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Regulation of Trophoblast Invasion – A Workshop Report

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Abstract

Trophoblast invasion during placental development helps to establish efficient physiological exchange between maternal and fetal circulatory systems. Trophoblast stem cells differentiate into multiple subtypes, including some that are highly invasive. Signalling to the trophoblast from decidua, uterine natural killer cells and vascular smooth muscle can regulate extravillous trophoblast differentiation. Important questions remain about how these cellular interactions promote trophoblast invasion and the signalling pathways that are involved. New and established biological models are being used to experimentally examine these interactions and the underlying molecular mechanisms.

Keywords

Trophoblast giant cells; Extravillous trophoblast; Invasion; Uterine NK cell; Spiral artery

1. Introduction

Considerable progress has been made in identifying regulatory mechanisms controlling commitment and specification of mouse trophoblast; however, key genes and signalling cascades involved in invasive differentiation of human trophoblast remain largely elusive. The goal of this workshop was to delineate critical mechanisms controlling development and differentiation of invasive murine and human trophoblast as well as to summarise recent progress in understanding specialised functions of trophoblast in the two species.

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8. Conflict of interest

The authors do not have any potential or actual personal, political, or financial interest in the material, information, or techniques described in this paper.

2. Invasive trophoblast subtypes

David Simmons reported that markers for a variety of mouse trophoblast subtypes have now been identified. The murine placenta contains two invasive cell types, trophoblast giant cells (TGC) and glycogen trophoblast cells (GlyT). The TGC population is now recognized to have several subtypes, two of which are invasive; TGCs that form a barrier between the maternal decidua and the underlying placenta (parietal TGCs) and TGCs that invade via an endovascular route (spiral artery-associated TGCs) [1]. In cell lineage-tracing experiments utilizing Cre-expressing intermediate cells of the TGC differentiation pathway, TGC subtypes were found to have multiple developmental origins [1]. However, all TGC subtypes require the transcription factor Hand1 for their proper differentiation. Interestingly, retinoic acid, known to promote TGC formation and invasion, promotes the formation of invasive TGC subtypes specifically while inhibiting the formation of others. GlyT, in contrast to TGCs, do not as yet have any characterized subtypes and reside within the spongiotropho-blast layer. They are interstitially invasive cells, migrating past the parietal TGC layer and deeply into the decidua during the second half of gestation. These cells have an uncertain developmental origin, although the prevailing hypothesis is that they arise from SpT precursors. This is in part due to the lack of GlyT markers, although recently *Pcdh12* (protocadherin 12) has been shown to be a specific marker in placenta [2].

3. Wnt—TCF signalling

Martin Knöfler reported that tissue-restricted bHLH factors may not play a predominant role in column formation and invasion of human extravillous trophoblast (EVT) since cells from the 8th to 12th week of gestation lack proteins such as Hand1 [3]. However, whole genome gene expression profiling of different trophoblast cell models led to the identification of Wntless (Wnt)-dependent transcription factors of the T-cell factor (TCF) family, which were shown to be predominantly expressed in p57/KIP2-positive, invasive trophoblasts [4]. Activation of the classical, canonical Wnt cascade using recombinant Wnt-3a provoked nuclear translocation of β -catenin, providing the activation domain of TCFs, and induced expression of a TCF luciferase reporter, as well as of TCF target genes. Moreover, Wnt-3a promoted trophoblast proliferation, migration and invasion through both canonical and non-canonical signalling, the latter through activation of the kinase PBK/AKT, which is also a well-known target of the invasion-promoting factors EGF and IGF-II in trophoblasts. In complete mole placentae, a large proportion of EVT were shown to contain nuclear β -catenin, suggesting that aberrant Wnt signalling could be involved in excessive trophoblast invasion. In summary, Wnt-dependent signalling pathways may play a critical role in the invasive differentiation process of human trophoblasts.

4. EGF signalling system

Randy Armant presented information on the role of the epidermal growth factor (EGF) signalling system in trophoblast invasion and placental pathology. Six EGF-like growth factors are expressed throughout gestation in villous and EVT populations of the human placenta. Immunohistochemical analysis of placentae from women delivering throughout the second and third trimesters revealed significant reductions in some EGF-like growth factors when pre-eclampsia occurred [5]. Expression in the basal plate implicates involvement of this pathway in EVT differentiation, and suggests that dysregulation in pre-eclampsia could contribute to the associated poor trophoblast invasion. It remains to be determined whether these pathological changes occur during placentation. Disruption of the EGF signalling system in human first trimester cytotrophoblast cell lines using antibodies against the growth factors or their receptors reduced trophoblast invasiveness *in vitro*, while supplementation with these growth factors increased invasiveness [6]. Integrin switching from $\alpha 6$ to $\alpha 1$ that occurs with

EVT differentiation was induced by EGF-like growth factors through activation of either the EGF receptor or ErbB4/HER4. The results of these mechanistic *in vitro* studies are consistent with a possible relationship between the observed pathological dysregulation of the EGF signalling system and inadequate trophoblast invasion.

