Placenta 33, Supplement A, Trophoblast Research, Vol. 26 (2012) S55–S62

Contents lists available at SciVerse ScienceDirect

Placenta

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

IFPA Award in Placentology Lecture: Molecular regulation of human trophoblast invasion

M. Knöfler*, J. Pollheimer

Department of Obstetrics and Fetal-Maternal Medicine, Reproductive Biology Unit, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

ARTICLE INFO

Article history:
Accepted 30 September 2011

Keywords:
Human placenta
Endovascular trophoblast
Interstitial trophoblast
Invasion
Migration
Spiral artery remodelling
Growth factor
Signal transduction
Transcription factor
Cell column
EVT
Differentiation

ABSTRACT

Invasion of extravillous trophoblast cell types into maternal uterine tissues is essential for successful human placental development and progression of pregnancy. Whereas endovascular trophoblasts migrate into the maternal spiral arteries, interstitial trophoblasts invade the decidual stroma, colonize the vessels from outside and communicate with diverse uterine cell types such as decidual stromal cells, macrophages and uterine NK cells. For example, interstitial trophoblasts expressing polymorphic human leukocyte antigen-C interact with uterine NK cells through binding to their killer immunoglobulin-like receptors which likely plays a role in trophoblast invasion and reproductive success of pregnancy. Both extravillous trophoblast subtypes are critically involved in the vascular transformation of the spiral arteries into dilated conduits ensuring appropriate blood flow into the intervillous space. Failures in this remodeling process are thought to be associated with severe forms of fetal growth restriction, preeclampsia and other pregnancy complications warranting studies on the molecular regulation of extravillous trophoblast differentiation. Moreover, interstitial trophoblast-derived hormones may regulate diverse biological functions in the decidua. In particular, human chorionic gonadotrophin has been shown to promote angiogenesis and to suppress apoptosis of endometrial stromal cells. In return, decidual cells produce a plethora of soluble factors controlling trophoblast invasion in a time- and distance-dependent manner. However, the underlying mechanisms have not been fully elucidated. Here, we will summarize autocrine as well as paracrine factors regulating invasion of extravillous trophoblasts and discuss critical signaling cascades involved. In addition, we will focus on key regulatory transcription factors controlling cell column proliferation and differentiation of the human extravillous trophoblast.

1. Introduction

1.1. General aspects

Development of placental extravillous trophoblasts (EVT) migrating into maternal uterine tissue is fundamental to successful placentation and fetal outcome. The trophoblast invasion process starts early in pregnancy and continues until the 20th week of gestation. Within the first weeks endovascular trophoblasts migrate into the maternal spiral arteries and plug those vessels, likely to prevent precocious onset of maternal blood flow into the intervillous space [1]. Abnormal trophoblast invasion, incomplete vessel occlusion and a premature rise in oxygen levels are thought to damage the placental villi due to oxidative stress potentially resulting in the development of early pregnancy complications such as miscarriage [2].

Little is known about the factors controlling trophoblast invasion in early human gestation. However, it is likely that factors secreted from endometrial glands such as epidermal growth factor (EGF), vascular endothelial growth factor (vEGF), and various cytokines controlling placental and embryonic growth during the first trimester are also critically involved in early trophoblast differentiation processes [3]. Once the placenta switches from histiotrophic to hemotrophic nutrition, trophoblast plugs are dissolved and extensive remodeling of the spiral arteries within the decidual and the inner part of the myometrium takes place involving both endovascular and interstitial trophoblasts, the latter colonizing the arteries from outside [1]. The transformation process is characterized by disruption of the vascular wall and elastolysis and involves diverse coordinated interactions between vascular smooth muscle cells, uterine natural killer cell (uNK) cells and invasive trophoblasts [4]. Conversion of the vessels into dilated conduits is thought to reduce contractility, pressure and rate of blood flow into the intervillous space supporting a constant delivery of oxygen and nutrients to the developing fetus [2].

* Corresponding author. Tel.: +43 1 40400 2842; fax: +43 1 40400 7842.
E-mail address: martin.knoefler@meduniwien.ac.at (M. Knöfler).

