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Differential Expression of Potassium Channels in Placentas from Normal and Pathological Pregnancies: Targeting of the K_{ir} 2.1 Channel to Lipid Rafts

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Abstract Potassium channels play important physiological roles in human syncytiotrophoblasts (hSTBs) from placenta, an epithelium responsible for maternal–fetal exchange. Basal and apical plasma membranes differ in their lipid and protein composition, and the latter contains cholesterol-enriched microdomains. In placental tissue, the specific localization of potassium channels is unknown. Previously, we described two isolated subdomains from the apical membrane (MVM and LMVM) and their respective microdomains (lipid rafts). Here, we report on the distribution of K_{ir}2.1, K_v2.1, TASK-1, and TREK-1 in hSTB membranes and the lipid rafts that segregate them. Immunoblotting experiments showed that these channels are present mainly in the apical membrane from healthy hSTBs. Apical expression versus basal membrane was 84 and 16% for K_{ir}2.1 and K_v2.1, 60 and 30% for TREK-1, and 74 and 26% for TASK-1. Interestingly, K_v2.1 showed differences between apical membrane subdomains: $26 \pm 8\%$ was located in the LMVM and $59 \pm 9\%$ in MVM. In pathological placentas, the expression distribution changed in the basal membrane: preeclampsia shifted to 50% and intrauterine growth restriction to 42% for TASK-1 and both pathologies increased to 25% for K_{ir}2.1 and K_v2.1, K_{ir}2.1 appeared to be associated with rafts that were sensitive to cholesterol depletion in healthy, but not in pathological, placentas. K_v2.1 and TREK-1 emerged in the nonraft fractions. The precise membrane localization of ion channels in hSTB membranes is necessary to understand the physiological events.

Keywords Potassium channel · Placenta · Apical membrane · Basal membrane · Lipid raft

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Introduction

Potassium channels exist in almost all epithelial and non-epithelial cells, and they are involved in several physiological functions, including membrane potential through the control of membrane permeability to K^+ ions, volume regulation, electrogenic solute transport, and hormone secretion, among many other functions (Warth 2003). Together with knowledge about the structure and function of K^+ channels, the possible role of lipid membrane composition in the regulation of channel localization and function has garnered increasing interest (Levitan et al. 2010). Human placental syncytiotrophoblasts (hSTBs) comprise the continuous epithelial layer that forms the main barrier for maternal–fetal exchange (Stulc 1997). The activity of K^+ channels in the hSTB includes similar functions to those mentioned above for epithelial cells. K^+ transport via the conductance pathway has been studied through flux experiments in vesicles prepared from membranes of placental hSTB (Illsley and Sellers 1992) and in isolated placental villi (explants) of hSTB (Birdsey et al. 1999). In addition, electrophysiological experiments performed in cytotrophoblast cells (Clarson et al. 2001) and on term placental membranes reconstituted in giant liposomes or transplanted in *Xenopus laevis* oocytes (Diaz et al. 2008) showed the presence of potassium conductance. Diaz et al. (2008) used single-channel recordings (patch-clamp method) and total current recordings (voltage-clamp method) to demonstrate the

existence of a Ba^{+2}-sensitive K^+ channel, a subpopulation of TEA-sensitive channels and some Na^+-sensitive K^+ channels in apical membranes purified from term placental hSTBs. Clarson et al. (2001) demonstrated that cytotrophoblast cells express an inwardly rectifying K^+ current that is typical of $K_{ir}2.1$, whose incidence increases with cytotrophoblast cell differentiation. Bai et al. (2005, 2006) described the expression and activity of TASK (1 and 2) and TREK, which are two pore domain K^+ channels (K2p), in the villous tissue from human placenta and in cultured human cytotrophoblast cells. Williams et al. (2008) suggest the presence of potassium channels sensitive to K_v channel blockers 4-AP and TEA in cytotrophoblast cells and human placental villous explants.

hSTBs, similar to all epithelial cells, polarize during differentiation, forming the apical and basal domains, which are two distinct plasma membrane domains with different protein and lipid compositions (Stulc 1997). In addition, there are two subdomains within the apical hSTB domain: the classical microvillous membrane (MVM), which has been used in our studies and by other authors to study transport mechanisms, and the light microvillous membrane (LMVM) (Jimenez et al. 2004; Riquelme 2011). In both purified fractions, we observed lipid rafts. These membrane microdomains are characterized by their resistance to detergent extraction and their ability to float in density gradient centrifugation (Godoy and Riquelme 2008; Riquelme 2011). The domains, subdomains, and microdomains were also characterized in placentas from pregnancy pathology, including preeclampsia (PE) and intrauterine growth restriction (IUGR) (Jimenez et al. 2004; Riquelme et al. 2011). PE is a hypertensive disease associated with proteinuria, sometimes with edemas (Lindheimer and Katz 1989), and affects up to 6% of all gestations beyond 20 weeks, especially in primiparous women. IUGR constitutes another group of complex diseases that affect 8–14% of pregnancies in which the fetus fails to achieve its genetically determined growth (Cetin and Alvino 2009).

