Syncytial Knots, Sprouts, Apoptosis, and Trophoblast Deportation from the Human Placenta

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SUMMARY

The syncytiotrophoblast (STB) that forms the epithelial covering of the placental villous tree has a unique cell biology on account of its syncytial nature. The tissue is in a terminally-differentiated, postmitotic state, and expands through the recruitment by fusion of underlying progenitor cytotrophoblast cells. This process occurs from the time of implantation until term, and so its nuclei will be of various ages, producing a spectrum of contrasting appearances; whilst some are euchromatic, others display dense condensations of heterochromatin, the latter often aggregating to form clusters referred to as syncytial knots. These appearances have led to the suggestion that knots are apoptotic, and a hypothesis has developed that the nuclei are transcriptionally inactive and transit through the STB before being shed into the maternal circulation. Here, we review the evidence for this hypothesis, looking at the morphology of the nuclei, their number throughout gestation, evidence of transcriptional activity, and trophoblast deportation. We conclude that there is little evidence to support the concept that turnover of syncytial nuclei takes place in the normal placenta, or that this occurs through an apoptotic-related process. Instead, we suggest that a proportion of syncytial nuclei are transcriptionally active, that epigenetic modifications underlie the changes in chromatin appearance, and that syncytial nuclei continue to accumulate until term. We recognize that apoptotic changes can occur in pathologic pregnancies, but consider the deportation of trophoblast that has been linked to preeclampsia to be most likely of necrotic origin following ischemic injury. [*Taiwan J Obstet Gynecol* 2009;48(1):28–37]

Key Words: apoptosis, chromatin, human placenta, transcription, trophoblast

Introduction

The syncytiotrophoblast (STB) of the placenta is a unique tissue in the human body. It is a multinucleated syncytium that forms the epithelial covering of the fetal villous tree, and extends over a surface area of 12–14 m^2 at term without apparent lateral cell boundaries [1,2]. The STB forms the interface with the maternal tissues, which in the human hemochorial situation is represented by maternal blood. As such, it performs many functions vital to a successful pregnancy, including active transport, immunologic defense and synthesis of peptide and steroid hormones. Another defining feature of the STB is that it is in a terminally differentiated postmitotic state. Mitotic figures have never been observed within the STB, and this may be an adaptation to reduce the risk of malignant change at the maternofetal interface. Instead, it is generated from the underlying population of mononuclear cytotrophoblast cells (CTB), which undergo proliferation, differentiation and finally fusion with the STB.

The extent and multinucleated nature of the STB raises a number of fundamental questions concerning its cell biology. Much attention has focused recently on the subsequent fate of the nuclei incorporated; indeed, it has been proposed that the nuclei transit through the STB, being released after a period of 2–3 weeks into the maternal circulation through apoptotic mechanisms

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[3–5]. Perturbations of this process have been linked to the pathogenesis of preeclampsia, where the debris is thought to cause activation of the maternal endothelial cells [6]. In this review, we assess the evidence on which these theories are based and suggest alternative hypotheses that should be tested.

Morphology of the STB

The STB is first formed when the blastocyst attaches to, and invades into, the uterine epithelium during implantation. It creates a mantle that surrounds the conceptus and forms the epithelial covering of the placental villi when these are elaborated [7]. During early pregnancy, this epithelium is a two-layered structure, with the outer STB lying on a complete layer of progenitor CTB cells. At this stage of gestation, the majority of CTB cells generally display a rounded profile and have a relatively large centrally located nucleus but relatively few cytoplasmic organelles [8,9]. The nucleus is euchromatic with a well-developed nucleolus, which is the site of ribosomal RNA synthesis [10]. As these cells differentiate, they come to resemble closely the overlying STB. Thus, the number and complexity of their organelles increase, and the nucleus takes on a more irregular shape with pronounced indentations (Figure 1A). Some small aggregations of heterochromatin may be observed, often located just under the nuclear membrane, but a nucleolus is still a prominent feature. Such cells are thought to be about to fuse with the STB soon, although the time course for this event is not known.

Nuclear appearances within the STB vary with gestational age. Early in pregnancy, the nuclei are randomly dispersed within the syncytioplasm and have a euchromatic appearance. A nucleolus is frequently present (Figure 1B). Towards term, however, many nuclei display dense aggregates of heterochromatin [11]. Such nuclei are often clustered together in groups referred to as syncytial knots [12,13] and have long been assumed to represent aged or effete nuclei (Figure 2A). This is supported by the finding that nucleoli are less frequent within STB nuclei from term placentas (8%) than in early pregnancy (55%) [11]. Furthermore, the nucleoli that are present at term are smaller in diameter and appear involuted, suggesting a decline in functional activity.