0143-4004
doi:10.1016/j.placenta.2011.09.019

Shallow invasion and failures in the vascular transformation process are thought to be associated with the development of gestational diseases such as severe forms of intrauterine growth restriction and preeclampsia [5]. Fluctuations in oxygen concentrations could be an underlying cause since hypoxia and re-oxygenation of placental tissue likely provokes stress-mediated secretion of harmful cytokines into the maternal circulation, which may result in endothelial dysfunction and the clinical symptoms of preeclampsia such as elevated blood pressure [2]. Besides their role in spiral artery remodeling interstitial trophoblasts are known to interact with uNK cells upon binding of human leukocyte antigen-C to maternal killer immunoglobulin-like receptors, thereby likely modulating maternal immune responses and, as a consequence, placental development [6]. Moreover, invasive trophoblasts could provide chemotactic signals to uterine leukocytes [4], and affect decidual angiogenesis and apoptosis by secreting human chorionic gonadotrophin (hCG) [7]. Interstitial trophoblasts may also aggregate and partly fuse to form placental bed giant cells which is considered as the end-stage of extravillous trophoblast differentiation [8]. The specific functions of giant cells, however, as well as the molecular mechanisms triggering their formation are mostly unknown. The two different differentiation pathways of extravillous trophoblasts as well as their predominant functions are summarized in Fig. 1.

1.2. Invasive differentiation program of the anchoring villus

Since blood flow to the placenta has to be precisely regulated throughout pregnancy, oxygen has been considered as a main regulator of trophoblast proliferation and differentiation. High oxygen levels promote invasion, whereas low oxygen causes hypoxia-inducible factor 1α (HIF-1α)-dependent villous trophoblast proliferation in accordance with rapid growth of the placenta in early pregnancy [9]. However, the role of oxygen remains controversial since it cannot explain early endovascular trophoblast invasion and plugging of spiral arteries which in early pregnancy may occur in the absence of blood flow and oxygen [1]. Along those lines, increased invasiveness of trophoblasts under hypoxia has also been reported by different authors [10]. In addition, oxygen concentrations as well as diffusion distance in vitro are still under discussion [11].

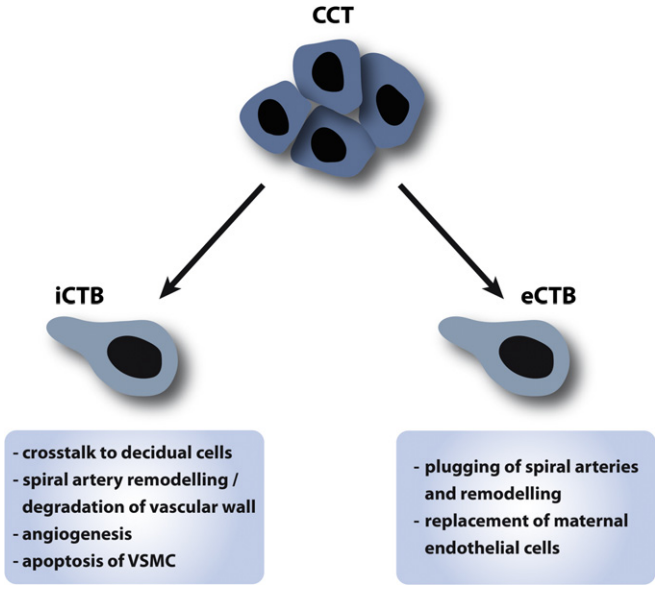

Fig. 1. Development of different extravillous trophoblast subtypes and their functions. Progenitors residing at the basement membrane of cell columns (cell column trophoblast, CCT) give rise to interstitial cytotrophoblasts (iCTB) invading the uterine decidua and endovascular cytotrophoblasts (eCTB) migrating into the maternal spiral arteries.

Cultivation of first trimester villous explant cultures under 20% oxygen induces matrix attachment, column formation and differentiation even in the absence of serum [12] suggesting that proliferation and invasion of EVT are triggered by an intrinsic differentiation program. Correct integrin switching, a hallmark of the differentiation process of the anchoring villus, takes places in EVT migrating on top of collagen I- or fibronectin-coated surfaces or upon invasion into Matrigel [13–15] suggesting that ECM composition is of minor importance for some key features of EVT differentiation. Hence, besides oxygen concentrations numerous growth factors released from trophoblasts and the villous stromal core, such as IGF-I [16], likely control the endogenous differentiation program of EVT. Along those lines, EVTs were shown to produce a wide range of receptors for cytokines, chemokines, and growth factors allowing for precise autocrine as well as paracrine control of trophoblast invasion [17].

