14 successful pregnancy ². In fact, endovascular trophoblast invasion is limited to superficial
15 decidua and the myometrial segments remain narrow in cases of preeclampsia and/or
16 intrauterine growth restriction ³.

17 Vascular infiltration by endovascular trophoblast is a unique phenomenon in primate
18 placenta ⁴. Moreover, in most of non-human primates, vascular remodeling is restricted to
19 the decidua, which in human has to be considered as pathological. Lack of proper
20 experimental model animal has hampered analysis of endovascular trophoblast invasion and
21 the mechanism is still largely unknown.

22

1 Platelet in human placentation

2

3 What directs extravillous trophoblasts towards the maternal spiral arteries? It is intuitive

4 to consider that some factor(s) derived from the endothelium or blood constituents direct this

5 movement. Relatively high oxygen tension in maternal arteries promotes trophoblast

6 differentiation toward invasive phenotype, which could be one of the mechanisms that

7 facilitate endovascular trophoblast invasion ⁵. This hypothesis is derived from the findings

8 that isolated cytotrophoblasts cultured under hypoxic conditions (2% O₂) continued

9 proliferating, whereas those cultured in 20% O₂ stopped proliferating and differentiated to

10 invasive phenotype. Another candidate that facilitates endovascular trophoblast invasion

11 could be maternal platelets ⁶. Histological examination of human placental bed revealed that

12 maternal platelets are trapped by endovascular trophoblast aggregates that are formed inside

13 the lumen of the spiral arteries. These platelets were attached to collagen deposited around

14 endovascular trophoblasts, suggesting that these platelets are activated. Indeed, these

15 platelets expressed P-selectin. P-selectin is one of activation markers of platelets that binds

16 to P-selectin glycoprotein ligand-1 (PSGL-1) on the surface of neutrophils, leading to

17 recruitment and activation of the neutrophils. The activated neutrophils, in turn, release

18 platelet-activating factor (PAF) that promotes platelet aggregation ⁷. In vitro, co-culturing

19 with platelets that were activated by collagen induced matrigel invasion of extravillous

20 trophoblasts isolated from early human placental tissue. After 48-hour culture, most of the

21 isolated extravillous trophoblasts exhibit elongated spindle-shaped morphology mimicking

22 interstitial trophoblasts, whereas they are transformed to round-shaped morphology

23 mimicking endovascular trophoblasts after 48-hour co-culture with platelets (Figure 2). The

24 round-shaped cells share similar molecular property with endovascular trophoblasts in vivo,

such as up-regulation of integrin $\alpha 1$ and CCR1^{8 9}. These changes do not require direct contact of platelets and isolated extravillous trophoblasts, suggesting that some soluble factor(s) derived from the activated maternal platelets direct trophoblast invasion towards maternal arteries and induce differentiation towards endovascular phenotype.

Then, what are the platelet-derived soluble factors that enhance trophoblast invasion? Besides regulating hemostasis, platelets contain a number of bioactive peptides, including growth factors (EGF, VEGF, PDGF-B, TGF- $\beta 1$, IGF-1, HGF, bFGF), cytokines (soluble CD40 ligand), chemokines (RANTES, β TG, PF4), and bioactive phospholipids (S1P). These mediators are stored in platelet granules and released upon stimulation¹⁰. The effect of platelet-conditioned medium to promote invasion of the isolated extravillous trophoblasts was completely abrogated by heat treatment, but not by charcoal stripping⁹. This suggests that some growth factors, cytokines, or chemokines, but not bioactive phospholipids contribute to the invasion-promoting effect of platelets. Immunocytochemistry revealed that isolated extravillous trophoblasts express a chemokine receptor, CCR1. Matrigel invasion assay revealed that RANTES, one of CCR1 ligands, enhances invasion of isolated extravillous trophoblasts, indicating that CCR1 expressed on isolated extravillous trophoblasts is a functional receptor. Interestingly, immunohistochemistry of the placental bed showed that CCR1 is predominantly expressed from the cell column through endovascular trophoblasts. These findings suggest that circulating maternal platelets are trapped by endovascular trophoblast aggregates and activated to release CCR1 ligands, which in turn attracts CCR1-positive extravillous trophoblasts into maternal spiral arteries to encourage the vascular remodeling (Figure 3).