Syncytial Knots

There is no precise definition of a syncytial knot, and so the term has been used rather loosely in the literature, leading to potential confusion. Aggregations of STB nuclei are a conspicuous feature of any normal term placenta, but the majority of these do not represent syncytial knots. Rather, these appearances either reflect the clustering of euchromatic STB nuclei at points on the villous surface not involved in diffusional transport, enabling the formation of vasculo-syncytial membranes [14], or they are the result of tangential sectioning through the STB. The latter may give the false impression of clumps of nuclei protruding from the villous surface or linking adjacent villi in the form of a trophoblastic bridge; but when followed in serial sections, they are seen to arise as glancing sections through the STB at a villous branching

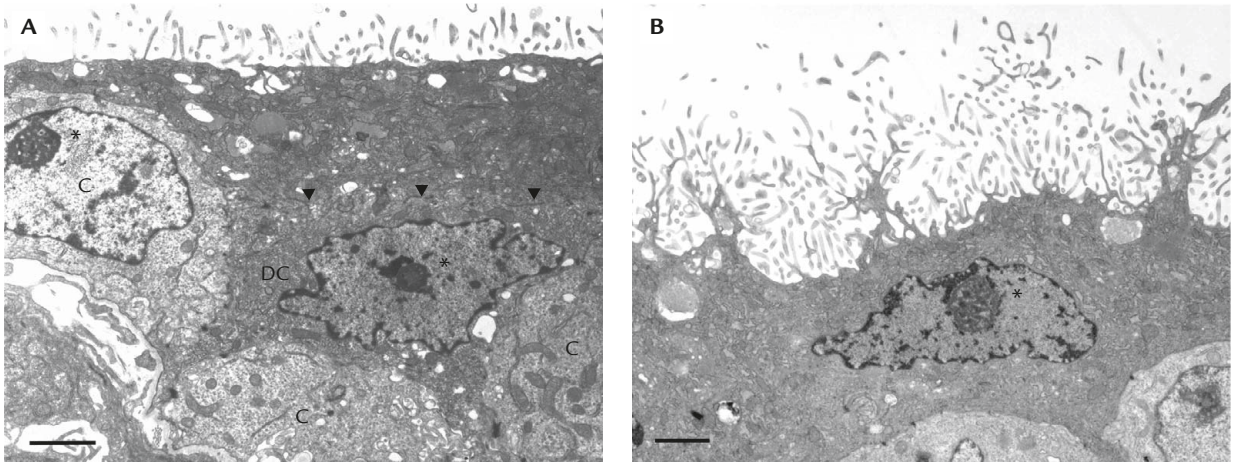

Figure 1. Electron micrographs of first-trimester villi illustrating nuclear appearances within the syncytiotrophoblast (STB). (A) Progenitor cytotrophoblast (CTB) cells (C) lie on a well-developed basal lamina and generally have a pale-staining cytoplasm and round nucleus with a prominent nucleolus (asterisk). When CTB cells differentiate (DC), the complexity of their cytoplasmic organelles increases, and they come to resemble the overlying STB. This example is still attached to the STB by a series of desmosomes (arrowheads). The nucleus shows pronounced indentations, and a nucleolus is still present (asterisk). Scale bar, 2 μm. (B) An example of an STB nucleus at 7 weeks of gestation displaying a prominent nucleolus (asterisk). Scale bar, 2 μm.

