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IFPA Award in Placentology Lecture: Biology of the placental syncytiotrophoblast – Myths and facts

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ABSTRACT

About 15 years ago apoptosis was attributed a role in the development of the human placenta. Since then an increasing number of publications has shown that programmed cell death plays an essential role in placental growth and differentiation, especially in the villous trophoblast. During the last ten years a concept was established linking the progress of apoptosis to differentiation of cytotrophoblasts and syncytiotrophoblast. Thus, development and maintenance of the syncytiotrophoblast depends on the precise orchestration of different processes and stages of the apoptosis cascade.

This review focuses on the maintenance and growth of the syncytiotrophoblast as well as the deportation of trophoblast material into the maternal circulation. Nuclear morphology is related to transcriptional activity, RNA protection and storage strategies are discussed and the differences between syncytial expression rates of RNA and protein are highlighted. Moreover, deportation of trophoblast fragments is related to the relevant morphological structures (syncytial knots) and to their effects on the maternal system. Finally, different modes of release of trophoblast fragments such as apoptotic, apo-necrotic and necrotic are discussed as being responsible for the maternal inflammatory response during pre-eclampsia.

1. Introduction

The villous trees of the human placenta are covered by a singular epithelial tissue, the villous trophoblast. This tissue is unique in that it is composed of a layer of mononucleated villous cytotrophoblasts covered by a layer generated and maintained by syncytial fusion. This villous syncytiotrophoblast does not contain lateral cell borders and thus is a typical syncytium with a multinucleated appearance and membranes only on the apical and basal side. It covers all villous trees of a placenta and hence there is a single syncytiotrophoblast in each placenta [1].

As the outermost layer covering the chorionic villi, the syncytiotrophoblast is the only villous tissue in a placenta that comes into direct contact with maternal blood throughout pregnancy. In this localization the syncytiotrophoblast is essential for the embryo and fetus in terms of immunological defense mechanisms, active transport and expression of proteins, hormones, cytokines and chemokines.

Recently a new hypothesis has been formulated in an attempt to shed some new light on the maintenance and turnover of the syncytiotrophoblast [2]. New ideas and hypotheses are crucial for the development of science. At the same time such hypotheses need to have a solid base and relevant experimental data to support the new idea. Thus, there seems to be a need to clarify the way the syncytiotrophoblast is maintained and how apoptotic material is deported by syncytial knots.

2. Syncytial growth and *Caenorhabditis elegans*

Volume growth of the syncytiotrophoblast may be achieved not only by incorporation of cytotrophoblasts; rather syncytial volume might further be gained by internal growth of the syncytiotrophoblast. Similar mechanisms have been discussed in volume gain of the hyp7 syncytium of *Caenorhabditis elegans* (*C. elegans*) [3]. This study found that fusing seam cells (the precursor cells of the hyp7 syncytium) contributed less than 10% to the total volume increase of hyp7. Knight et al. [3] speculated that the main function of seam cell proliferation and fusion may be to supply the hyp7 syncytium with additional genomes for the purpose of growth.

Growth of the syncytiotrophoblast in terms of increasing the number of nuclei is provided by continuous fusion of cytotrophoblasts with the syncytiotrophoblast throughout gestation. In the first half of pregnancy, the cytotrophoblast layer is a complete layer of cuboidal mononucleated cells. During the course of pregnancy, cytotrophoblasts change their cellular shape. In the second half of

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pregnancy the cytotrophoblasts adopt an octopoid shape with a rounded perinuclear body from which thin cytoplasmic processes extend towards neighbouring cells [4]. Thus, at term the distance between single cytotrophoblast nuclei underneath the syncytiotrophoblast is far greater than in the first half of pregnancy. In a section through a placental villus it now seems as if this layer has become incomplete.

The reduction of cytotrophoblasts per syncytial area can be explained by a reduced need of high cell numbers since the relative growth of the syncytiotrophoblast decreases during pregnancy. The area of the villous surface, i.e. the syncytiotrophoblast surface, changes between 1.8 and 3.6-fold every four weeks in the first half of pregnancy, while these changes are reduced to a 1.2–1.4-fold increase every four weeks after 30 weeks of gestation (fold changes calculated from data in Table 28.1 in [1]).

Interestingly, during gestation the ratio of syncytial nuclei to cytotrophoblast nuclei remains constant at a level of about 8 syncytial nuclei per cytotrophoblast nucleus [5]. Thus, the number of nuclei in both compartments shows a similar increase with a 6.0-fold increase in nuclear number in the cytotrophoblast compartment and a 6.5-fold increase in the syncytiotrophoblast (calculated from [5]). In the same time window the volume of the syncytiotrophoblast increases about 7.9 times from 10 to 41 weeks of gestation, while the cytotrophoblast volume increases only 4.3 times [6]. Thus, there is growing syncytial volume per syncytial nucleus during gestation (increase by 22%) while the volume per nucleus in cytotrophoblasts decreases during gestation (decrease by 28%). This reverse relation in volume growth of syncytiotrophoblast and cytotrophoblast during gestation further explains the reduction of cytotrophoblasts per syncytial volume/area.