2. Soluble factors controlling trophoblast motility

2.1. Paracrine effectors of trophoblast invasion and migration

Timing and extent of interstitial trophoblast invasion is thought to be controlled by a plethora of factors expressed in the diverse decidual cells. Soluble proteins secreted from luminal and/or glandular epithelia such as leukemia inhibitory factor (LIF), EGF, interleukin-11 (IL-11), activins and chemokines which likely promote blastocyst implantation are also expressed in different decidual cell types. In summary, uNK cells, decidual stromal cells, and macrophages produce growth factors, cytokines, interleukins, chemokines, prostaglandins and angiogenic growth factors which have been shown to regulate invasion and/or migration in different in vitro trophoblast model systems (Fig. 2). Moreover, each decidual cell type secretes factors promoting and inhibiting trophoblast motility. Whereas the role of individual factors in trophoblast invasion has been studied, the combinatorial effects of proteins as well as their concentrations at different gestational times remain largely unknown. Along those lines, the overall role of the decidua in trophoblast invasion is still a matter of discussion. Since trophoblast invasion does not occur beyond the upper part of the myometrium, the decidua is generally considered as an inhibitory tissue restraining and limiting trophoblast invasiveness. Recent in vitro experiments, however, suggest that decidual supernatants and cultures may actually increase trophoblast invasion. Whether this effect is due to the utilization of tumorigenic and immortalized trophoblasts in these studies, however, remains to be determined [18–20].

2.2. Autocrine factors regulating trophoblast invasion and migration

The occurrence of tubal pregnancies suggests that EVT exhibit a strongly activating, intrinsic differentiation program promoting cell invasion independently of the local environment. Similar to tumor cells, invasive EVT are equipped with different growth factor-dependent protease systems, i.e. matrix metalloproteinases (MMPs), and urokinase plasminogen activator (uPA) allowing for ECM degradation within the decidua [21–23]. Autocrine factors such as insulin-like growth factor-II (IGF-II), hyperglycosylated hCG (h-hCG), heparin-binding epidermal growth factor (HB-EGF), interleukins and others (Fig. 3) were shown to increase expression and activity of EVT-derived proteases of which the gelatinases

