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Review: An overview of molecular events occurring in human trophoblast fusion

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ABSTRACT

During human placentation, mononuclear cytotrophoblasts fuse to form a multinucleated syncytia ensuring hormonal production and nutrient exchanges between the maternal and fetal circulation. Syncytia formation is essential for the maintenance of pregnancy and for fetal growth. The trophoblast cell fusion process first requires the acquisition of cell fusion properties, then cells set up plasma membrane protein macrocomplexes and fusogen machinery that trigger cell–cell fusion. Numerous proteins have been shown to be directly involved in the initiation of trophoblast cell fusion. These proteins must expressed at the right time and in the right place to trigger cell–cell fusion. In this review, we describe the role of certain fusogenic protein macrocomplexes that form the scaffold for the fusogen machinery underlying human trophoblastic-lipid mixing and merging of cell contents that lead to cell fusion in physiological conditions.

1. Overview

Cell fusion processes are essential for fertilization, fetal and placental development, skeletal muscle formation and bone homeostasis [1–4]. Recently, cell fusion was also shown to play a role in metastasis [5]. Cell fusion involves processes different from those involved in the fusion of vesicles to the plasma membrane, in terms of the necessary proteins, macrocomplexes and cellular signaling pathways. However, the mechanical properties and biophysics of membrane lipids show certain similarities. The purpose of this review is not to provide a list of all proteins and signaling pathways involved in trophoblast fusion (in human primary cell cultures and cell lines), as these have already been reviewed [6]. Instead, we propose to describe for the first time molecular events underlying the main steps of the fusion process observed in the physiological model of cultured human primary trophoblasts, as supported by biochemical and molecular biology experiments.

Cell fusion and syncytia formation involves the mixing of plasma membrane components and cell contents between two or more cells. Two different types of cell fusion can be distinguished. In homokaryon formation, during placentation, skeletal formation and bone resorption for example, homotypic cells (cells of similar lineages) fuse together, while in heterokaryon formation (during fertilization and metastasis for example), heterotypic cells (cells of various origins) fuse together. Cell fusion processes occurring in a variety of biological contexts share many steps that are tightly regulated in space and time [7]. As described by Aguilar et al., these processes can be separated into three main steps [7,8]. The first "competence" stage involves a loss of proliferative activity, followed by induction and differentiation into fusion-competent cells. The second, "commitment" stage is characterized by cell migration, recognition of fusion partners and cell–cell adhesion, which initiate gap junction communication leading to synchronization and exchange of fusogenic signals. This triggers the organization of protein complexes necessary to promote cell–cell fusion, with apposition of the outer lipid monolayers of the two cell membranes before the opening of a fusion pore and progression to full fusion with mixing of cellular contents [9]. Exclusively and in some specific conditions, somatic cells can remain competent for a new round of fusion, generating giant syncytia and/or ensuring regeneration, as observed in muscle and placenta for instance.

2. Introduction

Human embryo implantation requires placentation, a process in which fetal cytotrophoblasts invade the maternal endometrium to

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form an interphase with the maternal circulation, ensuring effective exchange of gases and nutrients [10]. During human implantation, the blastocyst is composed of a trophoectoderm and embryoblast. The trophoectoderm (of trophoblastic origin) undergoes intercellular fusion to generate primitive syncytia, which promote implantation of the embryo into the maternal endometrium. Two weeks after conception, villi containing cytotrophoblasts and syncytiotrophoblast appear. Throughout human pregnancy, syncytia are maintained by continuous fusion of cytotrophoblasts with overlying syncytiotrophoblasts, in a regenerative process. These multinucleated syncytia in contact with maternal blood control all feto-maternal exchanges and produce and secrete pregnancy-specific hormones [11,12]. The cytotrophoblast plays an essential role during human pregnancy, through its ability to differentiate into syncytia. These syncytia allow feto-maternal exchanges necessary for fetal growth. For ethical reasons, studies of primitive syncytia are not allowed in certain countries. Thus, the molecular mechanisms underlying cell fusion processes presented in this review reflect syncytial repair processes and the fusion of mononuclear cytotrophoblasts into an overlying syncytiotrophoblast.

