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Gabor Than Award Lecture 2006: Pre-Eclampsia and Villous Trophoblast Turnover: Perspectives and Possibilities

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Abstract

Placental apoptosis is exaggerated in pre-eclampsia and cytotrophoblast proliferation is enhanced. This imbalance may be a primary pathogenic event, whereby excessive syncytiotrophoblast apoptosis counters cytotrophoblast fusion, promoting the liberation of syncytial material which perturbs the maternal vascular endothelium. We have previously shown that primary trophoblasts and explant cultured villous fragments from pre-eclamptic pregnancies elicit greater levels of terminal differentiation and apoptosis. This review considers current opinions in trophoblast cell turnover in normal pregnancy and pre-eclampsia. In the context of other findings, this review highlights: (i) the disparity in expression of pro-apoptotic transcription factor p53 in the syncytiotrophoblast in pre-eclampsia, (ii) the importance of reactive oxygen species and hypoxia in initiating villous trophoblast apoptosis and (iii) the concept that aberrant intervillous haemodynamics, as opposed to oxygen *per se*, initiates excessive syncytiotrophoblast shedding. Finally, therapeutic ways of restoring the syncytiotrophoblast in pre-eclampsia and preventing excessive placental apoptosis are considered, including a role for mitotic manipulators and growth factor replacement strategies.

Keywords: Apoptosis; Pre-eclampsia; Syncytiotrophoblast

1. The placenta and pre-eclampsia

Pre-eclampsia, a syndrome of vascular endothelial dysfunction and heightened systemic inflammatory response, is a severe disorder affecting up to 10% of primiparous women in industrialised countries [1]. The fact that pre-eclampsia occurs in molar pregnancies in the absence of a fetus, but presence of a placental mass, confirms the importance of the placenta as the crucial stimulus. Certainly this is verified in cases where the placenta is retained after delivery, as the maternal symptoms persist until the organ is removed. With respect to systemic inflammation, there is substantial evidence to support the concept that pre-eclampsia develops when the normal inflammatory response of pregnancy is exaggerated [2]. It is therefore hypothesised that the responsible factor is liberated from the placenta in moderation in normal pregnancy and to excess in pre-eclampsia. This is substantiated by an increased incidence in pregnancies of larger placental mass, i.e. those of multiple or molar pregnancies [3].

Numerous factors produced by the placenta are elevated in pre-eclampsia. Many are implicated in its pathogenesis but no factor, yet suggested, satisfactorily explains its clinical progression. When looking to identify such a factor and the basis for its release, the haemochorial nature of the placenta is undoubtedly important. Amongst other rationale, it is speculated that humans more than any species are predisposed to pre-eclampsia for the following reasons: (i) the extensive nature and depth of trophoblast invasion, (ii) the extent of trophoblast-induced uterine artery transformation, (iii) the complexity of the chorionic villous tree, and (iv) the surface area for transport and diffusion. Individually or in combination, these features may play a significant role, but the latter takes on the

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greatest importance when the causative factor is itself the villous trophoblast.

2. The syncytiotrophoblast and pre-eclampsia

Fragments of trophoblast are liberated from the surface of the villous placenta and readily enter the maternal peripheral circulation. These trophoblast remnants are detectable in uterine veins at Caesarean section and are more prevalent in pre-eclampsia than normal pregnancy [4]. In contrast to their presence in uterine veins, very few trophoblast fragments are found in the maternal peripheral circulation, suggesting that these alone are unlikely to cause maternal leukocyte activations or vascular endothelial complications. Focus has therefore turned to sub-cellular fractions of the syncytiotrophoblast, the outer trophoblast layer of the human placenta. In this context, Redman and Sargent propose that syncytiotrophoblast microparticles, liberated from the villous, circulate freely because of their diminished size and thus avoid entrapment and removal in the lungs [5]. The presence of these micro-particles has now been defined in plasma in normal pregnancy and shown to be significantly greater in pre-eclampsia [6]. Artificially generated syncytiotrophoblast membrane fragments, termed STBMs, can inhibit lymphocyte proliferation, induce apoptosis of T-lymphocytes and can disrupt endothelial cell monolayers and proliferation-cornerstones of pre-eclamptic pathogenesis [7]. Other components of the syncytiotrophoblast, circulating in increased quantities, include cytokeratin, a cytoskeletal protein of trophoblast origin, fetal proteins (e.g. activin-A, inhibin-A, placental alkaline phosphatase) and cell-free RNA and DNA of fetal origin [8–10]. Together, these culminate in robust evidence for the excessive deportation of syncytiotrophoblast in pre-eclampsia.