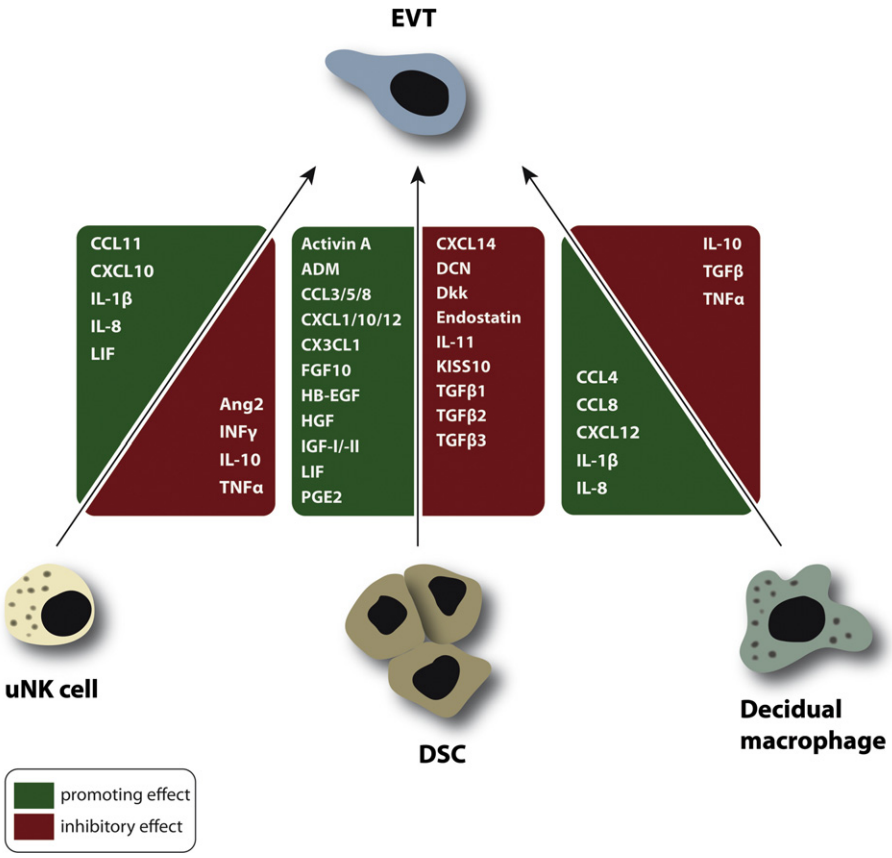

Fig. 2. The influence of decidual cell types on interstitial trophoblast invasion and migration. Predominant cell types of the maternal decidua, uterine natural killer (uNK) cells, decidual stromal cells (DSC) and decidual macrophages produce soluble factors promoting and inhibiting trophoblast motility.

MMP-2 and MMP-9 have been most studied. Since there is a considerable overlap in expression patterns of soluble factors secreted from EVT and the decidual cell types, it may not be surprising that many of the paracrine factors depicted in Fig. 2 also activate these protease systems. Both decidua and EVT, however, also produce the respective inhibitors, tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs) likely to fine-tune and limit trophoblast invasiveness [24]. Indeed, decidual (Fig. 2) as well as EVT-derived (Fig. 3) factors such as transforming growth factors (TGFs), Nodal, or tumour necrosis factor α (TNFα) were shown to elevate TIMP or PAI expression [24–26]. Besides degradation of ECM components invasive trophoblasts specifically express different integrins, for example α5β1 and α1β1 heterodimers known to promote trophoblast adhesion and migration by interacting with fibronectin and collagen/laminin, respectively [13].

2.3. Signalling cascades involved in trophoblast motility

Signaling pathways known to regulate growth and motility in other cellular systems also play fundamental roles in EVT. Whereas the specific effects of growth factors and sequential steps of signaling transduction cascades controlling trophoblast proliferation and invasion/migration were discussed in the past [22,27], we here briefly summarize the motility-promoting pathways which have been functionally evaluated by using chemical inhibitors, antisense oligonucleotides or siRNA-mediated gene silencing of critical signaling components (Fig. 4). As also recently mentioned [22] inhibition of the MAPK kinase (MEK)/extracellular regulated kinase (ERK) pathway or phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling affected hCG, EGF, IGF-II and hepatocyte growth factor (HGF)-dependent migration and invasion suggesting that these factors act through several cascades in EVT. Other proteins such as IGF-I and insulin-like growth factor binding protein-1 (IGFBP-1) may increase trophoblast migration through focal adhesion kinase (FAK) and integrin-linked kinase (ILK) activation since down-regulation of the kinases was shown to reduce stimulus-dependent trophoblast motility. Along those lines, gene silencing of components of the Rho-ROCK pathway, Rho, Rac1, CDC42 and ROCK also negatively affected prostaglandin E2 (PGE2) and IGF-II-dependent trophoblast migration. Leptin and IL-11 were shown to signal through Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) and silencing of STAT3 reduced trophoblast invasion. Moreover, cascades involved in developmental processes may also play critical roles in EVT differentiation. Wingless (Wnt) induces trophoblast motility through PI3K/AKT signaling as well as through the canonical Wnt pathway involving glycogen synthase kinase-3β (GSK-3β)-dependent stabilization of β-catenin and formation of nuclear, transcriptionally active T-cell factor-4 (TCF-4)/β-catenin complexes [28–30]. In addition, Notch signaling provoking generation of Notch intracellular domain (NICD) which acts as a co-activator of the transcription factor RBPJκ could be involved in endovascular trophoblast invasion [31]. Kinases known to promote trophoblast invasion might be negatively affected by inhibitory molecules present in EVT and/or decidua. For example endostatin, the C-terminal cleavage product of collagen XVIII is expressed in decidual stromal cells [32] and impairs IGF-II-mediated trophoblast migration by downregulating AKT/mTOR activity [15]. Endostatin is likely generated by EVT-derived

