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Isolation of basal membrane proteins from BeWo cells and their expression in placentas from fetal growth-restricted pregnancies☆

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ABSTRACT

Introduction: The syncytiotrophoblast, a key barrier between the mother and fetus, is a polarized epithelium composed of a microvillus and basal membrane (BM). We sought to characterize BM proteins of BeWo cells in relation to hypoxia and to investigate their expression in placentas from pregnancies complicated by fetal growth restriction (FGR).
Methods: We isolated the BM fraction of BeWo cells by the cationic colloidal silica method and identified proteins enriched in this fraction by mass spectrometry. We evaluated the effect of hypoxia on the expression and intracellular localization of identified proteins and compared their expression in BM fractions of FGR placentas to those from normal pregnancies.
Results: We identified BM proteins from BeWo cells. Among BM proteins, we further characterized heme oxygenase-1 (HO-1), voltage-dependent anion channel-1 (VDAC1), and ribophorin II (RPN2), based on their relevance to placental biology. Hypoxia enhanced the localization of these proteins to the BM of BeWo cells. HO-1, VDAC1, and RPN2 were selectively expressed in the human placental BM fraction. C-terminally truncated HO-1 was identified in placental BM fractions, and its BM expression was significantly reduced in FGR placentas than in normal placentas. Interestingly, a truncated HO-1 construct was predominantly localized in the BM in response to hypoxia and co-localized with VDAC1 in BeWo cells.
Discussion: Hypoxia increased the BM localization of HO-1, VDAC1, and RPN2 proteins. FGR significantly reduced the expression of truncated HO-1, which was surmised to co-localize with VDAC1 in hypoxic BeWo cells.

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1. Introduction

The human syncytiotrophoblast layer is a polarized epithelium composed of an apical microvillus membrane (MVM) and a basal membrane (BM), which plays a key role in regulating maternal–fetal communication. Diverse trophoblastic proteins are differentially expressed in maternal-facing MVM or fetal-facing BM [1,2]. Selected transports from mother to fetus are modulated by the trophoblastic BM. Examples of such transports include transplacental glucose transfer and important feedback that controls placental iron transport [3,4]. Although BM function is critical, our current understanding of this function is inadequate. Functional studies are hampered, in part, by the technical difficulty in isolating the trophoblastic BM [5]. Whereas proteomic analysis of the human syncytiotrophoblast has identified enrichment of specific proteins, such as annexin A2 and alkaline phosphatase, in MVM lipid rafts [6], proteomic analysis of the human trophoblastic BM has not been hitherto reported.

The cationic colloidal silica (CCS) method, first described by Chaney and Jacobson in the early 1980s to isolate plasma membranes from suspended cells, has undergone many modifications

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and has been applied for purifying the luminal endothelial cell membrane and basolateral membrane of adherent cells [7]. Recently, the efficiency of the CCS method for capturing the basolateral integral membrane in a colon adenocarcinoma cell line was validated, revealing minimal contamination from other membranes and basic proteins [8]. The trophoblastic BeWo cell line was identified as a polar cell model and thus was suitable for the study of MVM and BM fractions [3,9].

Using the CCS method, we here isolated the BM of BeWo cells and performed a proteomic analysis to identify proteins enriched in the BM. Among proteins enriched in the BM fraction as observed by mass spectrometry, we focused three proteins, HO-1, VDAC1, and RPN2, based on their relevance to maternal–fetal communication. We investigated the expression of these proteins in BeWo cells cultured in standard or hypoxic conditions and correlated their expression patterns in normal placentas or in placentas from pregnancies complicated by fetal growth restriction (FGR). We found that truncated HO-1 was constitutionally expressed in the BM fraction of human placenta, and we further studied the change in expression and intracellular localization of truncated HO-1 in response to hypoxia by using a truncated HO-1 construct (Flag-HO-1△C23), which has deletion in the C-terminal region of HO-1.

2. Material and methods

2.1. Cell culture

BeWo cells were obtained from Korean Cell Line Bank (KCLB No. 10098) and were maintained in Ham's F-12K (Kaighn's) Medium (Invitrogen, Life Technologies, USA) containing 10% fetal bovine serum and penicillin/streptomycin (Invitrogen). To provide a hypoxic environment, we used a hypoxia incubator chamber (1% O_2; Forma Anaerobic System).

2.2. Isolation of the BM fraction from BeWo cells

The BM fraction was isolated on the basis of a previously described method [8]. Briefly, BeWo cells were washed with cold PBS (pH 7.4) containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$, and then with plasma membrane coating buffer (PMCB; 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM MES, and 135 mM NaCl, pH 5.3). The apical membrane was coated with 5% CCS beads. The beads were then coated with negatively charged 0.5% polyacryl acid (PAA) (in PMCB buffer, pH 6–6.5) to form a stable pellicle. Finally, the pellicle and attached apical membrane were sheared from underlying cells by incubating with lysis buffer containing 2.5 mM imidazole and a protease inhibitor cocktail (Thermo Scientific). The cells were washed with 5 M NaCl solution followed by 100 mM sodium carbonate buffer (pH 11.4) to release intracellular organelles, while leaving basolateral membranes attached to the culture dish. The efficiency of BM isolation was assessed by western blot analysis for marker proteins of each cell component as follows: MVM, placental alkaline phosphatase (PLAP); BM, adenyl cyclase and plasma membrane calcium pump (PMCA); mitochondria, cytochrome C.