The interaction between the transporter proteins, including ion channels, and membrane lipids can be highly specific and is often essential for the functional and structural integrity of the membranes (Tillman and Cascio 2003). Many of these studies have demonstrated that membrane cholesterol is a major regulator of ion channels (Levitan et al. 2010).

In general, the organization and maintenance of ion channels within a specific plasma membrane domain, subdomain, and microdomain play an important role in determining the ion channels' physiological functions. For that reason, it is important to study the relationship between K^+ channels and these specific regions in the normal placenta and in pathological placentas and to determine whether alterations in their segregation can be related to pathology, which occurs in other cells and tissues (Michel and Bakovic 2007). The present work aimed to examine the expression of the K^+ channels $K_{ir}2.1$, $K_v2.1$, TASK-1, and TREK-1; their relationship to the domains, subdomains, and microdomains of hSTB membranes from normal, PE and IUGR placentas was also investigated.

Materials and Methods

Placenta Collection

At the San José Hospital Maternity Unit, placentas obtained from normal pregnancies and from pregnancies with moderate PE and IUGR were collected immediately after delivery and transported to the laboratory on ice. Diagnosis of moderate PE was based on the classic criteria of systolic and diastolic blood pressure $\geq$140/90 mmHg on at least two occasions and proteinuria $\geq$300 mg/24 h. Patients with severe PE, defined as diastolic blood pressure >110 mmHg and/or proteinuria >5 g/day, were excluded from our study sample (Centro de Diagnóstico e Investigaciones Perinatales, Chile; www.cedip.cl/Guias/Guia 2003). Placentas from patients with HELLP syndrome were also excluded, as well as placentas from patients with moderate PE accompanied by any other pathology. An idiopathic diagnosis of IUGR was established by the attending physician via the clinical estimation of the fetal weight corresponding to the growth rate under the 10th percentile for each sex and the gestational age according to the curve selected by the Ministry of Health of Chile (curve of intrauterine growth (Juez 1989)). Fetuses that were at the 10th percentile but had halted growth during a reasonable observation period of at least 14 days were also considered IUGR (Juez 1989). The IUGR exclusion criteria included fetal or maternal infections, maternal drug use or alcohol abuse, multiple pregnancies, fetal malformations, chromosomal abnormalities, maternal chronic hypertension, maternal cardiovascular or autoimmune diseases, diabetes, and moderate PE. Placentas with IUGR from PE pregnancies were excluded. All of the placentas we used were obtained from term pregnancies.

Preparation of Placental Membranes

Human placental apical or MVM, LMVM, and basal membrane (BM) vesicles were prepared from fresh human placenta by a method that we previously described and that enables the simultaneous isolation of apical and basal membranes from the same placenta (Jimenez et al. 2004). The purification method included the precipitation of non-microvillous membrane with magnesium ions, differential

centrifugation, and a sucrose step gradient; this assured that the isolated fractions were enriched and free of contamination (Jimenez et al. 2004). The solutions used were as follows: buffer A (in mmol/l), 250 sucrose, 0.7×10^{-3} pepstatin, 1.1×10^{-3} leupeptin, 80×10^{-6} aprotinin, and buffer B (in mmol/l), 300 sucrose; all solutions were buffered with 20 mM Tris-maleate, pH 7.4. The microvillous and basal enriched preparations containing about 10–15 mg and 6–8 mg of apical and basal protein, respectively, were overlaid on the sucrose gradient. Bands were obtained at the 10/37 and 37/45% sucrose interfaces, which correspond to the LMVM apical fraction and to the classical MVM apical fraction, respectively (Jimenez et al. 2004). The band at the 47/52% (w/v) sucrose interface was collected, corresponding to the BM fraction. These fractions were collected and diluted tenfold with 20 mM Tris-maleate (apical fractions) and 20 mM Tris-HEPES (basal fractions), both at pH 7.4, before centrifugation at 110,000×*g* for 30 min. The final pellet was resuspended in 300 mM sucrose and 20 mM Tris-maleate, pH 7.4 buffer, and stored in liquid nitrogen.

The protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL) for the colorimetric detection and quantification of total protein (Smith et al. 1985; Wiechelman et al. 1988). The purity and enrichment of the apical fractions were determined routinely by assaying for alkaline phosphatase activity, an apical membrane marker. Enrichment of alkaline phosphatase activity was over 20-fold for MVM and LMVM; both preparations were essentially free of basal membranes and mitochondrial membranes. The purity and cross-contamination of the membranes were similar to those previously observed (Jimenez et al. 2004). The purity and enrichment of the basal membrane fraction were routinely determined by assaying for classic marker protein activities as described by Jimenez et al. (2004). Placental alkaline phosphatase (PLAP) was used as an apical membrane contamination marker. The degree of cross-contamination of the purified basal membranes with apical membranes, quantified using a ratio of PLAP activity enrichment of the BM compared with the MVM (BM/MVM), was approximately 0.1 for the placentas used in this study; this ratio was lower than or equivalent to that from several other reports for single or paired apical and basal membrane preparations, as demonstrated by Jimenez et al. (2004).