3. Syncytiotrophoblast apoptosis

Apoptosis, programmed cell death, was first defined in the human placenta by Sakuragi and co-workers [11]. With more recent advances, this process has now been more tightly associated with cytotrophoblast differentiation (Fig. 1). Throughout gestation the functional and morphological maintenance of the multinucleated syncytiotrophoblast, including the supply of fresh mRNA, proteins and organelles, depends exclusively on the fusion of subjacent, post-mitotic cytotrophoblast cells [12]. These events are coordinated by the transcription factor GCM-1 [13] and other regulatory proteins, including syncytin-1 and -2 of retroviral origin, and ADAM-12, a recognised component of myoblast cell-cell fusion [14]. Besides these regulatory factors, fusing cytotrophoblasts undergo a necessary flip of phosphatidylserine from the inner to the outer leaflet of the plasma membrane. This loss of asymmetry is attributed to the activation of the cysteine aspartase, caspase 8, an initiator of the common apoptotic pathway [15]. Once fusion has occurred, these early but reversible phases of apoptosis are abruptly halted, thus restricting indiscriminate decline of the vasculo-syncytial membrane. At this stage, the inhibition of

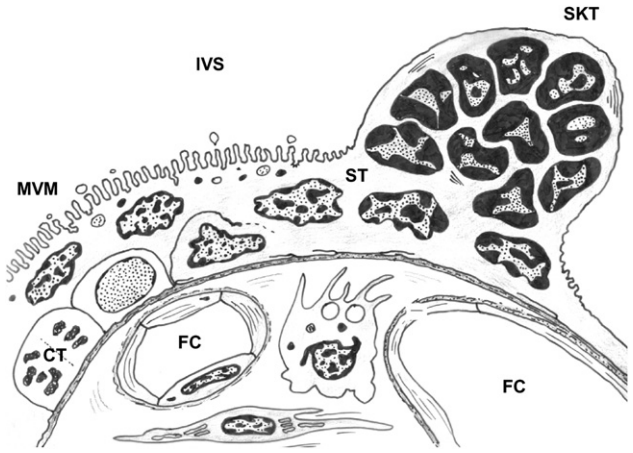

Fig. 1. Diagram of cell turnover of the villous trophoblast. Proliferation of stem cell cytotrophoblasts is followed by the induction of early stages of apoptosis culminating in the dissolution of their plasma membrane, fusion and incorporation into the overlying syncytiotrophoblast. Apoptotic progression leads to the accumulation of condensed nuclei into syncytial knots and the shedding of these aggregates into the intervillous spaces. CT, cytotrophoblast; FC, fetal capillary; IVS, intervillous space; MVM, microvillous membrane; SKT, syncytial knot; ST, syncytiotrophoblast. Diagram kindly provided by Dr Carolyn Jones of the Division of Human Development, University of Manchester, UK.

apoptosis is attributed to the abundance of cytoplasmic anti-apoptotic oncoproteins in the syncytiotrophoblast, including Bcl-2, Mcl-1 and Mdm2, the presence of X-linked inhibitor of apoptosis protein (XIAP) and profusion of Flice-like inhibitory protein (FLIP), a blocker of caspase 8 activity [16,17]. These focal inhibitions, which are often lost in areas of grouped and aggregated nuclei, are generally but not inevitably followed by the activation of effector caspases, including caspases 3 and 6. What confines these caspases to discrete areas of the syncytiotrophoblast is unclear, but upon restoration of caspase activity, nuclear proteins, such as PARP, lamin B and topoisomerase II, are reduced and annular chromatin condensation initiated [17]. Finally, aging nuclei accumulate within the tips of villi where they protrude from the apical membrane as syncytial knots. Although difficult to distinguish histologically, these knots are eventually shed as membrane sealed vesicles into the intervillous spaces [18].

4. Placental apoptosis and pre-eclampsia

The view of human villous trophoblast as a continuously renewing epithelium, promotes the idea of a steady state between cytotrophoblast and syncytiotrophoblast compartments. A consensus on the numeric ratio between cytotrophoblast and syncytiotrophoblast has been defined in the third trimester and described as 1:9, respectively [19,20], but uncertainty in early gestation prevents an agreement as to the maintenance and coverage of cytotrophoblasts throughout gestation [19–21]. Perturbations in trophoblast volume and thickness are induced by alterations in cytotrophoblast proliferation, fusion and/or syncytiotrophoblast loss. Investigations of placental apoptosis in pre-eclampsia have utilised morphological and biochemical features of apoptosis, typically heterochromatin condensation