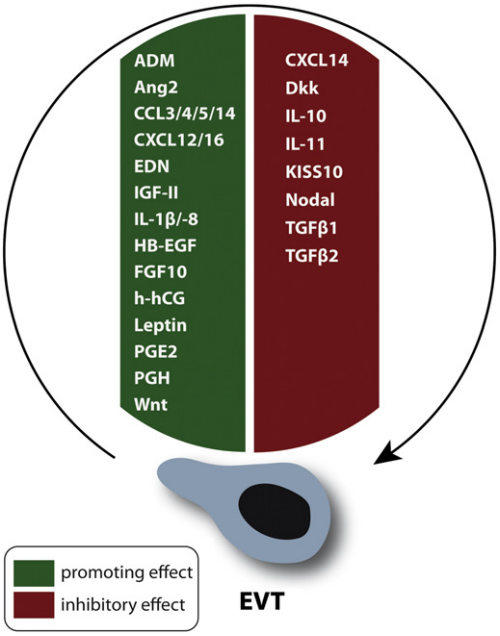

Fig. 3. Autocrine factors secreted from EVT promoting and inhibiting trophoblast motility.

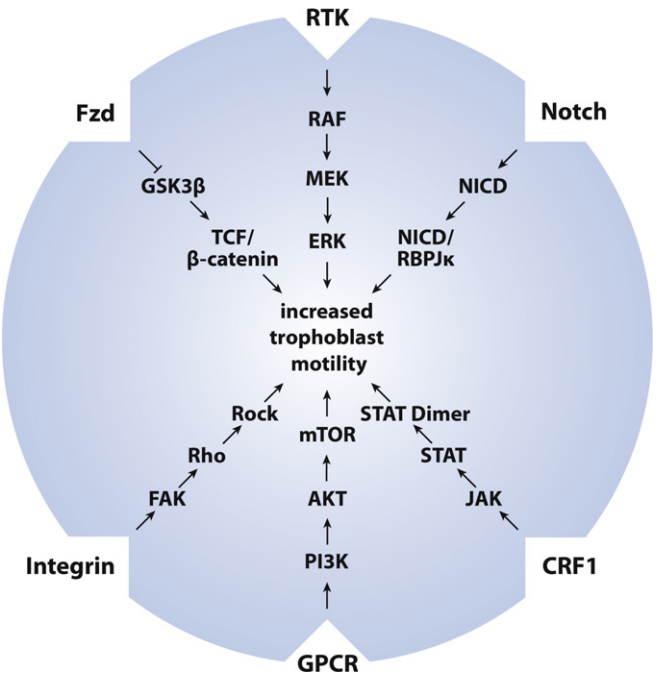

Fig. 4. Signaling pathways promoting trophoblast invasion and migration. Numerous soluble growth factors expressed at the fetal-maternal interface signal through receptor tyrosine kinases (RTK) and G-protein-coupled receptors (GPCR) to provoke activation of MEK/ERK, PI3K/AKT/mTOR and Rho/ROCK signaling. Besides the JAK/STAT pathway downstream of cytokine receptor family 1 (CRF1), Wnt-dependent activation of frizzled (Fzd) receptors as well as Notch activation could be involved in controlling trophoblast motility.

MMPs providing another mechanism to restrain trophoblast invasiveness [33].

3. Cell column formation and EVT differentiation

EVT differentiation in anchoring villi is characterized by transient proliferation in proximal areas of the cell column, cell cycle exit at more distal sites, loss of cell-cell contacts and local invasion into decidual stromal compartments. Therefore, it is likely that a complex network of key nuclear factors regulates maintenance of cell column progenitors, growth and stability of the column as well as development of distinct EVT subtypes. Little, however, is known about the transcription factors controlling commitment and differentiation of interstitial or endovascular trophoblasts. Similarly, upstream signaling pathways activating these factors as well as their putative target genes remain largely elusive.