Preparation of Apical Lipid Microdomains

Apical plasma membrane microdomains were isolated separately from MVM and LMVM enriched membrane fractions as detergent-resistant membranes (DRMs) through extraction with Triton X-100 using a modified protocol based on that described by Brown and Rose (1992). As we described in Godoy and Riquelme (2008) and Riquelme et al. (2011), normal placenta (NP)/PE/IUGR DRMs from isolated apical fractions (MVM and LMVM) were extracted with 1% Triton X-100 on ice and subjected to ultracentrifugation and sucrose flotation. After centrifugation, the gradients were divided into 10 fractions (0.5 ml each) from the top of the gradient and the pellet was resuspended in 0.5 ml MBS-buffered saline (25 mM morpholinoethanesulfonic acid, 150 mM NaCl, pH 6.5; fraction 11) for subsequent analysis. Throughout this article, we use the terms "lipid microdomains" and "lipid rafts" to refer to the membrane material that floats on the sucrose gradient around the 5/35% interface (fractions 1–5). All flotation fractions from MVM and LMVM were characterized by specific markers for protein, alkaline phosphatase as a positive marker for apical lipid microdomain fractions and human transferrin receptor (hTf-R) as a nonraft marker.

Depletion of Membrane Cholesterol by Methyl-β-Cyclodextrin Treatment

Cyclodextrin treatment was carried out as described previously by Danielsen and Hansen (2003). Placental apical vesicles (0.6 mg of total protein) were incubated with 2% w/v methyl β-cyclodextrin (mβ-CD) in MBS buffer at 37°C for 30 min and centrifuged at 21,000×*g* for 2 h at 4°C. The pellet was resuspended in 1 ml of 1% Triton X-100 in MBS-buffered saline, and microdomain preparation was carried out as described above.

Electrophoresis, Western Blotting, and Densitometric Analysis

Placental Membrane Fractions

For each potassium channels ($K_{ir}2.1$, $K_v2.1$, TREK-1, and TASK-1), 20 µg of total protein of LMVM, MVM, and BM were loaded on a 10% SDS–polyacrylamide gel. Routinely, all four proteins were probed in membrane fractions isolated from the same placenta.

Apical Lipid Microdomains

Aliquots of 50 µl each were incubated with 10% trichloroacetic acid (TCA, v/v) for 30 min on ice and centrifuged at 21,000×*g* for 30 min at 4°C. The pellet was resuspended in sample buffer, boiled for 5 min and sonicated for 30 min. For each potassium channel ($K_{ir}2.1$, $K_v2.1$, TREK-1, and TASK-1) and raft (PLAP) and nonraft markers (hTf-R), 20 µl of each flotation gradient fraction from LMVM and MVM were used.

These samples and the molecular weight marker (PageRuler™ Prestained Protein Ladder; Fermentas, Glen Burnie, MD) were loaded on a 10% SDS–polyacrylamide gel. Electrophoresis was performed at 100 V, and the gel was transferred to a nitrocellulose membrane (BioRad, Richmond, CA; 162-0115) for 2 h at 100 V. The nitrocellulose membrane was blocked for 2 h at room temperature with 3% non-fat milk in Tween/saline buffer (138 mM NaCl, 270 mM KCl, and 0.05% Tween-20) and washed in Tween/saline buffer. Membranes were incubated with primary antibody for 2 h at room temperature. Potassium channel antibodies were diluted as follows in bovine serum albumin (BSA) 1%: anti-$K_{ir}2.1$ 1:500, anti-$K_v2.1$ 1:100, anti-TREK-1 1:1,000, anti-TASK-1 1:500. Each antibody was probed with its control antigen. The specificity of the primary antibodies was evaluated in competition experiments (1:1 antibody/antigen) in which incubation with the corresponding antigens partially neutralized the antibody. The mark of the band identified by the weights provided in the data sheet for each antibody decreased in all the experiments. Such a control was done in the initial stage of this study with all potassium channel antibodies used (data not shown).

Raft and nonraft marker antibodies were diluted as follows in distilled water: anti-PLAP 1:1,000 and anti-hTf-R 1:500. After washing with Tween/saline buffer, membranes were incubated with specific horseradish peroxidase (HRP)-linked secondary antibody: anti-rabbit 1:5,000 or anti-mouse 1:10,000, both diluted in Tween/saline buffer and incubated for 1 h at room temperature. Bands were detected with the enhanced chemiluminescence Western Blotting Analysis System (EZ-ECL; Biological Industries, Kibbutz Beit Haemek, Israel), and measurements were performed according to molecular weight given by a data sheet for each antibody and the band affected by the antigenic peptide. Protein content was quantified with Image J 1.43i (Wayne Rasband, National Institutes of Health, Bethesda, MD).

