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Current opinion

Turnover of human villous trophoblast in normal pregnancy: What do we know and what do we need to know?

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ABSTRACT

How the turnover of villous trophoblast is regulated is important for understanding normal and complicated pregnancies. There is considerable accord that syncytiotrophoblast (STB) grows and is refreshed by recruiting post-mitotic cells from the deeper cytotrophoblast (CTB). Nuclei in STB exhibit a spectrum of morphologies and packing densities and, until recently, there seemed to be a consensus that this variation reflected a transition from an early undifferentiated CTB-like phenotype to a long pre-apoptotic and brief apoptotic phase. In these later phases, nuclei are sequestered in clusters (syncytial knots) prior to extrusion as part of normal epithelial turnover. Early in gestation, nuclear clustering and formation of protrusions (syncytial sprouts) also occurs as a preliminary to villous sprouting. Nuclei in these clusters have a CTB-like phenotype and some sprouts may also detach from STB and pass into the uteroplacental circulation. However, this apparent consensus has been challenged and new interpretations of events in the proliferative (CTB), terminal differentiation (STB) and deportation compartments have emerged. Several different types of STB fragment are deported in normal pregnancy: larger multinucleate STB fragments, smaller uninucleate elements with CTB-like morphology, anucleate cytoplasmic fragments, microparticles and nanovesicles. This review identifies points of agreement and disagreement and offers possible avenues of future research. An obvious need is to standardise best practice in several areas including choosing appropriate references for cell cycle phase labelling indices and combining immunolabeling of cell cycle and apoptosis markers (at LM or TEM levels) with design-based stereological estimates of absolute numbers of cells and nuclei in different compartments throughout normal gestation. This would also provide a surer foundation for interpreting results from different research groups and changes in normal and complicated pregnancies.

1. Introduction

> "The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed." (*Albert Einstein, 1879–1955*)

The conduct of good science and the advancement of its knowledge base depends not only on the application of rigorous study design, sound sampling principles, newer and more accurate technology and the use of precise and unbiased or minimally biased investigative tools. It depends also on the attributes of researchers and their ability and willingness to remain curious, critical, unprejudiced and receptive to different or new ideas. Thomas Huxley is reported to have said that the tragedy of science is the slaying of a beautiful hypothesis by an ugly fact. The measure of a scientific researcher and the community in which he/she operates is how one deals with 'ugly facts'. A researcher who is ready to accept the possibility of misinterpretation or error is better than one who always claims exclusivity of the correct interpretation. Sometimes, when extreme views are held, the correct interpretation might lie somewhere in between or require fresh investigations and experiments in order to clarify the situation. It is true to say that some elements of research are inductive, relying on the marshalling of facts to prove a general statement or support an idea. The hallmark of good science is the formulation of a hypothesis and the designing of an experiment that can test it.

As the philosopher Karl Popper stated "You cannot prove that all swans are white by counting white swans. But you can prove that not all swans are white by counting one black swan."

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Recently, in the biomedical area of villous trophoblast turnover in the human placenta, new interpretations of events in its proliferative and terminal differentiation compartments have emerged and an apparent consensus has been challenged [1–4]. The principal points of contention [1,3] can be summarised as follows: (i) There is little evidence to support the idea that turnover of syncytial nuclei occurs in normal placenta. (ii) There is little evidence that it occurs *via* an apoptosis-related process. (iii) Epigenetic modifications, rather than apoptotic events, underlie changes in the heterochromatin content of STB nuclei. (iv) A proportion of STB nuclei remain transcriptionally active throughout gestation. (v) Syncytial knots are more resistant to deportation than syncytial sprouts. (vi) Apoptosis is normally confined to CTB cells and areas of STB associated with fibrin-type fibrinoid. (vii) Apoptotic events seen in CTB may be mistakenly ascribed to STB.

This review is an attempt to address the points of agreement and disagreement concerning trophoblast turnover and to offer possible avenues of future research.

2. Growth of placental villi and villous trophoblast through gestation

From about mid-gestation, the surface area available for transplacental exchange expands enormously by formation of new terminal villi [5,6]. Early villous sprouting is initiated by cells in regions of CTB and villous stroma with high proliferative activity. Post-mitotic CTB cells are recruited into STB which creates attenuated protrusions called syncytial sprouts and these subsequently acquire a vascularised stroma to become mesenchymal villi. These initial forms develop into a variety of different villous types which include stem, intermediate and terminal villi [6]. Around mid-gestation, mesenchymal villi transform into mature intermediate villi that generate enormous numbers of well-vascularised and small calibre terminal villi. This process continues to term.

