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Cystic fibrosis transmembrane regulator (CFTR) in human trophoblast BeWo cells and its relation to cell migration

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ABSTRACT

Introduction: ENaC and CFTR are coexpressed in epithelia and have positive or negative functional interactions. In addition, ENaC and CFTR promote migration in placental trophoblastic cells and human airway cells, respectively. Here we tested the idea if CFTR is functionally expressed in BeWo cells, a trophoblastic cell line, and if it is involved in their migratory behavior.
Methods: CFTR expression was studied in BeWo cells with RT-PCR, biotinylation and Western blot. Ion currents were analyzed with patch clamp, and cell migration with the wound healing method.
Results: The mature CFTR 160-kDa band was present, and its localization at the surface membrane was confirmed. Forskolin (20 μM), an adenylate cyclase activator, was used for channel activation, and subsequently $CFTR_{inh}$-172 (2 μM) for its inhibition. The conductances in the presence of $CFTR_{inh}$-172 plus forskolin (16.0 ± 0.7 pS/pF and 32.6 ± 1.5 pS/pF) were significantly lower than in presence of only forskolin (29.7 ± 0.9 and 47.0 ± 2.0 pS/pF). The conductance of $CFTR_{inh}$-172 inhibited currents was 14.9 ± 0.7 pS/pF with a linear I-V relationship illustrating the nonrectifying properties of the CFTR. Cell migration was measured and covered 11.2 ± 0.4, 24.0 ± 1.7 and 13.9 ± 1.0% of the wound when cells were cultivated under control, forskolin, and forskolin plus $CFTR_{inh}$-172, respectively. Proliferation was not changed by any of the treatments.
Conclusions: Our results shows that BeWo cells functionally express the CFTR which plays a role in the wound healing increasing the cell migration process.

1. Introduction

The cystic fibrosis transmembrane regulator (CFTR) is a cAMP-activated, ATP-dependent Cl^- channel that mainly participates in the electrolyte and fluid transport in several epithelia [1,2]. CFTR is also involved in HCO_3^- transport from the intracellular to the extracellular side of the membrane [3] and controls the activity of other membrane transport proteins, such as ATP secretion, the outwardly rectifying anion channel (ORCC) and the epithelial sodium channel (ENaC) [4,5]. CFTR also responds to Ca^{2+} mobilizing secretagogues, and contributes to cholinergic and purinergic responses [6].

ENaC and CFTR are coexpressed at the apical surface of epithelia and have positive or negative functional interactions that are tissue specific [2]. In addition to function as an anion channel, CFTR is also involved in cell migration and proliferation [7,8]. Schiller et al. [1] demonstrated that the inhibition of CFTR transport produced delays in wound closure in human airway epithelial that correlated with a reduction in lamellipodia surface area.

CFTR is also found in the trophoblast from human normal placenta [9] and its expression as well as that of ENaC is diminished in preeclamptic placentas [10,11, see below]. Since we showed that ENaC and ORCC are present in trophoblast BeWo cells [12–15], we tested the idea if CFTR is also functionally expressed in BeWo cells and is involved in their migratory behavior.

The BeWo cell line is a human hormone-synthesizing trophoblastic cell line which displays many biochemical and morphological properties similar to those reported for the *in utero* proliferative cytotrophoblast during the last trimester of pregnancy and they have been used as a model to investigate the placental transport mechanisms [16]. Molecular biology and patch clamp techniques were used to study the expression and function of CFTR channels and the scratch wound assay for the migration process under normal conditions, and also the use of blockers of CFTR channels. Our results show that CFTR participates in the migration of BeWo

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cells for the following reasons: 1) CFTR is expressed functionally in BeWo cells; 2) there is an increase in wound healing in forskolin activated CFTR channels compared to cells in the absence of the drug, being this effect blocked by the use of $CFTR_{inh}$-172, a specific CFTR inhibitor. Cell proliferation, assessed with the reagent MTT, was not affected.

2. Methods

2.1. Cell culture

The BeWo and T84 (positive control, [17]) cell lines (American Type Culture Collection, USA) were maintained in Ham's F12 medium (Gibco BRL, Life Technologies, Buenos Aires, Argentina) with 10% fetal bovine serum (FBS, Natocor Biotechnology, Buenos Aires, Argentina) [14,15].

2.2. Reagents

Forskolin (Alomone Labs, Jerusalem, Israel) and $CFTR_{inh}$-172 (Calbiochem, San Diego, CA, USA) were dissolved in DMSO. DMSO final concentration per se did not affect the experiments [18].