Reagents and Antibodies

All chemicals were analytical grade. Buffers were made with distilled water, and pH values were determined at room temperature. The following polyclonal antibodies were used: anti-$K_{ir}2.1$, anti-$K_v2.1$, anti-TREK-1, anti-TASK-1 (Alomone Labs, Jerusalem, Israel); mouse monoclonal antibody against human alkaline phosphatase, PLAP (clone 8B6; Sigma, St. Louis, MO); hTf-R (clone H68.4; Zymed, San Francisco, CA); and HRP-conjugated secondary goat anti-mouse (Amersham, Aylesbury, UK) for monoclonal antibodies and rabbit (Santa Cruz Biotechnology, Santa Cruz, CA) for all potassium channels.

Statistical Analysis

Results are expressed as means ± standard deviation (SD). Statistical significance was measured using the one-way ANOVA plus Bonferroni's multiple comparison test and Student's *t*-test. $P < 0.05$ was considered significant.

Results

Expression of K^+ Channels in Domains and Subdomains from Placental hSTB

Apical (MVM, LMVM) and basal membranes (BM) from NP and from PE and IUGR placentas were isolated and purified using the protocol described in ''Materials and Methods'' section. We found similar enrichment markers and cross-membrane contamination markers as described fully in previous work (Jimenez et al. 2004; Riquelme et al. 2011). We purified the membranes from 11 NPs, 4 PE placentas, and 5 IUGR placentas. In these purified membranes, we performed expression experiments using western blotting with specific antibodies for $K_{ir}2.1$, $K_v2.1$, TASK-1, and TREK-1. Figure 1 shows the expression distributions of these four K^+ channels in LMVM and MVM apical subdomains and BM domains. The results are expressed as the percent relative to the expression associated with MVM + LMVM + BM (set at 100%). As shown in Fig. 1a, no significant difference was found in the distribution of $K_{ir}2.1$ in LMVM and MVM between normal and pathological placentas; however, the total expression of this type of channel was higher in apical membrane domains compared with BM. The LMVM + MVM results were 84, 76 and 75% for NP, PE, and IUGR, respectively, in contrast to the BM results, which were 16, 24 and 25%, respectively. For $K_v2.1$ (Fig. 1b), the difference between the total apical (MVM + LMVM) and BM was maintained: 85 vs. 15% in NP, 78 vs. 22% in PE, and 77 vs. 23% in IUGR. However, there was a large difference between the LMVM and MVM. Interestingly, approximately 60% of the total expression in placental hSTB was found in the LMVM apical subdomains from normal and pathological placentas. In contrast, the distribution of TREK-1 was approximately 33%, on average, for all of the membrane fractions from either normal or pathological placentas. As observed for $K_{ir}2.1$, $K_v2.1$, and TREK-1, there was greater expression in the apical domain compared with the basal domain, although there were differences in the distribution between the apical subdomains, as in the case of $K_v2.1$. In contrast, the expression of TASK-1 in PE was similar in both domains, with values of 50 and 49% for MVM + LMVM and BM, respectively. Interestingly, there was an increasing