In this theory, activation of platelets in the spiral arteries requires the preexistence of endovascular trophoblast aggregates. In this respect, platelet is not a primary initiator of

- 1 extravillous trophoblast invasion into the spiral arteries; rather it might provide a positive
- 2 feedback mechanism that facilitates endovascular trophoblast infiltration.
- 3

1 Platelet in mouse placentation

2

3 Gene knockout has generated several mice lineages that have quantitative or qualitative
4 platelet defect. The transcription factor NF-E2 is essential for megakaryopoiesis and
5 NF-E2-deficient mice have severe quantitative platelet defect ^{11 12}. NF-E2-null murine
6 embryos are able to complete intrauterine life, although they show significant growth
7 restriction at birth. The growth restriction is associated with abnormal vascularization of the
8 labyrinthine layer in NF-E2-null placenta. Since the number of blood vessels in the
9 labyrinthine layer is not reduced, abnormal vascularization is considered to be due to failure
10 in the maturation of preexisting vessels. These indicate that embryonic platelets are required
11 for normal placentation ¹³.

12 In mice lacking α -subunit of the heterotrimeric guanine nucleotide binding protein Gq
13 ($G\alpha_q$), platelet count is normal but these platelets cannot be activated in vitro with
14 physiological agonists including thrombin, ADP, and collagen ¹⁴. In contrast to NF-E2 null
15 mice, vascularization of $G\alpha_q$ -null placenta is not affected, suggesting that hemostatic platelet
16 function is not required for normal placentation. In other words, non-hemostatic function of
17 platelets, which is only revealed in mice with quantitative platelet defect, is essential for the
18 maturation of the fetus-derived vessels in the placenta ¹³.

19 Both quantitatively and qualitatively platelet-deficient mice are able to complete
20 intrauterine development and some of them even survive to reproductive age. These adult
21 female mice allow analysis of possible effects of maternal platelet defect on placentation. In
22 contrast to NF-E2 null embryos, the litter size of NF-E2 null mothers is not reduced as long as
23 embryo has normal platelet count. Detailed analysis of the placental histology showed that
24 massive placental hemorrhage occurs in approximately half of the placentas of NF-E2 null

- 1 mothers most probably due to maternal bleeding diathesis ¹⁵. Thus, maternal platelet is not
- 2 required for normal murine placentation.
- 3
- 4

1 Platelet and human pathological pregnancies

2
3 Primary hemostasis begins immediately after endothelial disruption. The exposed
4 subendothelial collagen recruits circulating von-Willebrand factor, which binds to platelet
5 surface glycoprotein Ib-IX-V complex and thus mediates the contact between collagen and
6 platelets. Collagen-activated platelets form pseudopods that stretch out to cover the injured
7 surface and express receptors for fibrinogen that mediates primary platelet aggregation.
8 Next, bioactive substances such as thromboxane A₂ are released from the activated platelets
9 (platelet degranulation) and further activate surrounding platelets. These activated platelets
10 express glycoprotein IIb-IIIa complex that can bind to von-Willebrand factor. Secondary
11 platelet aggregation is mediated by interaction between von-Willebrand factor and
12 glycoprotein IIb-IIIa complex, leading to primary thrombus formation. In the secondary
13 hemostasis, coagulation cascade efficiently progresses on the phospholipid exposed on the
14 surface of the aggregated platelets to solidify the primary thrombus (secondary thrombus
15 formation).

16 Low-dose aspirin has been used worldwide to prevent placenta-mediated obstetric
17 complications such as recurrent pregnancy loss, intrauterine growth restriction, and
18 preeclampsia ¹⁶. Aspirin inhibits platelet cyclooxygenase to suppress synthesis of
19 thromboxane A₂ that induces secondary platelet aggregation and systemic vasoconstriction.
20 Thus, theoretically, aspirin can inhibit thrombus formation in the placenta to help maintain
21 placental blood flow as well as ameliorate hypertension in preeclampsia. In fact, recent
22 meta-analysis established moderate but consistent preventative effect of low-dose aspirin
23 therapy on preeclampsia ¹⁷. Since aspirin does not interfere with platelet adhesion to
24 collagen or subsequent platelet degranulation, platelet release of bioactive soluble factors

other than thromboxane A₂ should be maintained during low-dose aspirin therapy. Therefore, in terms of maternal vascular remodeling, aspirin does no harm to endovascular trophoblast invasion enhanced by platelet-derived soluble factors.

Secondary thrombus formation generally ensues after platelet activation. Given that maternal platelets trapped by the endovascular trophoblasts are activated, it seems peculiar that no fibrin deposition is detected among the endovascular trophoblast aggregates. Although the actual mechanism for this discrepancy remains to be elucidated, abundant thrombomodulin¹⁸ and tissue- and urokinase-type plasminogen activators^{19 20 21} expressed by trophoblasts could inhibit the coagulation cascade and/or facilitate rapid degradation of the fibrin at the surface of the endovascular trophoblasts. In pathological pregnancies such as preeclampsia, it is fascinating to consider that inhibitory mechanism against the secondary thrombus formation around the endovascular trophoblasts is defective, thus leading to excessive placental thrombosis. In this situation, aspirin could be particularly beneficial, because it can inhibit platelet secondary aggregation that triggers the initiation of the coagulation cascade without affecting endovascular trophoblast invasion.