2.3. RT-PCR

RT-PCR was performed as described before [18] with 5 μg of total RNA. PCR (35 cycles) was performed at 94 °C for 60 s, 59 °C for 60 s, and 72 °C for 60 s, followed by a final extension of 10 min at 72 °C. The reaction was carried out using two specific oligonucleotide primers coding for nucleotides 4354–4374 and 4650–4630, respectively, of CFTR exon 24 (COOH terminus) sequence (sense 5′-ACTATTGCCAGGAAGCCATT-3′, antisense 5′CACCGGAACTCAAGCAAGTG-3′).

2.4. Immunoblotting

Total proteins (75 μg) from the cells were resolved on 6% polyacrylamide gel and electrotransferred onto nitrocellulose membrane. We employed polyclonal antibodies against CFTR (Alomone Labs. or Santa Cruz Biotechnology (CA, USA), dilution 1:2500 overnight) and a goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (1:5000) (Vector Lab., Burlingame, USA) [15].

2.5. Cell surface biotinylation

Cell-surface expression levels of the CFTR protein in BeWo and T84 cells were examined using the membrane-impermeant biotinylation reagent (Pierce Chemical, Rockford, IL, USA). Cells were seeded onto T75 cm^2 flasks up to 90–95% confluence. After this, the medium was removed and the cells were washed twice with ice-PBS, pH 8.0. Each flask of cells was incubated with 10 ml of sulfo-NHS-SS-biotin (0.5 mg/ml in PBS; Pierce) and gently agitated on ice for 30 min at 4°C. The reagent was freshly prepared for incubation. After biotinylation, each flask was incubated with 500 μl of quenching solution (192 mM glycine, 25 mM Tris-Cl [pH 7.4]) for 20 min on ice to ensure complete quenching of the unreacted sulfo-NHS-SS-biotin. Cells were scraped into solution and pelleted at 500 rpm for 5 min. Pellets were solubilized for 30 min in 150 μM of lysis buffer (500 mM NaCl, 50 mM Tris-Cl, 1% Triton X-100 and 5 mM EDTA) containing protease inhibitors. Unlysed cells were removed by centrifugation at 13,000 rpm at 4°C. Biotinylated proteins were precipitated with NeutrAvidin-agarose beads (50 μl, Pierce Chemical), added to the supernatant to isolate cell membrane protein and incubated overnight in an end-over-end mixing rotator. Membrane proteins were dissolved in loading buffer with 50 mM DTT. The CFTR was detected in the pool of membrane proteins by polyacrylamide gel electrophoresis and immunoblotting as described above with CFTR H-182-Santa Cruz Biotechnology antibody. The immunoblots were quantified by densitometric analysis. As input controls 75 μg of nonbiotinylated cell lysates proteins were used (input). The CFTR expression at the cell surface (same protein loading) was quantified by densitometric analysis. The intensities of the surface signals (each band) were normalized to the corresponding intensities of nonbiotinylated lysate signals (input). The calculated values for immature CFTR were set as 100%. After normalization the values were plotted as intensity of the biotinylated mature CFTR with respect to immature CFTR (mean percent change ± SE). Each experiment was repeated five times in BeWo cells and 3 times in T84 cells.

2.6. Whole-cell recordings

Electrical activity was recorded in the whole cell configuration as described previously [15,18] with standard patch clamp technology. The protocol consisted in pulses from −80 to +100 mV in steps of 20 mV, from a holding potential of 0 mV and with a 10 ms interval between each pulse. Solutions: Pipette solution (intracellular) contained (mM): 125 CsCl, 1 $MgCl_2$, 10 HEPES, 0.2 BAPTA (pH 7.4). The bath solution contained (mM): 150 NaCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4). CsCl was the main salt in the intracellular solution in order to diminish K^+ conductances. The osmolarity was 260 ± 5 mOsm/l to the solution of CsCl, and 292 ± 6 mOsm/l for NaCl solution.

Current–voltage (I–V) plots were obtained from currents measured in a stationary state and were expressed as current densities (current per unit cell capacitance) [14,15]. The capacitance of the cells (C_m) was measured applying 100 ms, 10 mV, depolarizing pulses from a holding potential of 0 mV. The series resistances (Rs) were determined (14.7 ± 1.1 MΩ, $n = 4$), the currents were fitted to an exponential function and the time constant (τ) was measured; thus $C_m = \tau/Rs$. The Rs and capacitances were compensated during experiment using the analog circuit of the amplifier. Although, not been taken note of the exact values of the used seal resistances, only patches with seal resistances greater than 1 GΩ were recorded. All experiments were done at room temperature (20–24 °C). Cells were exposed to 20 μM forskolin for 10 min and then to 2 μM $CFTR_{inh}$-172 (in the presence of forskolin) until maximal blockage was achieved.

2.7. Wound-healing assay

Wound healing assay was performed as previously [1,15]. Briefly, monolayers were manually scraped and Ham's F12 + 1% FBS was added to attenuate cellular proliferation without impairing cell survival, with different treatments at pH 7.4: (1) a control group, (2) 20 μM forskolin, (3) 20 μM forskolin plus 2 μM $CFTR_{inh}$-172, and (4) $CFTR_{inh}$-172. At this time was considered time 0. All treatments were incubated for 5–6 h straight and images were taken.