2.3. Mass spectrometric analysis and protein identification

Isolated BM proteins were separated on a 10% SDS-PAGE gel

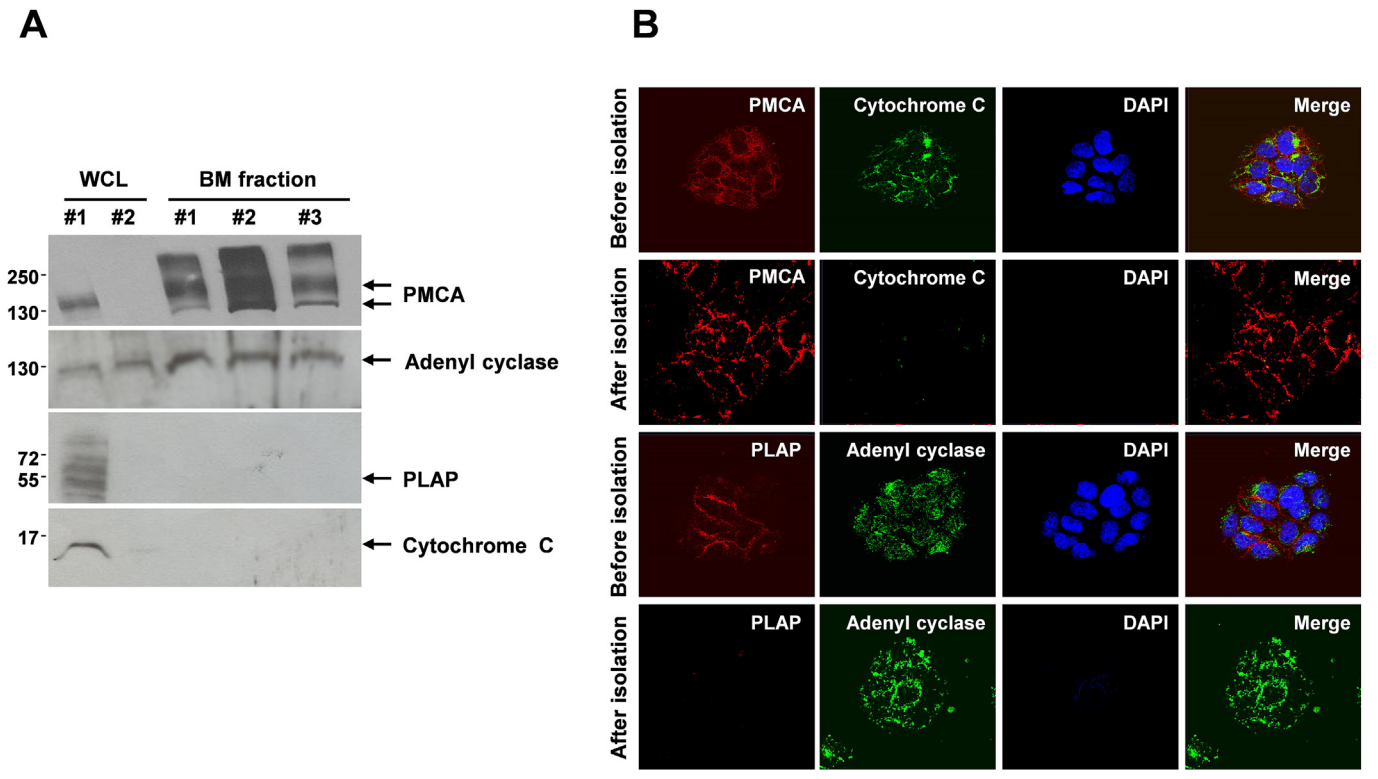

Fig. 1. Isolation of the BM fraction from BeWo cells by using the CCS method. (A) Isolated BM fractions of BeWo cells were compared with whole cell lysates (WCL) in western blots for marker proteins of the BM, MVM, and cytoplasm. Numbers indicate three different preparations of the BM fraction. Plasma membrane calcium pump (PMCA) and adenyl cyclase are markers of BM. Placental alkaline phosphatase (PLAP) is a marker of MVM and cytochrome C was used as a marker of the cytoplasmic mitochondria protein. WCL1 and WCL2 represent whole cell lysates by different lysis buffers which are RIPA and imidazole buffer, respectively. (B) Immunofluorescence staining for marker proteins of each compartment was performed before and after isolation of the BM of BeWo cells. Marker proteins of each intracellular compartment were visualized by Alexa Fluor-labeled secondary antibodies, as described in the Material and methods section. Nucleus was stained with DAPI. These experiments were performed 3 times and a representative data was shown.

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