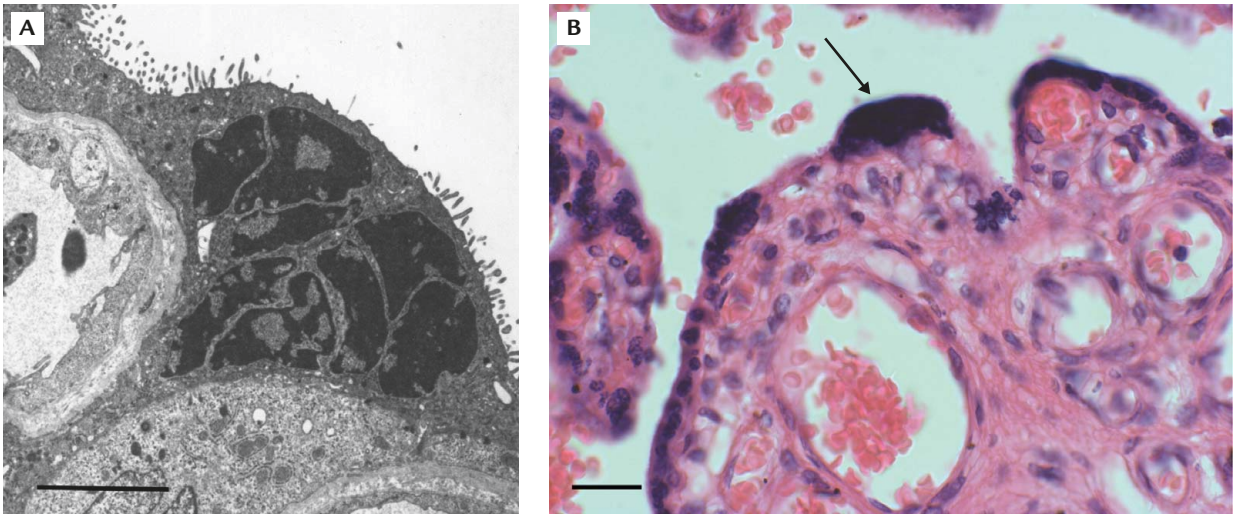

Figure 2. Syncytial knots in normal term placentas. (A) An electron micrograph illustrating a cluster of syncytiotrophoblast nuclei displaying highly condensed chromatin. Scale bar, 5 μm. (B) A light micrograph of what we take to be a true syncytial knot (arrow). Note that the superimposition of the nuclei makes it very difficult to assess the chromatin pattern. Scale bar, 20 μm.

point or sharp bend [15,16]. Since the topology of the villous tree can be influenced by the prevailing intrauterine conditions, these artifacts are often more common in complicated pregnancies, such as at high altitude when villous branching is increased [17].

In contrast, true syncytial knots consist of closely packed STB nuclei displaying heavily condensed chromatin, and frequently gently protrude from the villous surface (Figure 2B). However, there are no quantitative descriptors of how many nuclei must be involved or the percentage of the nuclear profile that has to be occupied by heterochromatin. The latter may be difficult to discern by light microscopy, since the STB nuclei frequently stain very intensely. True knots are, therefore, best identified by electron microscopy. The apical membrane of the villus bounding the knot is often unchanged; but in a proportion of cases, there may be a loss of microvilli and associated coated pits. This may reflect physical interactions with neighboring villi, for when they do come into contact, fusion of the membranes leads to the formation of intervillous bridges [13]. The nuclear profiles are smooth and sometimes closely interlocking, but there is no evidence of blebbing of the nuclear membrane or karyorrhexis. Between the nuclei, there may be large bundles of cytoplasmic filaments, which may serve to aggregate them into the knots, as well as annulate lamellae [13].

Quantitative assessments have demonstrated that syncytial knots are very infrequent before 32 weeks of gestation, and that their frequency increases towards term when they are found on 10–30% of villi in most normal placentas [12]. In terms of their correlation with placental pathologies, the strongest association is with postmaturity; at 42 weeks, there is a sudden increase in their frequency. No relationship exists with maternal age or parity, birth weight or fetal distress, but a slight increase is seen in preeclampsia, and more so in cases of fetal stem artery occlusion [12,18].

Syncytial knots may, therefore, be viewed as a way of sequestering aged syncytial nuclei in an area of the villous membrane where they do not interfere with diffusional exchange between the maternal and fetal circulations. In any consideration of trophoblast biology and pathology, they should be clearly distinguished from syncytial sprouts which, as Fox and Sebire [18] lamented, has not always been the case. In part, this reflects that some authors use the terms interchangeably, for example Aladjem [19], whereas others have made a clear distinction between the two, for example Boyd and Hamilton [20,21].

Syncytial Sprouts

Syncytial sprouts, as defined by Boyd and Hamilton [21], are aggregations of STB nuclei that differ in several key respects from syncytial knots. Firstly, they are associated with proliferation of the villous tree and the formation of new villi. Thus, they are most commonly seen in early pregnancy, although they still occur in the term placenta, in particular in the more hypoxic regions at the periphery of a lobule and near the chorionic plate [22]. Secondly, the nuclei within a sprout are euchromatic, often containing a prominent nucleolus, and show no signs of degeneration. This can be difficult to determine by light microscopy, particularly in the mature placenta when the nuclear profiles are often superimposed and stain intensely (Figures 3 and 4).