These changes are associated with temporal variation in the supply of oxygen and nutrients across gestation, as reviewed recently [7]. Before 10–12 wk, the intervillous oxygen tension is less than 3 kPa and rises to 5–11 kPa before steadying to about 5 kPa at term. Early on, expression of HIF-1a (which regulates responses to oxygen levels) is high in villi and affects cell proliferation and apoptosis. The onset of maternal perfusion of the intervillous space is also accompanied by increases in the activities of antioxidant enzymes [8].

An important component of villi is the trophoblast. Early in gestation, the CTB layer appears continuous but, as gestation progresses, it becomes discontinuous as cells alter shape and disperse more widely [6,9–11]. The apparent sparsity of cells on histological sections is explained by the fact that increases in cell number do not match the expanding trophoblast surface so that CTB cell bodies become more widely separated [12]. Proliferation of CTB cells and incorporation into STB are elements of the growth in villous surface area and both are sensitive to hypoxia which also affects apoptosis in CTB cells [7].

The fact that villous trophoblast has a syncytial compartment is unique for a human epithelium. Whilst it may have evolved to allow invasion of maternal tissues without compromising the integrity of the maternofetal intervascular barrier, a syncytium offers other benefits. For instance, a syncytium allows redistributions of STB cytoplasm to produce locally thick and thin regions. Together with changes in villous surface area and the peripheralisation and dilation of fetoplacental capillaries in the stroma, these improve the efficiency of gas and nutrient transfer by passive diffusion by reducing the harmonic mean intervascular distance [13]. STB is also sensitive to oxygen tensions which can induce a form of apoptosis different from that in CTB [7].

Overall, the increase in surface area outpaces growth in trophoblast volume within which compartments and STB regions respond differently [14]. After 10 wk of gestation, volume growth of CTB lags behind whilst, within STB, growth in volume and surface area of regions of nuclear clustering and in the surface area of sites of de-epithelialisation exceed that of other regions. In addition, deposition of perivillous fibrin-type fibrinoid correlates well with villous surface area.

We now examine these events in more detail in the context of trophoblast proliferation, differentiation and loss during normal pregnancy.

3. Villous trophoblast – a continuously renewing epithelium

Like cells of the epidermis, intestinal epithelium and blood-forming tissues, villous trophoblast is continuously renewing [15]. CTB cells lie on a basal lamina and exist in one of the phases (G_0, G_1, S, G_2 or M) of the mitotic cell cycle. Overlying CTB is the syncytium. CTB cells divide continuously throughout gestation and some of their progeny are recruited into STB by membrane fusion and cytoplasmic confluence. Estimates of numbers of CTB and STB nuclei from 13 wk to term are consistent with the notion that CTB cells undergo several divisions before fusing into STB [15]. Indeed, fusion into the syncytium makes trophoblastic epithelium uniquely different from epidermis, intestinal and other epithelia where cells retain their individuality as they migrate and differentiate between proliferation and extrusion sites.

Once a CTB cell has been incorporated into STB, a tightly-controlled sequence of differentiation occurs. An early phase of maturation and continuing transcription is followed by one of terminal differentiation in which some nuclei show substantial changes in shape, packing density, chromatin condensation and nuclear envelope integrity and loss of transcriptional activity [16–18].

4. CTB – the proliferation and recruitment compartment

Although the CTB layer becomes discontinuous during gestation, its cells are not truly independent. Ultrastructural and confocal studies on term placentas [11] have shown that they possess fine processes which are contiguous with those of surrounding cells. At this stage, CTB cells and their processes cover about 40% of the basal lamina surface [11] and 10% of trophoblast volume [14].

4.1. CTB cells and 'proliferation markers'

Measures of proliferative activity are complicated by the fact that 'proliferation markers' are usually applied uncritically. Actually, they mark one or more phases of the cell cycle [19]. Simple counts of mitotic figures on tissue sections are used to estimate a mitotic index which is taken to represent the fraction of cells in M phase. In fact, this index is not unbiased because it is based on an incorrect implicit assumption, viz. that relative numbers seen on sections equate to relative numbers in 3D. It is also an approximation because it accounts for only some of those phases of mitosis in which mitotic figures are discernible (e.g. metaphase and anaphase).

S phase, which involves synthesis of DNA and the activity of associated proteins, can be labelled also (autoradiographically with tritiated thymidine or immunochemically with bromodeoxyuridine, BrdU) to obtain the fraction of cells or nuclei which are in S phase. Other phase markers include the Ki-67 family (including MIB-1) and proliferation associated nuclear antigen, PCNA. Ki-67 protein is present during all active phases of the cycle (G_1, S, G_2