3.1. Regulatory transcription factors controlling cell column proliferation and EVT differentiation

Many different nuclear factors were shown to be expressed in cell columns and/or EVT [34,35], and changes in gestational diseases such as fetal growth restriction were noticed [36]. However, few of them have been functionally analyzed. Regulatory transcription factors could be divided into two classes, proteins that are differentially expressed between proliferative cell column trophoblasts (CCT) and non-growing EVT, and proteins that are uniformly expressed. Since the latter also display distinct functions in CCT and EVT, it is likely that their activity is differentially regulated by post-translational modifications and/or co-activator recruitment. The expression patterns of transcription factors which have been functionally tested in different trophoblast model systems as well as their roles in EVT differentiation are depicted in Fig. 5. H2.0-like homeobox (HLX), HIF-1α, and the winged helix protein Storkhead box 1 (Stox1) promoted trophoblast proliferation, and gene silencing of HIF-1α and Stox1 increased trophoblast invasion suggesting an inhibitory role of the two factors in EVT differentiation [9,36,37]. Both HIF-1α and Stox1 could be involved in the pathogenesis of preeclampsia. TGFβ3, an inhibitor of EVT differentiation controlled by HIF-1α, was shown to be elevated in placentae of women suffering from this particular disorder [9]. Downregulation of TGFβ3 restored the invasive capacity of preeclamptic villi in vitro suggesting that correct oxygen sensing via HIF-1α could be affected in the gestational disease. Stox1, on the other hand, has been identified as a susceptibility gene for familial preeclampsia in a Dutch population [37]. The identified mutation in Stox1, *Y153H*, may increase binding activity of the factor to target genes such as αT-catenin and thereby maintain trophoblast adhesion and proliferation instead of promoting EVT differentiation. Similarly, inhibitor of DNA binding 2 (Id2) was shown to act as a negative regulator of trophoblast invasiveness [38]. Downregulation of its expression during differentiation likely provokes elevated activities of differentiation-promoting basic helix-loop-helix (bHLH) genes in EVTs [39]. Similarly, ligand-dependent activation of proliferator-activated receptor γ (PPARγ), controlling invasion-promoting genes such as hCG, interferes with trophoblast motility [40]. In contrast, glial cells missing 1 (GCM1) and TCF-4 are induced in non-proliferating EVT and stimulate trophoblast migration and invasion [28,41]. Nuclear localization of the co-activator of TCF-4, β-catenin, was observed in a subset of EVT and complete hydatidiform mole placentae displayed elevated expression of the protein suggesting abnormal Wnt signaling in these tissues [28]. Besides GCM1 and TCF-4, siRNA-mediated downregulation of an isoform of Ikaros (Ikx), of STAT3 and of EGF-induced activating protein-2α (AP-2α) also diminished trophoblast motility [42–44]. With respect to downstream effects of these factors, MMPs and uPA could be the prime target genes. Indeed, EGF/AP-2α and Wnt/TCF were shown to induce uPA and/or MMP-2 expression in EVT [29,42].