5. Uterine natural killer cells

It has been proposed that one of the functions of uterine natural killer (uNK) cells in early pregnant decidua may be to regulate EVT invasion via secretion of cytokines. However, stimulating, as well as inhibitory, effects of uNK cells on EVT invasion and migration have recently been published [7,8]. Gendie Lash reported that several cytokines secreted by uNK cells (i.e. TGF- β 1, TNF- α and IFN- γ) inhibit EVT invasion *in vitro*, whereas IL-8, another uNK cell cytokine, can stimulate EVT invasion. It was also demonstrated that uNK cell secretion of IFN- γ and IL-8 increases with increasing gestational age. Lash and coworkers investigated the effect of uNK cell supernatants at 8–10 and 12–14 weeks gestational age on invasion through Matrigel basement membrane by EVT from placental explants of the corresponding gestational age. In addition, the role of TGF- β 1, TNF- α , IFN- γ and IL-8 were studied by the use of neutralising antibodies. uNK cell supernatants had no effect on EVT invasion at 8–10 weeks gestation, but increased the number of invading cells at 12–14 weeks gestation, via a mechanism associated with an increase in secreted gelatinases and decreased EVT apoptosis. Neutralising antibodies to TGF- β 1, TNF- α and IFN- γ had no effect on uNK stimulation of EVT invasion. In contrast, neutralising antibody to IL-8 partially abrogated uNK stimulation of EVT invasion. Based on these findings, they propose that at 8–10 weeks gestation EVT are naturally highly invasive. However, at 12–14 weeks gestation when invasiveness is reduced, the uNK cells are involved in enhancing EVT invasion to maintain their cell number in the placental bed and replace those lost by apoptosis. However, these studies were conducted *in vitro* and may not reflect the *in vivo* situation where local regulation of EVT invasion by different subsets of uNK or decidual cells may occur. This data also do not explain why invasion is limited to the inner third of the myometrium; although at that site EVT may be no longer under decidual influences and, therefore, potentially stimulating factors.

6. Trophoblast- and smooth muscle cell-derived proteases

Remodelling of the human spiral arteries during pregnancy is poorly understood. To attain a permanent increase in luminal diameter, elastic fibres within the vessel media must be degraded [9]. However, the source and identity of the enzymes responsible is unknown. Lynda Harris proposed that vascular smooth muscle cells (SMC) may be a potential source of elastolytic enzymes. She demonstrated that SMC exhibited constitutive, membrane-associated elastase activity, which was attributable to matrix metalloproteinases (MMP). Intracellular elastase activity was stimulated by the presence of elastin fragments, and both MMP and nitric oxide (NO) were required for optimal elastase activity *in vitro*. Furthermore, MMP-7 and -9 were strongly expressed by SMC within unmodified spiral arteries in first trimester decidua and MMP-12 expression was induced in artery segments perfused with trophoblast-conditioned medium *ex vivo*. First trimester trophoblasts also utilized MMP to degrade elastin. Enhanced expression of MMP-9 was noted at the leading edge of migrating trophoblasts, which co-localised with a region of inducible NO synthase expression. Inhibition of MMP or NO impeded trophoblast invasion *in vitro*. These findings suggest that generation of free elastin fragments and release of soluble factors by EVT may stimulate elastase activity in vascular SMC. Hence, the process of trophoblast invasion may stimulate cooperative elastin breakdown by spiral artery SMC during arterial remodelling.

7. Conclusion

The workshop provided insights into the complex differentiation program of murine trophoblast subtypes. Moreover, novel functions and control mechanisms of invasive human trophoblasts were discussed. However, molecular circuits specifying and regulating EVT differentiation are only just beginning to be elucidated. Much remains to be learned about how critical signalling pathways and placental—maternal cell types interact in an integrated manner in order to control decidual invasion and spiral artery remodelling in a spatial and temporal manner.

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