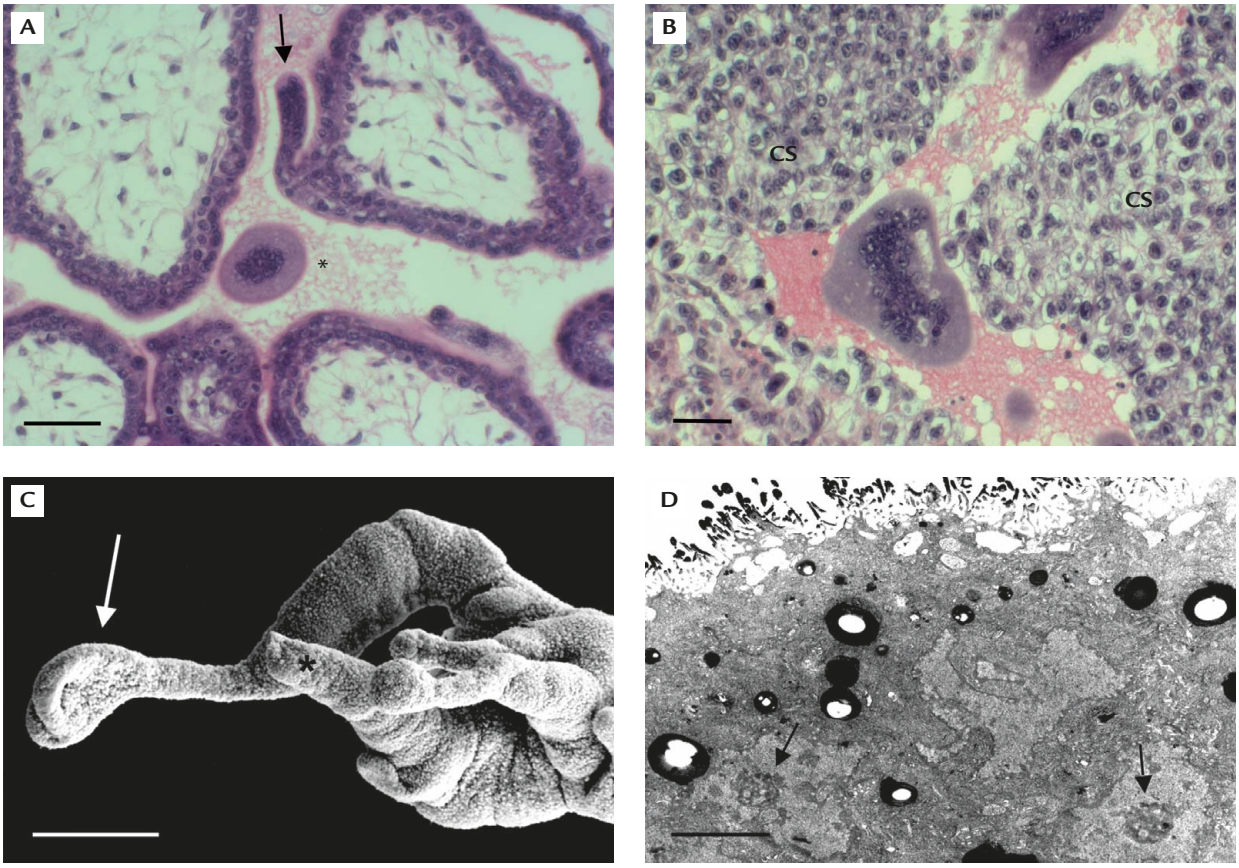

Figure 3. Syncytial sprouts in first-trimester placentas. (A) A sprout (arrow) arising from a villus of 6 weeks' gestational age. Note that the nuclei have the same chromatin appearance as those remaining in the syncytiotrophoblast and in the head of another sprout (asterisk) that appears free-floating within the intervillous space, suggesting deportation. Scale bar, 50 μm. (B) The head of a sprout lying in a venous opening through the cytotrophoblastic shell (CS). Note that the nuclei display a euchromatic appearance, and that nucleoli can still be distinguished. Scale bar, 20 μm. (C) Scanning electron micrograph of syncytial sprouts arising from a first-trimester villus. Note the often-expanded head and that the neck of one sprout (asterisk) is broken, suggesting deportation. This villus was embedded and the sprout (arrow) was sectioned. Scale bar, 100 μm. (D) Transmission electron micrograph of the head region identified in (C), showing the presence of three euchromatic nuclei (N) containing nucleoli (arrow). Scale bar, 5 μm. (C) and (D) adapted from Burton et al [23].

However, their euchromatic nature has been confirmed at the ultrastructural level [21], and by using correlative scanning and transmission electron microscopy [23] (Figures 3C and 3D). Thirdly, sprouts are usually pedunculated, extending either from the tip of a villus in early pregnancy (Figure 3A), or after 24 weeks of gestation more often from the lateral aspect (Figure 4A) [19]. The sprout is attached to the parent villus by a strand of syncytioplasm that may be several tens of micrometers in length, particularly in early pregnancy, and can become very attenuated (Figure 4C). Consequently, the head of the sprout containing the nuclei can easily break away and enter the maternal circulation [21].

The deportation of trophoblastic material into the maternal blood has been recognized for many years [21], and it has been estimated that approximately 100,000 sprouts enter the maternal circulation per day (Figures 3B and 4D). These aggregates of STB nuclei surrounded by a small amount of cytoplasm often have a tear-drop shape, with a short cytoplasmic tail reflecting the original connecting stalk to the parent villus. They may be found in the uterine venous blood and accumulate in the capillary bed of the maternal lungs, where they cause no apparent reaction. Hence, they are not detected in the peripheral circulation.

Syncytial sprouts are, therefore, a mark of trophoblast and villous proliferation. Their function is unknown, and one needs to exercise particular care when interpreting immunohistochemical data since, in our experience, many antibodies bind nonspecifically to sprouts, but they may serve as a means of reducing the number of nuclei within the STB.