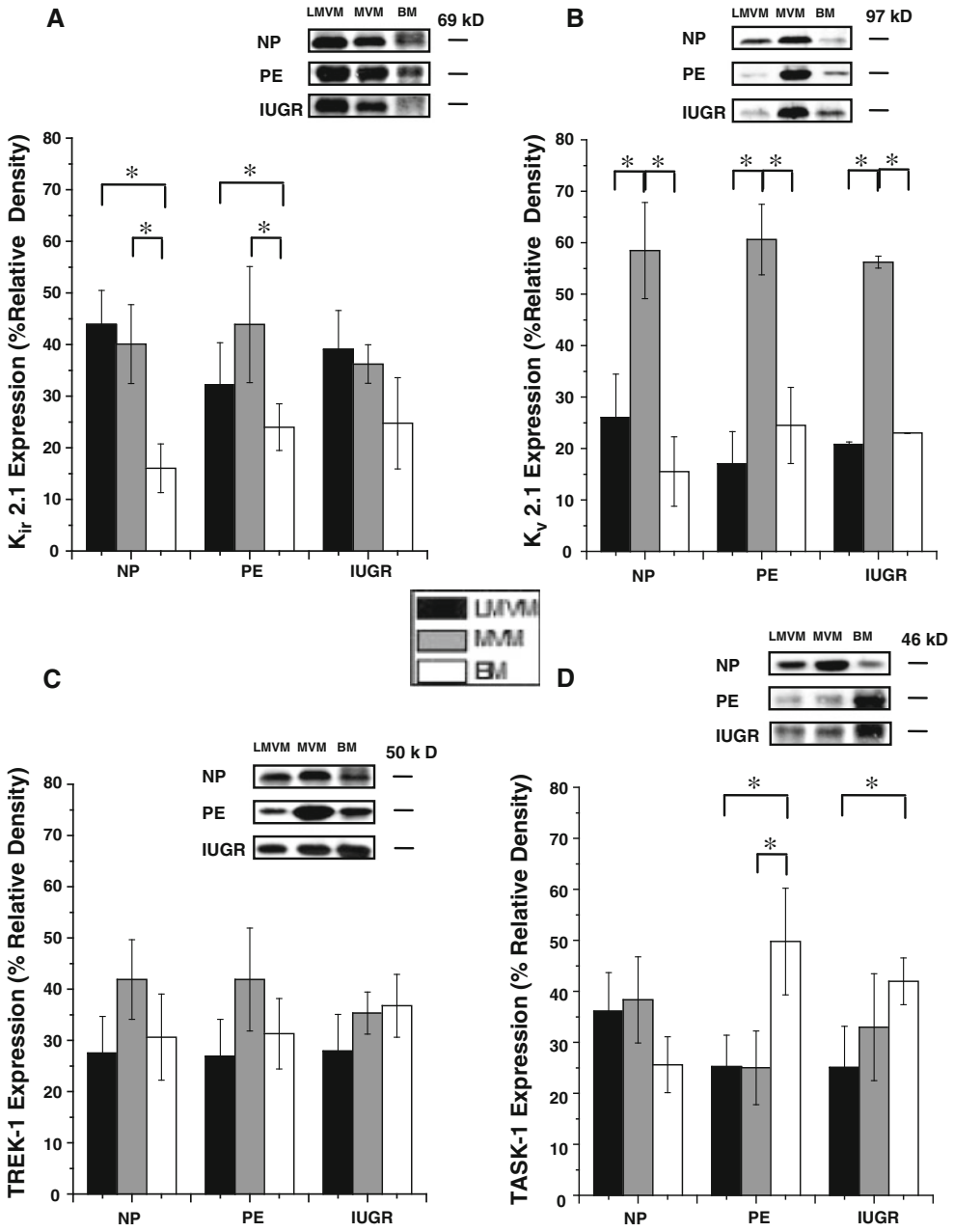

Fig. 1 Expression and distribution of potassium channels ($K_{ir}2.1$, $K_v2.1$, TREK-1, and TASK-1) in hSTB membranes from NP, PE, and IUGR placentas. **a** $K_{ir}2.1$, **b** $K_v2.1$, **c** TREK-1, and **d** TASK-1, tested in LMVM, MVM, and BM purified membranes from NP ($n = 8$), PE ($n = 4$), and IUGR ($n = 4$) placentas (means ± SD, $^*P < 0.05$). The density of the signal in each membrane fraction is expressed as a percentage of the sum of densities (MVM + LMVM = 100%). Representative western blots are shown in *insets*

trend in the expression of these channels in the BM domain from pathological placentas.

Partition of $K_{ir}2.1$ into Lipid Rafts in the Apical Domains of Normal and Pathological Placentas

Apical membranes from normal and pathological placentas were incubated with 1% Triton X-100 and separated by flotation in a discontinuous sucrose gradient. They were tested for the presence of specific raft and nonraft markers as described in Godoy and Riquelme (2008) and Riquelme et al. (2011). We confirmed the quality of our preparations using immunoblotting. All of the fractions in each raft preparation were probed for PLAP, which is a specific apical and raft marker. In Fig. 2a, b the representative western blot and graphic show the distribution of this marker in the flotation gradient fractions. PLAP was present in the first five fractions corresponding to the lipid