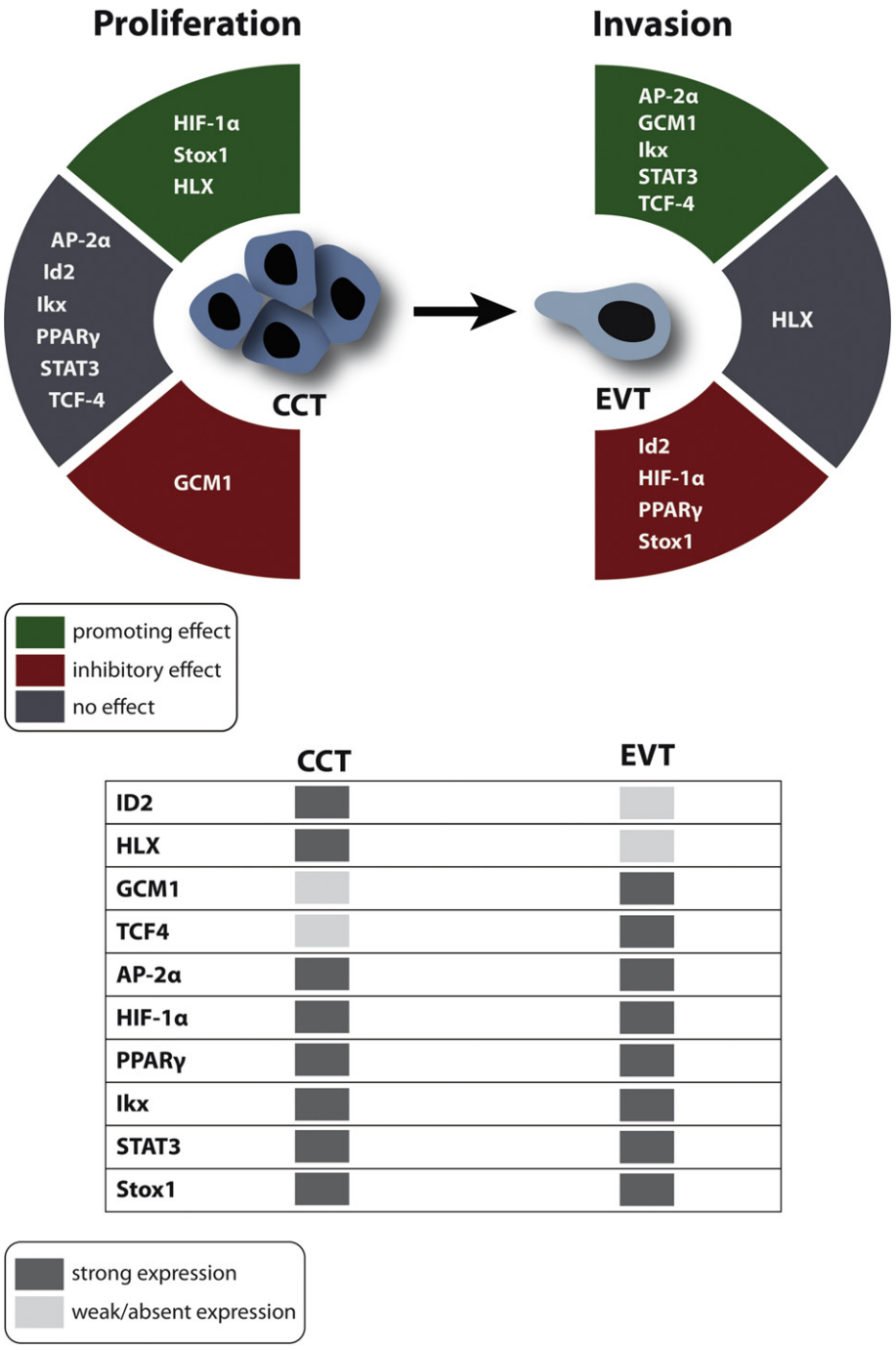

Fig. 5. Expression patterns and roles of regulatory transcription factors in EVT differentiation. Cell column trophoblasts (CCT) and extravillous trophoblasts (EVT) produce activators as well as inhibitors of trophoblast proliferation and invasion. Factors such as GCM1 and TCF-4 are absent from CCT progenitors and proliferative CCTs, respectively, corroborating their positive functions in EVT differentiation and invasion.

4. Summary

Trophoblast invasion and migration are controlled by a complex network of soluble autocrine and paracrine factors, signaling pathways and regulatory transcription factors. Besides the endogenous differentiation program of the anchoring villus promoting local invasion, maternal decidual cell types, i.e. decidual stromal cells, macrophages and uterine NK cells modulate trophoblast invasiveness. Despite the fact that an increasing number of regulatory factors are being unraveled, combinatorial effects, gestation-dependent changes of the regulatory network as well as the mechanisms controlling depth and timing of trophoblast invasion remain largely elusive. Along those lines, numerous unsolved questions and controversial views exist in the literature which should be answered in the future using appropriate trophoblast model systems and experimental approaches (Fig. 6). It is hoped that a better understanding of the molecular processes regulating EVT differentiation and invasion will improve our understanding about the role of the placenta in gestational diseases such as intrauterine growth restriction, preeclampsia and other pregnancy complications with incomplete spiral artery remodeling.