Number of STB Nuclei

As formation of the STB starts at the time of implantation and its expansion is reliant on the fusion of CTB

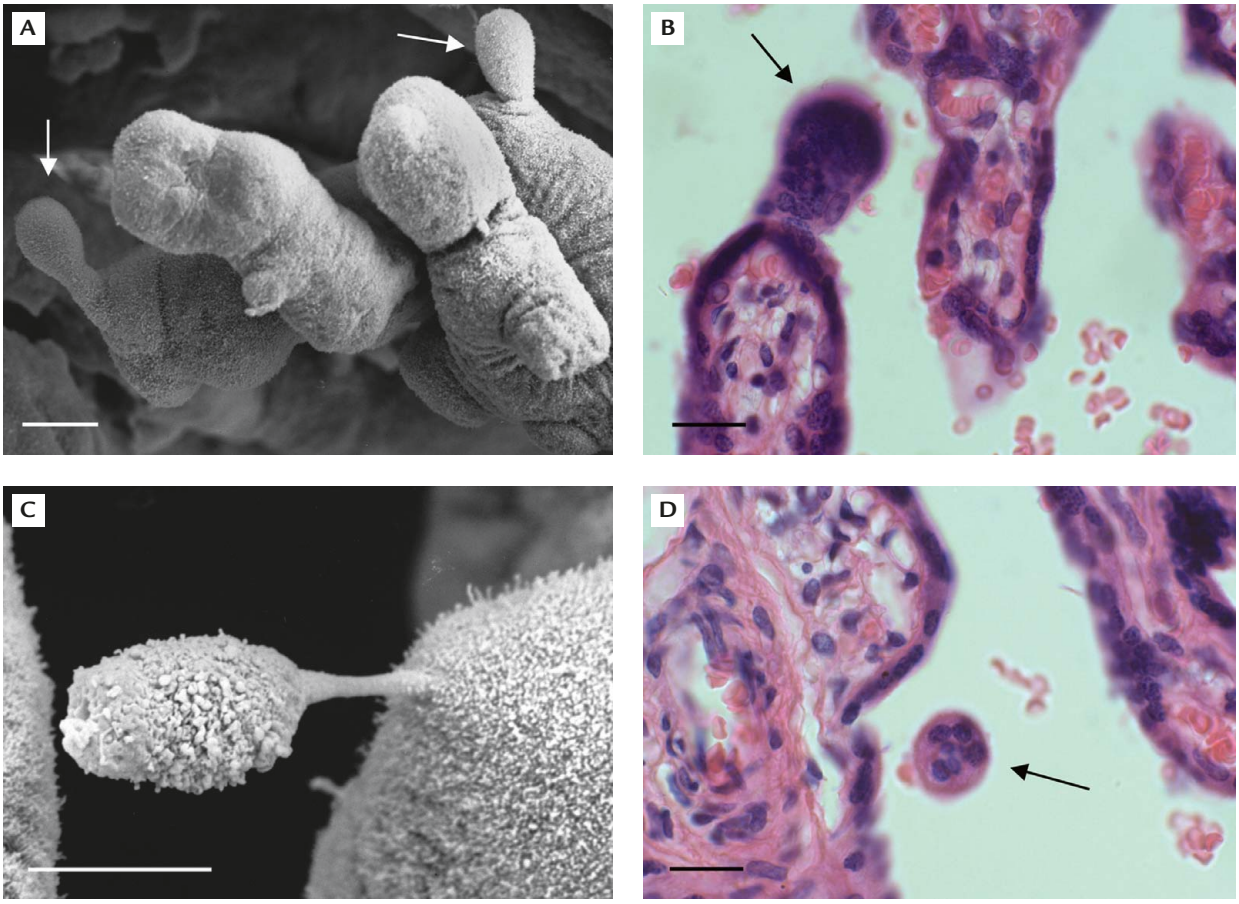

Figure 4. Syncytial sprouts in term placentas. (A) Scanning electron micrograph illustrating sprouts (arrow) arising from the lateral surface of an intermediate villus. Scale bar, 20 μm. (B) Light micrograph of a sprout showing that the nuclei at the proximal end have a euchromatic appearance. Scale bar, 20 μm. (C) An example of a sprout in which the neck region has become extremely attenuated. It is not difficult to imagine how this may break, resulting in deportation. Scale bar, 10 μm. (D) A sprout (arrow) apparently lying free in the intervillous space, from where it will be swept into the maternal circulation. Scale bar, 20 μm.

cells, it is clear that STB nuclei will vary in the time elapsed since their incorporation. This most likely accounts for the range in morphologic appearances of the nuclei, although there is at present no experimental evidence to confirm the hypothesis. For many years, it was believed that the number of progenitor CTB cells reduces as gestation advances, based on the fact that they are observed less frequently in any single histologic section towards term. This would imply that the capacity to renew the STB is impaired, leading to the concept that the placenta has a limited lifespan. However, quantitative assessments using the gold standard disector technique have revealed that the number of both CTB and STB nuclei increases exponentially from 0.06×10^{10} and 0.62×10^{10} at 13–15 weeks to 0.58×10^{10} and 5.81×10^{10} at 37–39 weeks, respectively [24]. The explanation for the apparent demise of the CTB cells is that villous surface area also expands rapidly towards term, and so the nuclei become more dispersed [25]. Thus, while they may appear less frequent in any one section towards term, the total number of sections to view increases greatly.