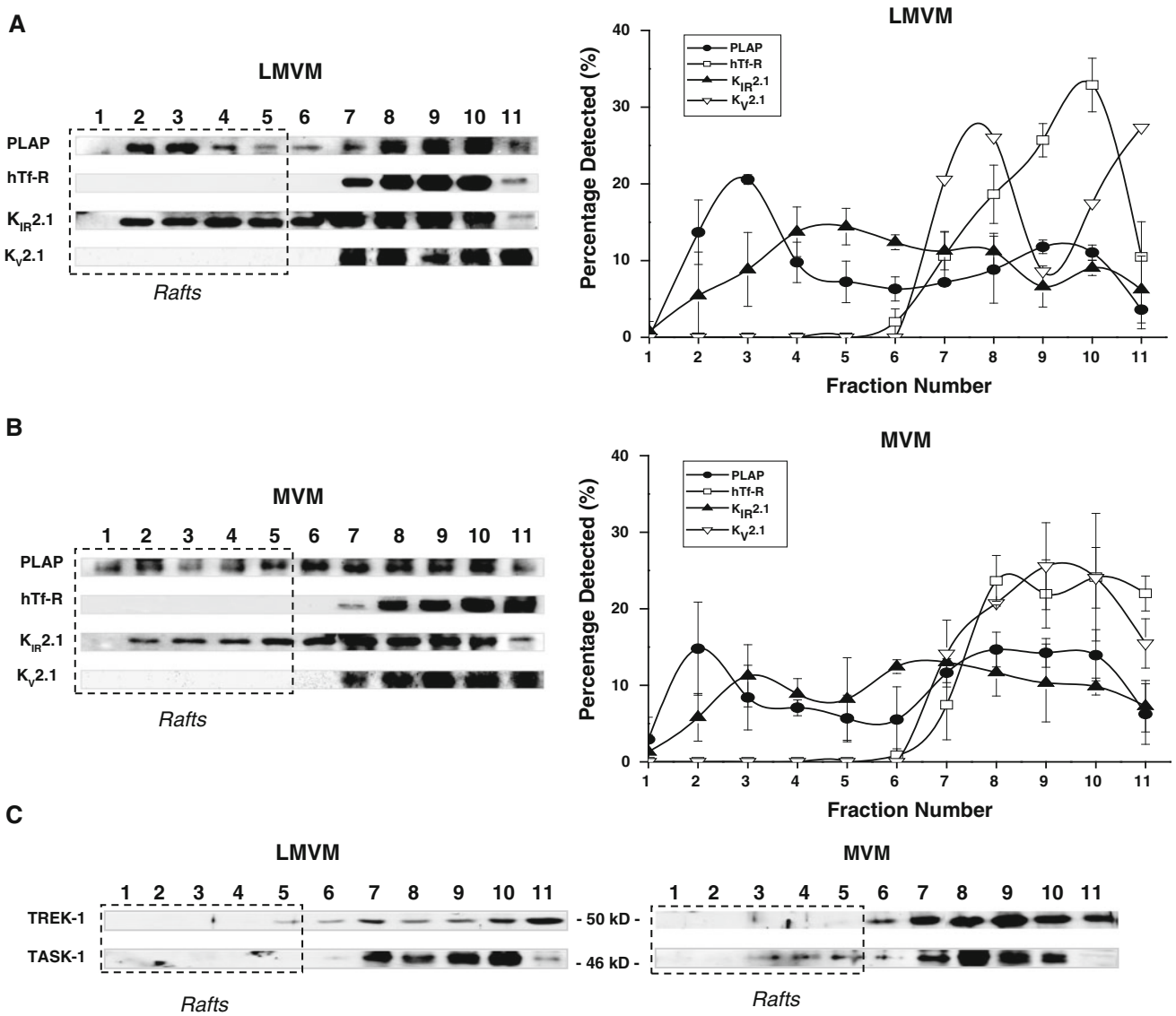

Fig. 2 Differential distribution of potassium channels in the flotation gradient fractions of LMVM and MVM subdomains from normal placentas. Quantification of western blot analysis, and representative images are shown for PLAP (raft marker, $n = 4$), hTf-R (nonraft marker, $n = 4$), $K_{ir}2.1$ ($n = 4$), and $K_v2.1$ ($n = 9$) of LMVM (**a**) and MVM (**b**). **c** Representative western blots for TREK-1 and TASK-1 corresponding to LMVM and MVM

rafts from normal LMVM and MVM (Godoy and Riquelme 2008). To ensure that these lipid rafts were free of nonraft fractions, we routinely probed these fractions for hTf-R, which is a protein known to reside in nonraft areas. As Fig. 2a, b show, this protein was not found in fractions 1–6 in both LMVM and MVM, indicating that those fractions were free of nonraft components.

$K_{ir}2.1$, $K_v2.1$, TASK-1, and TREK-1 antibodies were used to study the possible segregation of these channels in raft and nonraft fractions. Immunoblots of the sucrose density gradient–derived fractions prepared from LMVM and MVM showed that some $K_{ir}2.1$ protein from NPs was localized in the low-density fractions, which corresponded to the first five fractions (Fig. 2a for LMVM and Fig. 2b for MVM). In contrast, $K_v2.1$ was present only in the nonraft microdomains. The behaviors of TASK-1 and TREK-1 are illustrated in Fig. 2c. TREK-1 in LMVM and MVM was found mainly in nonraft areas, 75 and 100%, respectively ($n = 8$ experiments from six independent placentas). For TASK, either in LMVM or in MVM, the results were not conclusive ($n = 6$ from three independent placentas). In general, the western blot suggested mainly nonraft markers for this type of channel; however, a weak band appeared in some of the raft areas.

In summary, the results described here suggested that some $K_{ir}2.1$ was localized in the first five fractions (the raft fractions).

Cholesterol-Depletion Effects

As a control, LMVM and MVM were treated with mβ-CD, a specific cholesterol-removal agent, to deplete cholesterol levels prior to detergent extraction and density ultracentrifugation, as described in "Materials and Methods". As shown in Fig. 3a, this treatment eliminated $K_{ir}2.1$ immunoreactivity from the low-density fractions, as expected for a protein localized to cholesterol-rich areas, such as lipid rafts.

Additionally, immunoblots of fractions separated by sucrose density gradients from LMVM and MVM of PE and IUGR placenta were assayed for $K_{ir}2.1$, and partitioning into raft fractions occurred in both pathologies. However, the association between $K_{ir}2.1$ and the low-density fractions from MVM and LMVM was not as clear and reproducible as in NPs and was not affected by the removal of cholesterol (Fig. 3b, c).