Bernard-Soulier syndrome is characterized by deficiency of platelet glycoprotein Ib-IX-V complex that mediates contact of platelets with collagen²². In this pathological condition, collagen-activation of platelets is severely impaired and most of the patients exhibit spontaneous bruising, epistaxis, and bleeding after minor trauma. In pregnancy, entrapment of maternal platelets by collagen-deposited endovascular trophoblasts and the subsequent activation should be restricted, resulting in insufficient maternal vascular remodeling. However, pregnant women with Bernard-Soulier syndrome as well as those with congenital thrombocytopenia generally complete uneventful pregnancy despite an increased risk of postpartum hemorrhage²³⁻²⁵. This suggests that maternal platelets are not an essential

- 1 component of human placentation process including maternal vascular remodeling.
- 2 Although in vitro data suggest importance of maternal platelets in endovascular trophoblast
- 3 infiltration, their actual role in vivo still needs to be defined.
- 4

1 Conclusion and future direction

2

3 In human placenta, maternal platelets are deposited in trophoblast clusters that have
4 invaded the uterine spiral arteries. These platelets are likely to be activated and release
5 various soluble factors, which as whole enhance invasive capacity of extravillous trophoblasts
6 in vitro. Thus, maternal platelets might be a candidate that attracts extravillous trophoblasts
7 into the spiral arteries and encourages maternal vascular remodeling. As demonstrated in
8 gene-knockout mice with quantitative or qualitative platelet defects, however, maternal
9 platelets are not required for murine placentation. The fact that pregnant women with severe
10 platelet defects are compatible with uneventful pregnancy suggests that maternal platelets are
11 not an essential component of human placentation.

12 The maternal vascular remodeling that ensures adequate placental perfusion is an essential
13 component of human reproduction and should be secured by several complementary
14 mechanisms. In this respect, promotion of endovascular trophoblast infiltration by maternal
15 platelets could be one of the mechanisms that regulate the vascular remodeling, although the
16 vascular remodeling might occur in the absence of maternal platelets.

17 Precise characterization of endovascular trophoblasts, which is a crucial step for
18 clarification of the mechanism(s) of maternal vascular remodeling, has been hampered by the
19 extreme difficulty in their isolation from human placenta. As mentioned above, co-culture
20 with platelets could induce differentiation of isolated extravillous trophoblasts towards
21 endovascular phenotype in vitro. These endovascular-like trophoblasts are potentially useful
22 to characterize endovascular trophoblasts, leading to identification of novel mechanisms of
23 maternal vascular remodeling.

24

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- 17
- 18

1 Figure legend

2

3 Figure 1 Schematic representation of early human placenta.

4 In floating villus, cytotrophoblasts (light gray cells) differentiate into multinucleated
5 syncytiotrophoblast (dark gray cells) and form the syncytial layer, where exchange of gas and
6 nutrients takes place. At villus-anchoring sites, cytotrophoblasts differentiate into extravillous
7 trophoblasts (spotted cells) and form the stratified structure called cell column. Extravillous
8 trophoblasts acquire invasive activity in the cell column and begin to invade the decidual
9 tissue (interstitial trophoblasts) or the spiral arteries (endovascular trophoblasts).

10

11 Figure 2 Platelet-derived soluble factors induce endovascular differentiation of isolated
12 human extravillous trophoblasts.

13 Isolated human extravillous trophoblasts were cultured for 48 hours in the absence (A,
14 control) or in the presence of human platelets (B, platelet co-culture) that were plated in the
15 collagen type I-coated upper chamber. Note that most of extravillous trophoblasts exhibit
16 round-shaped morphology (arrows) mimicking endovascular trophoblasts after platelet
17 co-culture (B).

18

19 Figure 3 An illustration showing possible chemokine gradient produced by platelets in
20 spiral arteries and its estimated effects on endovascular trophoblast infiltration.

21 In the spiral artery undergoing vascular remodeling, platelets are deposited among the
22 endovascular trophoblasts. These platelets are likely to have been activated by extracellular
23 matrix secreted from endovascular trophoblasts and to have released various soluble factors
24 including CCR1 ligands, forming local chemokine gradient. The chemokine gradient directs

- 1 CCR1-positive extravillous trophoblasts into the spiral artery, providing a positive feedback
- 2 cascade for trophoblastic arterial infiltration. Note that CCR1 is expressed from the cell
- 3 column through endovascular trophoblasts.

Spiral artery under vascular remodeling

CCR1-positive
trophoblasts

Chemokine gradient

Platelet

Endothelial cell