3. Morphology of villous trophoblast nuclei

The cells residing in the layer of the cytotrophoblast display various stages of differentiation from undifferentiated and proliferative cells to highly differentiated cells just prior to fusion with the syncytiotrophoblast. All nuclei within the latter have left the cell cycle and thus the syncytiotrophoblast is terminally differentiated and no longer capable of undergoing mitosis.

Cytotrophoblasts change their appearance from proliferation to high differentiation, which can be traced by looking at the nucleus. It starts as a large, ovoid and mostly euchromatic nucleus surrounded by few organelles. It ends just prior to fusion as a slightly darker and more heterochromatic nucleus [1] (see also Fig. 1A in [2]). These latter cells contain numerous mitochondria and free ribosomes as well as large amounts of rough endoplasmic reticulum. The high activity of these cells was proven by the incorporation of large amounts of ^{3}H-uridine [7,8]. Moreover, Hoshina et al. [9] have shown that these cells contain high copy numbers of mRNA such as that for the α subunit of human chorionic gonadotropin.

Mayhew et al. [5] have quantified the proportion of nuclei that are mostly euchromatic or heterochromatic, with 80.2% of all cytotrophoblast nuclei being mostly euchromatic and 17.4% being mostly heterochromatic. The more heterochromatic group may represent nuclei of highly differentiated cytotrophoblasts just prior to fusion with the overlying syncytiotrophoblast. The changes in cytotrophoblast nuclear morphology can be visualized throughout gestation.

Interestingly, a nucleolus is present throughout all stages of cytotrophoblast differentiation, may be because ribosomal RNA is not only needed for the mononucleated stage but needs to be transferred to the syncytial stage as well.

The morphology of syncytial nuclei at early stages of pregnancy is similar to that of the majority of syncytial nuclei later in gestation. As has been nicely depicted by Burton and Jones [2], already at seven weeks of gestation the syncytial nuclei display a much darker and electron denser appearance than cytotrophoblast nuclei and already start to show indentations with signs of heterochromatin aggregations.

At the end of pregnancy the syncytial nuclei still display the same dark appearance and clear indentations as early in pregnancy. In regions of the syncytiotrophoblast with no signs of accumulation of aged nuclei (termed non- syncytial knot regions; [5]), 60.7% of the nuclei are less heterochromatic while in syncytial knot regions 63.4% of the nuclei display high levels of heterochromatin aggregation [5].

Although most of the syncytial nuclei may display a dark but still not very strong heterochromatin appearance, there are two specific subsets of syncytial nuclei displaying a different morphology. (1) Those nuclei freshly incorporated by syncytial fusion with a cytotrophoblast look "younger" with less frequent indentations, are more euchromatic and contain a prominent nucleolus [10]. (2) The second subset of nuclei within the syncytiotrophoblast has already undergone changes that resemble those of nuclei in late apoptotic cells. They display increasing chromatin aggregations and finally show annular chromatin condensation with very intense staining using any DNA dye. Such "older" nuclei tend to accumulate at specific sites within the syncytiotrophoblast, the syncytial knots. The increasing number of "older" nuclei later in gestation may explain the finding that syncytial nuclei containing a nucleolus decline from about 55% early in pregnancy to about 8% at term [11].

4. Nuclear morphology and its relation to transcriptional activity

Gene expression during cellular differentiation is regulated by the availability of regulatory proteins and the accessibility of the DNA to the transcriptional apparatus. Position-effect variegation may place euchromatic genes adjacent to regions of heterochromatin and thus result in inactivation of euchromatic genes by heterochromatin spreading [12]. Hence, increased heterochromatization as found in nuclei of the syncytiotrophoblast could well lead to rearrangement of euchromatic chromosomal regions into heterochromatic nuclear compartments, in turn leading to repression of the expression of specific genes. There is a dynamic interplay between the mechanisms resulting in heterochromatin development (modifications of histones, alterations of structural nuclear components) and the mechanisms generating euchromatin resistant to heterochromatization [12].

Heterochromatization involves the development of constitutive heterochromatin as well as facultative heterochromatin. Constitutive heterochromatin is independent of the cell's state of differentiation and is mostly associated with gene-poor highly repeated DNA sequences. Facultative heterochromatin is especially abundant in terminally differentiated cells and is accompanied by general nuclear condensation and large-scale gene repression [13].

During blood cell differentiation many genes that are inactivated become relocated to heterochromatin regions, mostly at the nuclear periphery. Ikaros is an example of a protein forming protein bridges between repressed genes and heterochromatin, thus tethering such genes to regions of no expression [14]. Transcriptional repressors such as HDAC-1 and MeCP2 can be found in the heterochromatin regions of the nuclear margin in highly differentiated cells [15].

So far we are just at the beginning to understand the interplay between the morphological changes of the syncytiotrophoblast nuclei and the regulation of gene expression in such nuclei. Interestingly, the three proteins mentioned above in blood cell differentiation have all been shown to be expressed in the placenta/trophoblast. Ikaros and HDAC-1 are expressed in choriocarcinoma