Discussion

This study is the first to demonstrate the expression distribution of potassium channels in the domain and subdomain regions of placental hSTB from normal and pathological pregnancies. The four potassium channels examined ($K_{ir}2.1$, $K_v2.1$, TREK-1, and TASK-1) were previously characterized in multiple placenta studies (Clarson et al. 2001; Bai et al. 2005, 2006; Williams et al. 2008). In addition, this study is the first to demonstrate that $K_{ir}2.1$ is

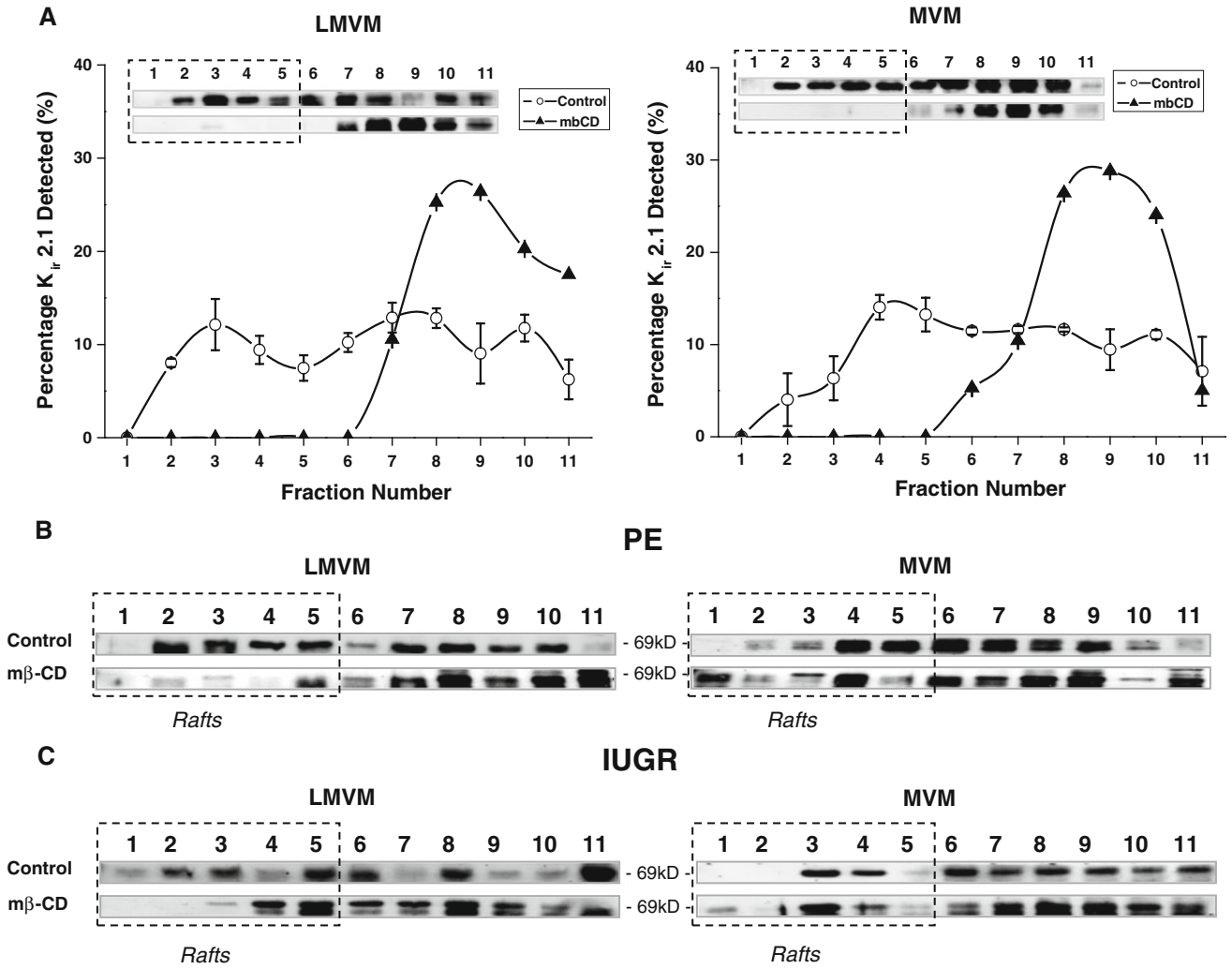

Fig. 3 Effect of cholesterol depletion on $K_{ir}2.1$ segregation into the lipid rafts in LMVM and MVM from NP, PE, and IUGR placentas. **a** Graphical quantification and representative image of western blot for $K_{ir}2.1$ in the flotation gradient fractions of LMVM and MVM subdomains from normal placenta before (control) and after the apical fractions were preincubated with 2% mβ-CD ($n = 2$). **b**, **c** Representative western blots reflecting the effect of cholesterol depletion on the association between $K_{ir}2.1$ (**b**) and the rafts from PE and IUGR apical membrane fractions ($n = 3$ for each condition)

partitioned into lipid rafts in the apical membranes (LMVM and MVM) in both normal and pathological term placentas.