Not only do the numbers of both sets of nuclei increase throughout gestation, the ratio of the two sets is maintained constant at approximately nine STB nuclei to one CTB nucleus. Furthermore, the volume of trophoblast associated with each nucleus also remains constant, being 1,100 μm^3 at 13–15 weeks and 970 μm^3 at 37–39 weeks [24]. This implies that the trophoblast continues to expand in a hyperplastic fashion from the start of the second trimester through to term, and dispels any notion of inbuilt senescence.

Transcription within the STB

The large number of STB nuclei, coupled with the syncytial nature of the tissue and their contrasting morphologic appearances, has raised questions concerning their activity. Is it necessary for all the nuclei to transcribe

messenger RNA (mRNA) when the latter is able to freely diffuse through the syncytioplasm and, if not, how is activity regulated? Surprisingly, it has been reported that all nuclei within the STB are transcriptionally inactive, as they fail to incorporate [^{3}H]uridine [26,27]. Instead, it is thought that the mRNAs necessary to sustain the activities of the STB are carried in from the CTB cells at the time of fusion. This arrangement would seem to leave the STB, a tissue vital for the maintenance of pregnancy, highly vulnerable to sudden changes in the intrauterine environment, for changes in the transcript profile would require gene expression in the CTB cells followed by fusion into the STB. Given the potential dilution effect, a high number of cell fusions would be required to make a significant impact on the STB transcriptome, and this could soon lead to depletion of the CTB population unless division also occurred. The latter would add further delay to the STB's response.

The apparent lack of transcriptional activity is also not consistent with the observation that some mRNAs, for example those encoding human placental lactogen, are only observed in the STB and not in CTB cells [28].

The experimental evidence demonstrating low or absent STB transcriptional activity was derived from villous explants sampled from either first-trimester or term placentas and maintained in medium containing [^{3}H]uridine under an atmosphere of 95% O_2/5% CO_2 at 2.3 bar for 1 hour [26,27]. No incorporation was seen within the STB nuclei, although it was intense within the CTB nuclei. It should be recognized, however, that these culture conditions are highly unphysiological, particularly the oxygen concentration which, *in vivo*, will be in the order of 3–8% depending on gestational age [29]. We have shown that the STB is particularly sensitive to hyperoxia [30], and so, although the authors reported that the tissue remained viable throughout, it is possible that transcription was inhibited through an as yet unidentified stress.

To address some of these concerns, we have performed pilot studies to assess STB transcription using three different techniques [31]. Firstly, we immunostained sections of first-trimester and term placental villi for the phosphorylated active form of RNA polymerase II (RNAP II). Many CTB nuclei and a proportion of the STB nuclei reacted positively, with a greater proportion being positive in the early than in the late pregnancy samples. Secondly, we immunostained for the dimethylated histone H3K4Me2, a marker of open euchromatin that is associated with active transcription. A widespread positive reaction was observed in the stromal, CTB, and STB nuclei. In the STB, it often co-localized with active RNAP II, but while some STB nuclei were positive for H3K4Me2 and not RNAP II, the opposite was not observed. Thirdly, we performed an *ex vivo* nucleoside incorporation assay using fluorouridine [32]. Villi from first-trimester and term placentas were cultured at 37°C under 2.5% or 5% O_2 for 1 hour with the nucleoside, which was detected using anti-BrdU antibodies. Incorporation into the STB nuclei was observed and was again greater in the early placental tissues than in the term samples. Incorporation was blocked by addition of α-amanitin, a specific inhibitor of RNAP II, and tissue viability was confirmed using the MTT assay.

Although our results require confirmation, they indicate that a proportion of the STB nuclei are transcriptionally active. Furthermore, our data suggest that the proportion of active nuclei reduces as gestational age advances. This is consistent with the morphologic studies of Martin and Spicer [11] that quantified the presence of a nucleolus within STB nuclei. Although we recognize that the nucleolus is the site of ribosomal rather than messenger RNA synthesis, we suggest that transcription of both types of RNA would be equally affected by chromatin condensation. However, we should not fall into the same trap that befell those investigating CTB cell number described in the past. It is possible that the total number of active STB nuclei increases to term, but that they merely become more widespread. Further quantitative measurements using the disector technique are required to test this hypothesis.

