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Review: Human trophoblast fusion and differentiation: Lessons from trisomy 21 placenta

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ABSTRACT

The syncytiotrophoblast layer plays a major role throughout pregnancy, since it is the site of numerous placental functions, including ion and nutrient exchange and the synthesis of steroid and peptide hormones required for fetal growth and development. Inadequate formation and regeneration of this tissue contributes to several pathologies of pregnancy such as intrauterine growth restriction and preeclampsia, which may lead to iatrogenic preterm delivery in order to prevent fetal death and maternal complications. Syncytiotrophoblast formation can be reproduced *in vitro* using different models. For the last ten years we have routinely purified villous cytotrophoblastic cells (CT) from normal first, second and third trimester placentas and from gestational age-matched Trisomy 21 placentas. We cultured villous CT on plastic dishes to follow the molecular and biochemical aspects of their morphological and functional differentiation. Taking advantage of this unique collection of samples, we here discuss the concept that trophoblast fusion and functional differentiation may be two differentially regulated processes, which are linked but quite distinct. We highlight the major role of mesenchymal-trophoblast cross talk in regulating trophoblast cell fusion. We suggest that the oxidative status of the trophoblast may regulate glycosylation of proteins, including hCG, and thereby modulate major trophoblast cell functions.

1. Introduction

The syncytiotrophoblast layer plays a major role throughout pregnancy, since it is the site of numerous placental functions, including ion and nutrient exchange and the synthesis of steroid and peptide hormones required for fetal growth and development [1,2]. Among them, progesterone and human chorionic gonadotropin (hCG), are essential for the maintenance of human gestation. The multinucleated syncytiotrophoblast is regenerated throughout pregnancy by a continuous turnover process including proliferation of underlying mononuclear cytotrophoblasts, fusion of these cytotrophoblasts into syncytiotrophoblast and progression toward apoptosis. Inadequate formation and regeneration of this tissue leads to several pathologies of pregnancy such as intrauterine growth restriction and preeclampsia, which may lead to iatrogenic preterm delivery in order to prevent fetal death.

2. Trophoblast fusion *in vitro*

Syncytiotrophoblast formation can be reproduced *in vitro* using different models. Choriocarcinoma cells, *ie* BeWo cells, are able to fuse in the presence of cAMP to form a multinucleated syncytium. However, the last step of differentiation, the gathering of nuclei into a central mount, is missing. In addition, these cells are transformed and some trophoblastic functions are not present [3]. Placental explant cultures *in vitro* are useful for studying cell function [4] and more recently denuded villous explants from early placenta have been used to follow syncytiotrophoblast repair *in vitro* [5]. In these models, cell–cell interactions are present, but cell–cell communication studies are difficult as is quantification of trophoblastic hormones. Purified villous cytotrophoblastic cells (CT), cultured on plastic dishes, allow us to follow morphological and functional

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differentiation of villous CT [6]. In this model, mononucleated villous CT leave the proliferative stage, adhere to the plastic dishes, aggregate, establish efficient communication and signal exchange, and fuse to form a multinucleated syncytiotrophoblast with pregnancy specific hormone production. This model allows accurate biochemical studies. Villous CT fuse *in vivo* with the overlying syncytiotrophoblast. The ability of isolated primary trophoblasts to fuse with each other *in vitro* has been recently discussed [7].

During the last ten years we have routinely used these primary cultures to study human trophoblast fusion and differentiation. We isolated and cultured villous CT from normal first trimester ($n = 1250$) and term placentas ($n = 540$) as well as from second trimester trisomy 21 placentas ($n = 77$) and normal karyotype gestational age-matched control placentas ($n = 44$). Syncytium formation was followed by fixing and immunostaining cells so that the distribution of desmoplakin and nuclei in cells (DAPI staining) could be observed [8]. Desmoplakin staining is present at the intercellular boundaries in aggregated cells but progressively disappears as the syncytium is formed. After 72 h of culture, mononuclear cells and the number of nuclei in syncytia are counted and then the fusion index $(N - S)/T$ is determined, where N is the number of nuclei in the syncytia, S is the number of syncytia, and T is the total number of nuclei counted.

The expression of beta and alpha hCG subunits was studied by quantitative RT-PCR [9] and secretion of total hCG and its subunits to the culture medium routinely evaluated using specific immunoassays [10].

In this review we summarize our experience of these large series of normal and T21 primary trophoblast cells. We discuss how these primary cultures of villous CT are helpful in understanding the molecular biology and biochemical aspects of human trophoblast fusion and differentiation.

3. Trophoblast abnormalities in trisomy 21-affected placentas

Trisomy of chromosome 21 (T21), which causes the phenotype known as Down syndrome, is the major known genetic cause of mental retardation and is found in around 1:800 live births [11].