3. Human trophoblast fusion models

The fusion process observed in the human placenta is reproducible *in vitro* by using purified cytotrophoblasts (Fig. 1), which aggregate and then fuse to form non proliferative, multinucleated, hormonally active syncytiotrophoblasts [13]. Villous explants containing the whole villous structure and cell types are often used to study placental physiology. This model has the advantages of closely matching human placental physiology but is limited for intensive biochemistry and molecular biology studies. Taking this into account, and in view of our own work on human trophoblast cell fusion, we believe that human primary trophoblast culture remains the most suitable and robust model for studying human trophoblast cell fusion, syncytiotrophoblast formation and regeneration. Studies of primary human trophoblasts require validation of cell purity by means of immunolocalization [14]. Trophoblast fusion is quantified in fusion assays, where fusion indices are calculated as the ratio of the number of nuclei in the syncytia divided by the total number of nuclei. A syncytium is defined as at least three nuclei surrounded by a cell membrane, as shown by discontinuous desmoplakin immunostaining [15,16]. Choriocarcinoma cell lines (the trophoblast-like cell lines BeWo, JAR and JEG3) are commonly used to study human placental functions. However, only BeWo cells are able to fuse and form syncytia in a cAMP-driven process. Interestingly, JEG3 cells could recover a cell fusion capacity under cAMP stimulation by overexpressing connexin-43 gap junction protein (Cx43) or cadherin-11 [17,18]. We consider that BeWo cells should only be used with care to study trophoblast cell fusion. Indeed, they are transformed carcinoma-like cells missing some trophoblast functions [19]. Moreover, BeWo cells fuse after activation of the cAMP signaling pathway. Stimulation by non physiologic agents such as forskolin or cAMP analogs cause uncontrolable and profound modifications of gene and protein expression, which lead directly or indirectly to fusion. Finally, a recent study showed a very weak correlation between gene expression in human cytotrophoblasts and BeWo cells [20]. BeWo cells thus represent a good fusion model but cannot serve as a physiologic model of fusion in the human placenta.

4. Competence stage

Not all cells are fusion-competent. Competent cells commit to fusion in a space- and time-regulated manner. The competence stage is the first step in the complex fusion process. Cells must first exit the cell cycle, which is incompatible with cell fusion. Cytotrophoblasts purified from human placenta are non proliferative [13]. A recent study showed that contacts between the syncytiotrophoblast and cytotrophoblasts are necessary to maintain cytotrophoblasts in a proliferative state [21]. Methods used to purify cells from human placenta probably trap competent cytotrophoblasts, due to their extraction from an environment rich in hormones and other molecules with high potency for competence induction, such as hCG. As observed in other models, satellite cells located close to syncytia are also non proliferative until the syncytium triggers the regeneration process. The fusion-competent stage is characterized by a succession of complex processes which initiate the fusion procedure, including cell migration and morphological changes, and also secretion and response to extracellular signals such as growth factors, cytokines and hormones. Many of these factors, of maternal, placental or fetal origin, have been reported to influence human trophoblast cell fusion: EGF/EGF-receptor (epidermal growth factor; [22]), GM-CFS

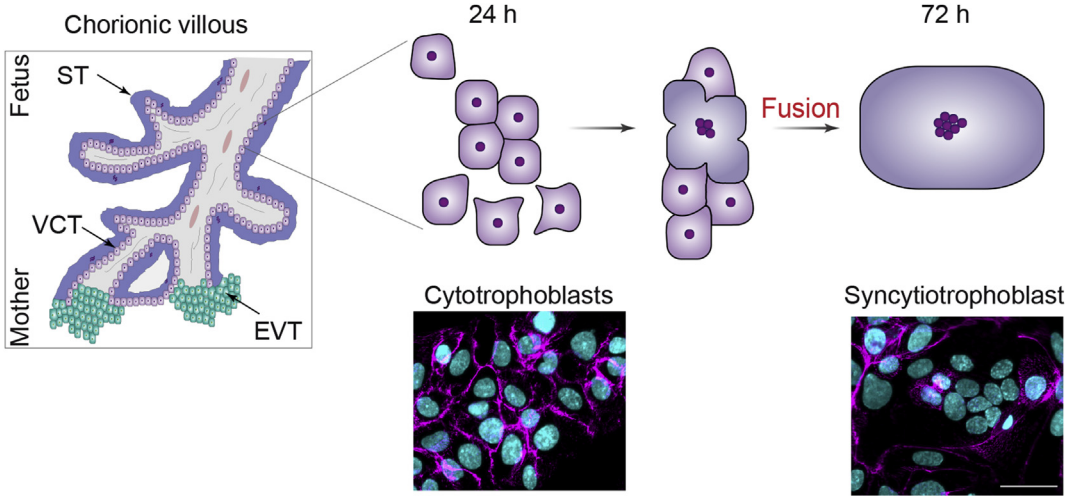

Fig. 1. Model of cultured villous trophobasts purified from human placenta. (left panel) Schematic view of human chorionic villi. VCT for villous cytotrophoblast, ST for syncytiotrophoblast and EVT for extravillous trophoblast. (Upper right panel) Model of trophoblast fusion. Cytotrophoblasts (CT) aggregate after 24–48 h of culture, and fuse into a syncytiotrophoblast (ST) after 72 h. (Lower right panels) Human trophoblast stained at 24 h and 72 h of culture for desmoplakin (magenta) and nuclei (DAPI, cyan). Scale bar: 15 μm.