Comparisons of the channel expression distribution between the apical and basal membranes from healthy hSTB revealed that $K_{ir}2.1$, $K_v2.1$, TASK-1, and TREK-1 channels were significantly more abundant in apical than in basal membranes. Expression of $K_{ir}2.1$ and $K_v2.1$ showed that approximately 84 vs. 16% were present in the apical membranes versus basal membranes, respectively; for TREK-1, the expression was 60 vs. 30% and for TASK-1, 74 vs. 26%. However, there was a difference between MVM and LMVM, which are the two subdomains from apical membranes, in that $K_v2.1$ expression was $26 \pm 8\%$ in the LMVM and $59 \pm 9\%$ in the MVM. In previous reports (see brief review in Riquelme 2011), we suggested that the two subdomains from the apical hSTB membranes were correlated: MVM corresponded to the microvillous finger-like region of the apical subdomain, and LMVM corresponded to the apical subdomain that constituted the base of the finger-like projections (Godoy and Riquelme 2008; Riquelme et al. 2011; Riquelme 2011). Then, we determined that most of the K_v was located in the microvillous finger-like region. The differential cell-surface distribution of these K^+ channels is due to the isoform-specific mechanisms that exist for the localization of these proteins (Tikku et al. 2007; Martens et al. 2001; Melnyk et al. 2002; O'Connell and Tamkun 2005).

The results of our equivalent studies in membranes from pathological placentas showed a tendency to maintain the distribution among the apical membrane subdomains for all types of channels studied. However, expression of these channels in the BM increased compared to that in the entire apical membrane. Expression of $K_{ir}2.1$ and $K_v2.1$ increased from 16 to 25% in the BM of PE and IUGR placentas. The most dramatic change was observed for TASK-1, the expression of which was shifted in the BM from 25% in NP to 50% in PE and to 42% in IUGR.

The preferential distribution of these potassium channels in one or more domains of this epithelium as well as their distribution in the apical membrane subdomains are indicative of their role in the transplacental transport model. These compartments are also composed of lipids and proteins, which optimize the environment surrounding these channels for their function, as observed in other epithelia (Levitan et al. 2010). Of note, the distribution of some of these channels is altered in pathologies such as PE and IUGR. These results are consistent with other studies that detected expression changes in proteins, such as syncytin, between the apical and basal membranes in PE (Lee et al. 2001). Those changes are associated with dysfunction of the PE placenta; potassium channels described herein could have an impact on transport through the placenta.

However, the current knowledge about the membrane lipid heterogeneity with the existence of lipid rafts as the specialized membrane microdomains rich in sphingolipids and cholesterol permits a closer examination of the relationship between the channels and the lipids. There was no information about the localization of these placental potassium channels or their membrane partitioning. The immunodetection of $K_{ir}2.1$, $K_v2.1$, TREK-1, and TASK-1 proteins using specific antibodies showed that only $K_{ir}2.1$ appeared to be associated with lipid rafts, whereas $K_v2.1$ and TREK-1 emerged in the nonraft fractions. The data on the TASK-1 channels were not conclusive.

The results, which demonstrate the segregation of placental $K_{ir}2.1$ to raft microdomains, represent a novel finding in the placenta. These results are consistent with several studies that showed that a variety of ion channels, including potassium channels, from other cells and tissues are regulated by the level of cholesterol in the membrane (Levitan et al. 2010). In addition, these studies showed that the depletion of membrane cholesterol by mβ-CD removed several channels from the raft fractions to the nonraft fractions (Tikku et al. 2007; Romanenko et al. 2004). The inwardly rectifying potassium channel (K_{ir}) is a ubiquitous channel that is expressed in many tissues (Kubo et al. 2005; Nichols and Lopatin 1997; Reimann and Ashcroft 1999); in particular Mylona et al. (1998) reported expression of the $K_{ir}2.1$ gene in the human placenta and in cultured cytotrophoblast cells at all different stages of differentiation. This family of potassium channels constituted a major type of ion channel involved in the maintenance of the resting potential in several cells; in particular, there are many reports about the link between the alteration of this type of channel and pathologies. For example, human mutations on $K_{ir}2.1$ may induce Andersen disease, and the targeted disruption of $K_{ir}2.1$ in mice induces death within a few hours after birth (Zaritsky et al. 2000), among other pathological syndromes associated with K_{ir} (Tristani-Firouzi and Etheridge 2010; Decher et al. 2007; Plaster et al. 2001).

Tikku et al. (2007) reported in 2007 that $K_{ir}2.1$ from the Chinese hamster ovary (CHO) K1 cell line had a double distribution between cholesterol-rich (raft) fractions, which are sensitive to cholesterol removal by mβ-CD, and nonraft fractions, indicating that the channels exist in two different types of lipid environment. Our results from normal placental membranes are comparable with those described by Tikku et al. (2007). When we studied this type of channel from PE and IUGR membranes, the segregation into rafts was similar to that obtained from healthy membranes; however, $K_{ir}2.1$ lost its cholesterol sensitivity, and mβ-CD did not remove the channels from the raft fractions. This result was very important because, for these channels and others, it is known that cholesterol enrichment modulates

channel activities (Tikku et al. 2007; Levitan et al. 2010). In general, the organization and maintenance of ion channels within a specific plasma membrane domain, subdomain, and microdomain play an important role in determining their physiological functions (Riquelme 2011). In placentas, the physiological relevance of the localization of potassium channels has not been established, so the relationship between the K^+ channels with these specific regions in normal and pathological placentas may explain whether alterations in their segregation may be related to pathology, as is explained in other cells and tissues (Michel and Bakovic 2007).

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