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# Morphological and Electrical Properties of Human Trophoblast Choriocarcinoma, BeWo Cells

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**Abstract**

The syncytiotrophoblast of the human placenta arises from fusion of stem cells called cytotrophoblasts. The molecular mechanisms associated with cell fusion and syncytiation of cytotrophoblastic cells remain largely unknown. In the present study, we investigated the morphological and electrical properties of BeWo cells, a human choriocarcinoma-derived trophoblast cell model, with several features of the human cytotrophoblast. Cultured cells tended to cluster, but only fused into small, multinucleated syncytia in the presence of cAMP (72 h). The morphological features of both the actin and microtubular cytoskeletons indicated that within 72 h of constant exposure to cAMP, intracellular cortical actin cytoskeleton disappeared, which was the most prominent inducing factor of multi-nucleation. The presence of the cation channel protein, polycystin-2 (PC2), a TRP-type cation channel, associated with placental ion transport in term human syncytiotrophoblast, co-localised with acetylated tubulin in midbodies, but was found non-functional under any conditions. Different electrical phenotypes were observed among control BeWo cells, where only 26% (8 of 31 cells) displayed a voltage-dependent outwardly rectifying conductance. Most quiescent BeWo cells had, however, a low, slightly outwardly rectifying basal whole cell conductance. Acute exposure to intracellular cAMP (<15 min) increased the whole cell conductance by 122%, from 0.72 nS/cell to 1.60 nS/cell, and eliminated the voltage-regulated conductance. The encompassed evidence indicates that the early events in BeWo cell fusion and syncytiation occur by cAMP-associated changes in ionic conductance but not morphological changes associated to chronic exposure to the second messenger. This suggests a tight regulation, and important contribution of cation conductances in cytotrophoblastic cells prior to syncytiation.


*Keywords:* Human placenta; Cytotrophoblast; Human syncytiotrophoblast; TRP channels; Polycystin-2; TRPP2

## 1. Introduction

The placenta is a transient organ that serves many important functions during the development of the fetus. Most of the specialised transport functions of the human placenta are carried out by the syncytiotrophoblast (hST), which is a syncytial epithelium of multinucleated cytoplasm with a distinctive brush border on the surface facing the maternal blood. The syncytiotrophoblast arises from the fusion of underlying stem cells called cytotrophoblasts. BeWo cells, a human trophoblast choriocarcinoma-derived cell line with cytotrophoblastic properties, preserve several placental differentiation markers, and were originally characterised because of their ability to produce human chorionic gonadotrophin (hCG), alkaline phosphatase, and placental lactogen [1,2]. BeWo cells have been used as a model for the investigation of placental transport, including amino acids [3], immunoglobulins [4,5],

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and fatty acids [6,7]. The ion transport mechanisms implicated in the electrodiffusional properties of BeWo cells, however, remain largely unknown. Previous studies by Lafond's group characterised $Ca^{2+}$ transport properties in BeWo cells under basal conditions [8]. This group reported that the basal $Ca^{2+}$ uptake into BeWo cells is mostly voltage-independent, and uninfluenced by L-type $Ca^{2+}$ channels and capacitative $Ca^{2+}$ conductance modulators. This is interesting in view that ion channels responsible for $Ca^{2+}$ homeostasis, including CaT1/ECaC2 (TRPV5, and TRPV6, respectively) and L-type $Ca^{2+}$ channels, have been identified in placental tissues [9]. $Ca^{2+}$ channels with L-type pharmacological characteristics, however, have been functionally identified in trophoblasts in relation to the regulation of hormonal secretions [10–12].

We previously determined that the TRP- (transient receptor potential) type cation channel, TRPP2 (polycystin-2, PC2) is abundantly expressed in the brush border membrane of term human syncytiotrophoblast [13]. Based on its conductance and perm-selectivity properties, PC2 likely represents a relevant transepithelial $Ca^{2+}$ transport pathway in the human syncytiotrophoblast and it is thus possible for such ion transport pathway to be developmentally expressed and controlled during gestation. To characterise the electrodiffusional transport pathways of BeWo cells, we decided to characterise the electrodifussional properties, particularly the presence of a functional PC2 in BeWo cells that fuse into functional syncytiotrophoblasts [14].