Little is known about placental development in this aneuploid condition despite the fact that the trophoblast and the mesenchymal core of the villi carry the genetic abnormality. Different histological observations have indicated that T21 may be associated with villous hypovascularity [12], intrastromal cytotrophoblastic cells, persistence of nucleated red blood cells and abnormalities of the trophoblastic layer [13]. Indeed an increased percentage of two-layered trophoblast is observed in T21-affected placentas, suggesting a delay in villous maturation and trophoblast differentiation [13,14].

Cultured CT isolated from T21 placentas aggregate but fuse poorly or belatedly [15,16] (Fig. 1). In our unique experience of primary culture of human CT isolated from second trimester control (normal karyotype) gestational age-matched placentas ($n = 44$) and T21 placentas ($n = 71$), we observed that abnormal trophoblast fusion occurs in more than 90% of the primary cultures of T21 cells. In addition, this *in vitro* defect or delay in syncytiotrophoblast formation is characterized by a dramatic decrease in the synthesis and secretion of syncytiotrophoblastic pregnancy-associated hormones [17]. This is illustrated in this large series of primary cultures by a significant decrease of hCG secretion into the culture medium (Fig. 1).

4. Abnormal oxidative status of T21 trophoblastic cells impairs cell fusion and differentiation

Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide ion (O_2^-), and hydroxyl radical ($^{\bullet}OH^-$) are generated in cells in response to stimulation by various hormones, growth factors, and cytokines. The oxygen radicals generated appear to act as second messengers in transmembrane signaling pathways to modulate cellular functions such as cell proliferation and differentiation [18,19].

Cellular oxidative status is determined by the balance between ROS production and their destruction by a variety of antioxidant enzymes. The primary antioxidant activity in the cell, which regulates the level of O_2^- and its reactive progeny, is due to the superoxide dismutases (SODs). Mammalian cells have a mitochondrial Mn-SOD (SOD-2), a cytoplasmic Cu, Zn-SOD (SOD-1), which also is found in peroxysomes, and an extracellular SOD (SOD-3), which is a Cu, Zn-SOD that is immunologically distinct from the classical Cu, Zn-SOD. These metalloenzymes act to dismute generated superoxide radicals to oxygen and H_2O_2. In turn, catalase, along with peroxidases such as glutathione peroxidase, catalyzes the decomposition of H_2O_2 to water and oxygen [20].

Both cytosolic Cu, Zn-SOD and mitochondrial Mn-SOD are expressed in human cytotrophoblasts [21,22]. We demonstrated a modulation of SOD-1 expression and activity with *in vitro* differentiation of human villous CT [23].

It is known that SOD-1 is located on human chromosome 21, and that it is overexpressed in different T21-affected cell types [24]. In T21 villous CT we demonstrated an overexpression of SOD-1 expression and activity, in accordance with a gene dosage effect [23,25], and a subsequent abnormal oxidative status of the villous CT as shown by a large increase in catalase activity [17].

Overexpression of SOD-1 in normal cytotrophoblasts impairs syncytiotrophoblast formation. This is associated with a significant decrease in mRNA transcript levels and secretion of hCG and of other hormonal markers of syncytiotrophoblast. We confirmed abnormal cell fusion by overexpression of GFP-tagged SOD-1 in cytotrophoblasts. In addition, a significant decrease of syncytin-1 transcript levels was observed in SOD-1 transfected cells [25].

Taken together the results in this genetic model confirm the major role of the oxidative status of the trophoblastic cells in fusion and differentiation. In addition, as illustrated in Fig. 2, treatment of T21 villous CT in culture by an antioxidant such as *N*-acetylcysteine (NAC) overcame the abnormal T21 fusion. A significant increase of the fusion index was observed after exposure to NAC. However the ability of T21 villous trophoblast to secrete hCG was not restored or even decreased.

5. Trophoblastic hCG glycosylation

Chorionic gonadotropin is specific to humans and anthropoid primates. HCG is a glycoprotein composed of an alpha subunit, which is common to other peptide hormones such as LH (luteinizing hormone), FSH (follicle stimulating hormone) and TSH (thyroid stimulating hormone), and a beta subunit, which confers the biological specificity of the hormone. HCG alpha is encoded by a single gene on chromosome 6 and hCG beta by a cluster of six genes on chromosome 19, the CGβ5 and CGβ8 genes being those most actively transcribed and contributing about 60–80% to the total pool of β-subunit mRNA transcripts in the placenta [26-28].

In normal human placenta, hCG is produced by the trophoblast – not only by the syncytiotrophoblast but also by the invasive trophoblast [29].

HCG is one of the first trophoblast signals detected in maternal blood and is used in pregnancy diagnosis. HCG and its free beta subunit are detected in the maternal blood from the second week of pregnancy and their levels increase until reaching a peak at ten to twelve weeks and then decrease gradually, whereas hCG alpha levels increase progressively up to term [30]. Maintenance of pregnancy during early gestation depends on the synthesis of hCG,