How is endovascular invasion of early pregnancy controlled, does it occur under high or low oxygen conditions, and may the effects of hypoxia vary throughout gestation? Trophoblast invasion takes place before and after the rise in oxygen levels. Which molecular mechanisms then determine the decline in trophoblast invasiveness after the mid second trimester of pregnancy? [3,10]

Which mechanisms control differentiation of progenitors into either endovascular or interstitial trophoblasts? Can interstitial trophoblasts trans-differentiate into endovascular trophoblasts and enter the spiral artery by intravasation? Vice versa can endovascular trophoblasts develop into interstitial trophoblasts? Which trophoblast cell type is the progenitor of intramural trophoblasts colonizing the spiral arterial wall? [1]

What are the temporal changes of the network of soluble factors controlling trophoblast invasion and migration? What are the critical signalling pathways and their downstream targets controlling trophoblast invasion and can we identify master regulatory transcription factors in EVT differentiation? What mechanisms control the expression patterns of critical transcription factors across the cell column, how is the induction of nuclear proteins promoting EVT differentiation achieved? [22,27,35]

How is the precise boundary between proliferating cell column trophoblasts and non-cycling EVT within anchoring villi established? Assuming that EVT differentiation is not merely a default pathway upon matrix contact, how are growth of cell columns into the matrix in vitro as well as into the decidual stroma in vivo achieved? Could a gradient of growth factors produced in the villous mesenchymal stroma be involved in controlling EVT differentiation? [16,45]

Are synctiotrophoblast and EVT derived from the same progenitor, or can we distinguish distinct precursor cells for the two differentiated trophoblast cell types? [46,47]

Since in vitro maintenance of trophoblast stem cells derived from placental villi has not been achieved so far, can we use other sources such as the chorion for the establishment of trophoblast stem cell lines and an human EVT differentiation model? [48,49]

Fig. 6. Open questions concerning regulatory mechanisms controlling human EVT differentiation and invasion. References indicate additional review articles and original publications where the respective research problem has been brought up, discussed or recently tackled.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

Acknowledgements

Trophoblast research in the laboratory of M. Knöfler is currently supported by grant 12487 of the Jubiläumsfonds of the Austrian National Bank, by grant P-22687-B13 of the Austrian Science Funds and by grant APP00323OFF of the Herzfelder'sche Familienstiftung.

Abbreviations

ADM	adrenomedullin
Ang2	angiopoietin 2
AP-2α	activating protein-2α
bHLH	basic helix-loop-helix
CCL	chemokine (C-C motif) ligand
CXCL	chemokine (C-X-C motif) ligand
CCT	cell column trophoblast
CRF1	cytokine receptor family 1
DCN	decorin
Dkk	Dickkopf
DSC	decidual stromal cell
ECM	extracellular matrix
eCTB	endovascular cytotrophoblast
EDN	endothelin
EGF	epidermal growth factor
ERK	extacellular regulated kinase
EVT	extravillous trophoblast
FAK	focal adhesion kinase
FGF	fibroblast growth factor
Fzd	frizzled
GCM1	glial cells missing 1
GPCR	G-protein-coupled receptor
GSK-3β	glycogen synthase kinase-3β
h-hCG	hyperglycosylated human chorionic gonadotrophin
HB-EGF	heparin-binding epidermal growth factor
HGF	hepatocyte growth factor
HIF-1α	hypoxia-inducible factor 1α
HLX	H2.0-like homeobox
iCTB	interstitial cytotrophoblast
Id2	inhibitor of DNA binding 2
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL	interleukin
JAK	Janus kinase
ILK	integrin-linked kinase
Ik	Ikaros
KISS	kisspeptin
LIF	leukemia inhibitory factor
MAPK	mitogen-activated protein kinase

MMP	matrix metalloproteinase
mTOR	mammalian target of rapamycin
NICD	Notch intracellular domain
PAI	plasminogen activator inhibitor
PG	prostaglandin
PGH	placental growth hormone
PI3K	phosphoinositide 3-kinase
PPAR	proliferator-activated receptor
ROCK	Rho-associated kinase
RTK	receptor tyrosine kinase
STAT	Signal Transducer and Activator of Transcription
Stox1	Storkhead box 1
TCF	T-cell factor
TGF	transforming growth factor
TIMP	tissue inhibitors of metalloproteinase
TNFα	tumour necrosis factor α
uPA	urokinase plasminogen activator
uNK	uterine natural killer cell
vCTB	villous cytotrophoblast
vEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cells
Wnt	wingless

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