Role of Apoptosis in the Cell Biology of the STB

The apparent lack of transcriptional activity, coupled with the dense heterochromatin seen in many STB nuclei, has led to the hypothesis that trophoblast nuclei undergo a series of prolonged apoptotic changes during their incorporation into and time within the STB. This process is thought to culminate with the shedding of end-stage apoptotic nuclei into the maternal circulation [3–5], which has been estimated to amount to 3 g per day at term [33].

It has been postulated that activation of the apoptotic cascade is an essential part of the CTB fusion process, but that completion of apoptotic changes in the incorporated nucleus is suspended for a period of 3–4 weeks by high concentrations of Bcl-2 carried in from the CTB cytoplasm [34]. However, several aspects of this hypothesis are unclear. Firstly, it is difficult to comprehend how locally high concentrations of Bcl-2 are maintained around the nucleus in the syncytial situation, or how those concentrations affect only one nucleus. Localized immunostaining of apoptotic proteins

has been reported in the STB [27]. However, it is possible that these patches represent apoptotic CTB cells which, as we have demonstrated at the ultrastructural level, loose contact with the basement membrane and become engulfed by the STB [35]. This distinction would be extremely difficult to make at the light microscope level. Secondly, the half-life of Bcl-2 is only a fraction of the supposed period of suspension [36], so it is not clear how it may function for so long. Thirdly, even if apoptosis can be suspended for the suggested period, significant numbers of apoptotic STB nuclei should accumulate during the first and second trimesters. However, as stated earlier, aggregations of nuclei displaying condensed chromatin are rare before the last few weeks of pregnancy [12]. Lastly, despite their depiction in many diagrams within the literature, syncytial knots do not have the pedunculated appearance of sprouts, and so will be much more resistant to deportation.

Therefore, the apoptosis hypothesis does not match with morphologic observations, and so, like Smith [37], we question whether these nuclei are truly apoptotic. Despite their dense heterochromatin, they never show the blebbing of the nuclear membrane characteristic of apoptosis. Furthermore, the hypothesis assumes that the number of trophoblastic nuclei remains constant during the last trimester [33], whereas, as described earlier, we now know that this is not the case [24]. We do not doubt that the nuclei within syncytial knots are aged and most likely effete; indeed, we have observed evidence of autophagic vacuoles in the vicinity of the knots, suggesting that the constituents of the nuclei may be recycled within the STB [13]. However, we suggest that there may be other explanations for the dense heterochromatin observed.

Over recent years, it has been recognized that chromatin modifications are powerful regulators of gene activity, influencing the accessibility of the DNA for transcription [38]. Heterochromatin can be either constitutive around the centromeres or facultative, when it is generated in a developmentally regulated manner [39]. The classic example of the latter is the inactivation of one X chromosome to form the Barr body, which is associated with global hypoacetylation of histones H3 and H4. Thus, not all condensed chromatin is apoptotic. We, therefore, question whether the changes in chromatin condensation seen in the STB nuclei are the result of similar mechanisms, such as histone methylation, phosphorylation or acetylation. If so, is this related to the age of the nucleus, does it reflect oxidative or other damage to the DNA, and how is it manifested at the molecular level? Further work is required to answer these and other questions.

Syncytiotrophoblastic Apoptosis in Pathologic Pregnancies

Although apoptosis may not play an integral role in the cell biology and general turnover of the STB, there is no doubt that the process can and does occur in pathologic pregnancies. This may be a localized effect or more widespread. Thus, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive nuclei have been reported in the immediate vicinity of breaks in the STB apical membrane, at the sites of fibrin deposition [40], but whether this is cause or effect remains to be determined.

Equally, a more generalized increase in the number of apoptotic nuclei has been reported in placentas from cases of intrauterine growth restriction, and even more so in preeclampsia. Indices for nuclei of all cell types of 0.17%, 0.24% and 0.39% have been reported for normal, growth restricted and severe preeclamptic placentas, respectively, on the basis of their light and transmission electron microscopic appearances [41,42]. Similar values were obtained for normal and preeclamptic placentas using the TUNEL technique by Allaire et al [43], whereas Ishihara et al [44] obtained values of approximately 1%, 4% and 8% in the STB alone. These differences in reported rates most likely reflect the specificity and sensitivity of the techniques used, and differences in the severity of the pathologies studied. Thus, Smith and coworkers [45] found that TUNEL produces incongruous results for apoptosis in normal first-trimester villi compared with the gold standard of electron microscopy, and concluded that apoptosis is indeed a rare event in these tissues.