The early mechanisms responsible for the formation of the syncytiotrophoblast from cytotrophoblast stem cells include the cessation of cell growth and the commitment to differentiation, followed by cell fusion and nuclear clustering [14]. Development of microvillous surface markers and the production of hCG and progesterone are some of the functional consequences of the process of syncytiation. Syncytiation-related molecular markers have recently been identified. Syncytin is a membrane-associated protein, which is only expressed in the syncytiotrophoblast of the human placenta [15]. Matrix-associated proteins such as CD98 [16], which are linked to integrins and thus the actin cytoskeleton, also seem to be linked to the process of syncytiation. Syncytiation is also associated with dramatic changes in the cytoskeleton, including the disappearance of E-cadherin and desmoplakin [17]. The release of hormones and regulatory substances by placental cells implicates responses to signals that raise cAMP [18]. Actually the response to cAMP by cytotrophoblastic cells seems to be one key effector process in cytotrophoblastic syncytiation. In rat placental explants, for example, Soares et al. [19] observed that addition of cAMP-raising compounds including forskolin, dibutyryl cAMP, and other cAMP analogues as well as cholera toxin, changed cell morphology, inhibited DNA synthesis and increased the release of progesterone, all consistent with syncytial formation. Forskolin and dibutyryl-cAMP also regulate the production of leptin in cultured human trophoblasts [20], and CRH in term placenta primary cultures [21]. Human choriocarcinoma, BeWo cells, which remain as single cytotrophoblast-like cells *in vitro*, can also be induced to fuse by signals that increase cellular cAMP. Incubation of BeWo cells with a high concentration of forskolin (100 μM), for example, results in extensive morphological differentiation, closely resembling that of normal syncytiotrophoblast [14,22,23]. In this process, forskolin-treated cells undergo massive fusion, nuclei clustering, and the development of numerous vacuoles, as well as placental-like microvillar brush border on their apical surface. This phenomenon resembles those observed in primary cultures of human and rat placental explants [14]. The process of cell fusion and syncytiation seems to require other cues to develop. Syncytiation can be extensively modified by oxygen tension, which changes the degree of cell fusion, the secretion of hCG and other cytotrophoblastic properties [24]. Interestingly, higher oxygen tension is associated with the lowest release of hCG and progesterone. This would be consistent with the potentially hypoxic environment under which the syncytiotrophoblast develops. Thus, signals that modify the rate of cellular division, including cAMP and other non-cAMP related signals such as the effect of methotrexate result in morphological differentiation towards syncytiotrophoblast-like morphology [25]. Similar findings are observed in placental explants that are chronically exposed to ethanol [26].

To gain further insight on the initial steps linked to cell fusion and the formation of the syncytiotrophoblast, we explored the electrical properties and morphological features of cultured BeWo cells after cAMP treatment. We observed that the ionic conductance of control BeWo cells segregated into two distinct populations, without apparent morphological differences, one of which seems to express a voltage-dependent cation conductance. We also explored the morphological features, particularly cytoskeletal re-modelling after cAMP treatment. Our data indicate that despite the fact that the channel protein PC2 is expressed in these cells, its location is intracellular, and thus does not contribute to the electrical phenotype. Our study further indicates that acute treatment (<15 min) with cAMP does not, itself modify the morphology of BeWo cells, but instead elicits the earliest changes in the ionic conductance.

## 2. Materials and methods

### 2.1. Cell culture

BeWo cells were obtained from the American Type Culture Collection (ATCC) and maintained in Ham's F12 medium containing 10% FBS, 1% penicillin, 2 mM L-glutamine, and 0.2% D-glucose. Cells were maintained in culture at 37 °C in an atmosphere containing 5% $CO_2$ and normal oxygen tension. Imaging of live cells was conducted under DIC microscopy (see below).

### 2.2. Reagents and immunochemistry

The polyclonal anti-PC2 antibody (0.2 mg/ml stock solution) was obtained from PolyFast (Zymed Laboratories Inc., South San Francisco, CA) and used at 1:100 dilution as reported [27]. The mouse monoclonal anti-acetylated α-tubulin antibody was obtained from Sigma-Aldrich (St. Louis, MO, 2.8 mg/ml stock solution) and used at 1:1000 dilution. The CY3 conjugated affinity pure donkey anti-mouse IgG was from Jackson Immunoresearch Labs. (West Grove, PA). The anti-rabbit IgG-FITC was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). For immunocytochemical studies, cells were grown on glass coverslips to confluence, rinsed twice with phosphate-buffered saline (PBS), and fixed for 10 min in freshly prepared para-formaldehyde (4%)