Apoptosis in the STB can also be induced *in vitro* by various treatments, providing a model system in which the intermediary signaling pathways can be studied [46]. Our experience has mainly been with hypoxia-reoxygenation, which leads to activation of the p38 and SAP MAPK pathways and cleavage of caspases 9 and 3 [47,48]. This is accompanied by loss of cytochrome C from the mitochondria, and the formation of a novel Mtd/Bok splice variant (Mtd-P) [47,49]. The end result is an increase in cleaved PARP and TUNEL-positive nuclei within the STB. Antioxidants, such as vitamins C and E, or carbon monoxide, can reduce this effect, confirming that oxygen free radicals play a key role in the process [48,50]. Oxidative stress also leads to the release of proinflammatory cytokines such as TNF-α [48], and application of TNF-α to villous explants induces trophoblastic apoptosis [51], providing an alternative autocrine pathway. These changes replicate those seen within the STB following labor, when the placenta is subjected to acute ischemia-reperfusion injury [52].

Placental malperfusion has been implicated as the initiating insult in the pathophysiology of preeclampsia, and it is widely held that deportation of apoptotic debris released from the placenta activates the maternal endothelial cells [53].

Trophoblastic Deportation

As described earlier, clumps of STB nuclei are deported into the maternal circulation from the earliest stages of pregnancy through rupture of the connecting stalks of syncytial sprouts (Figures 3B and 4D). Boyd and Hamilton [21] felt that there should be some function to this phenomenon, as it is so universal in the human. In the past, it has been suggested that it may serve to induce immune tolerance, although this is now difficult to envisage given the absence of expression of major histocompatibility complex class I or class II antigens.

STB fragments containing varying numbers of nuclei have been detected in the uterine venous blood of normal term pregnancies sampled at the time of elective caesarean section, and to a greater extent in cases of preeclampsia [54]. This increase is commonly attributed to increased apoptosis in preeclampsia, but again the morphologic evidence is not very compelling.

Most often, the STB fragments have a tear-drop shape and a cytoplasmic tail, and whilst the nuclei are described as pyknotic with dense heterochromatin, it is impossible to determine whether this condensation occurs pre- or post-release. Because of these appearances, the authors refer to such examples as syncytial sprouts [54]. Occasionally, more squamous clumps are observed, which would appear to match better the morphology of syncytial knots. It is notable that circulating CTB cells were also observed in preeclampsia, but only very rarely in normal pregnancies [54].

Most of the deported fragments containing nuclei would, therefore, appear to be syncytial sprouts, which have never been implicated in the apoptotic turnover of the STB. There are at least two reasons why sprouts may be deported to a greater extent in preeclamptic placentas. Firstly, sprouts are more common in preeclamptic placentas [55]. Secondly, the deficient conversion of the spiral arteries that is associated with preeclampsia means that maternal blood enters the placenta intervillous space with greater velocity than normal. This is sufficient to cause placental lakes devoid of villi to develop opposite the arterial openings, and turbulent flow is often seen in these cavities [56]. It is, therefore, not unreasonable to predict that sprouts would be dislodged more frequently under these conditions. Some evidence that this might be the case is provided by the fact that the burden of microparticulate STB debris is increased when higher placental perfusion pressures are used *in vitro* [57].

The fact that CTB cells are also deported in preeclampsia suggests that more severe damage is occurring to the villous covering rather than apoptotic changes. A characteristic of the latter is that cell integrity is retained, and so, if the STB nuclei are being extruded through the release of syncytial knots, then the underlying CTB cells should not be exposed. Ischemic damage is a prominent feature of the preeclamptic placenta, however, ranging from isolated areas of trophoblast necrosis to frank infarcts [18], the latter being closely associated with atherotic changes in the supplying spiral arteries [58]. Thus, one might expect areas of degenerating and necrotic trophoblast to be sloughed and deported in these circumstances. This seems a more likely explanation for the origin of the placental debris deported into the maternal circulation, and fits better with the pathophysiology observed. In particular, the debris is proinflammatory, activating maternal immune and endothelial cells [53,59]. In contrast, a key aspect of apoptosis is that the process does not stimulate an inflammatory response.

Overview

There can be no doubt that CTB cells are constantly recruited into the STB during gestation at what appears to be a uniform rate, at least between 13 weeks and term. Consequently, nuclei of different ages and functional activity accumulate within the STB. The appearances of these nuclei vary; some are euchromatic and contain a prominent nucleolus, whereas others display dense heterochromatin with an involuted nucleolus. Whether these appearances reflect epigenetic changes secondary to oxidative damage or developmental programming is currently uncertain, but we conclude that there is little evidence to support the current hypothesis that apoptosis and subsequent shedding underpin STB nuclear turnover. Clumps of STB nuclei are undoubtedly deported from the placenta into the maternal circulation, but their healthy morphology indicates that the majority arise from the rupture of syncytial sprouts. In pathologic pregnancies, this deportation may increase owing to the greater frequency of sprouts and the greater force with which maternal blood enters the intervillous space. In addition, ischemic damage to the villous tree, secondary to atherosis in the maternal spiral arteries, can cause localized necrotic changes and the release of more irregular STB and CTB masses. These, along with microparticulate debris, most likely cause the heightened

inflammatory response that has been implicated in the pathophysiology of preeclampsia.

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