Cell proliferation was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) assay as described previously [15] at the same conditions of wound healing experiments.

2.8. Statistical analysis

Data were expressed as mean values ± standard error (SE) (n = number of cells and repetitions). We used either a Student's test or one-way and repeated measures ANOVA for multiple data comparison, followed by a *post hoc* test. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. CFTR expression in BeWo cells

To determine whether CFTR is expressed in BeWo cell line, total RNA was extracted and RT-PCR analysis was performed using primers to amplify human CFTR (see Methods). Cells from the T84 line from a colonic human cancer were used as a positive control [17,19]. An expected band of 295 bp corresponding to CFTR product was obtained in both, BeWo and T84 cells. No product was detected in the absence of reverse transcriptase (negative control). A band of 289 bp was detected, corresponding to β-actin, showing positive cDNA integrity (Fig. 1A).

Further determination of CFTR protein in BeWo cells was conducted by Western blot analysis of whole cell lysates. Fig. 1B shows blots of BeWo cells. The membranes were incubated with two types of antibodies generated against different parts of the protein. One of them was a polyclonal antibody generated against aminoacids 1-182 mapping at the N-terminus of human CFTR (H-182, Santa Cruz Biotechnology). A band of about 160 kDa was observed, that corresponds to mature CFTR. In addition, another band of a lower molecular weight was evident representing an immature, deglycosylated form of CFTR. This is in agreement with previous results indicating that the immunoreactive bands in the 140- to 180-kDa regions represent various forms of the CFTR protein [20,21]. A unique band of 160 kDa was observed in T84 cells used as positive control, where CFTR is constitutively expressed [17] (left panels). It is consistent with the idea that the maturation of CFTR protein is complete in T84 cells, so with an efficient processing of endogenous CFTR [22]. The other used antibody was against aminoacids residues 1468–1480 corresponding to C-terminal part of human CFTR (Alomone Labs LTD). The 160-kDa band was present in BeWo and T84 cells (right panels). No bands were detected with nonimmune mouse IgG (data not shown).

Biotinylation of the BeWo membrane was used to estimate whether the CFTR is expressed on cell-surface. The biotinylated CFTR protein was detected as the two forms, mature glycosylated and immature unglycosylated CFTR. A unique band corresponding to mature glycosylated CFTR was found in T84 cells used as positive

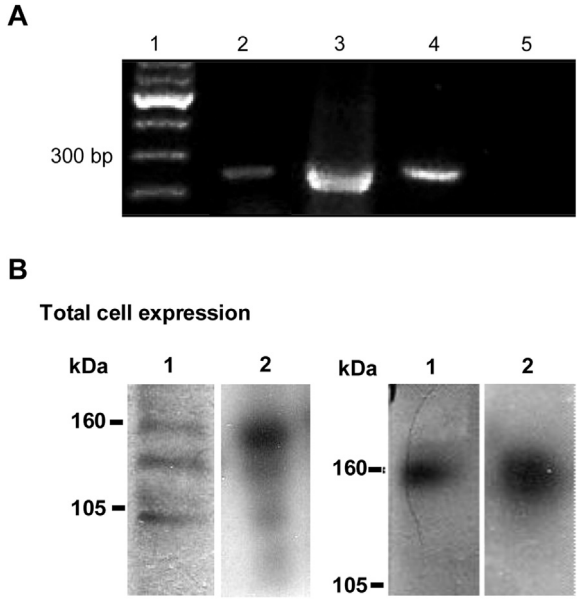

Fig. 1. CFTR expression in BeWo cells. A. Detection of CFTR mRNAs in BeWo cells. Lanes 1 to 5: One hundred-bp ladder; CFTR in BeWo cells (295 bp); β-actin in BeWo cells (289 bp); human colon carcinoma cells (T84) used as a positive control; BeWo cells samples without reverse transcriptase used as a negative control (absence of genomic DNA amplification). The experiment was repeated 3 times. B. Representative *immunoblots* for CFTR in total extracts from BeWo cells. Membranes were incubated overnight with antibodies directed toward different parts of the protein: anti-CFTR (Alomone, Jerusalem, Israel) and another (H-182, from Santa Cruz Biotechnology, Ca, USA). With the first one, a unique band of 160 kDa was observed in both, BeWo (line 1) and T84 cells (line 2) (right panels). With the second antibody (H-182) two bands were detected in BeWo cells (line 1): a band about 160 kDa was seen corresponding to mature CFTR protein and another band of a lower molecular weight consistent with an immature, deglycosylated form of CFTR (left panels). A unique band of 160 kDa was observed in T84 cells (line 2) used as positive control.

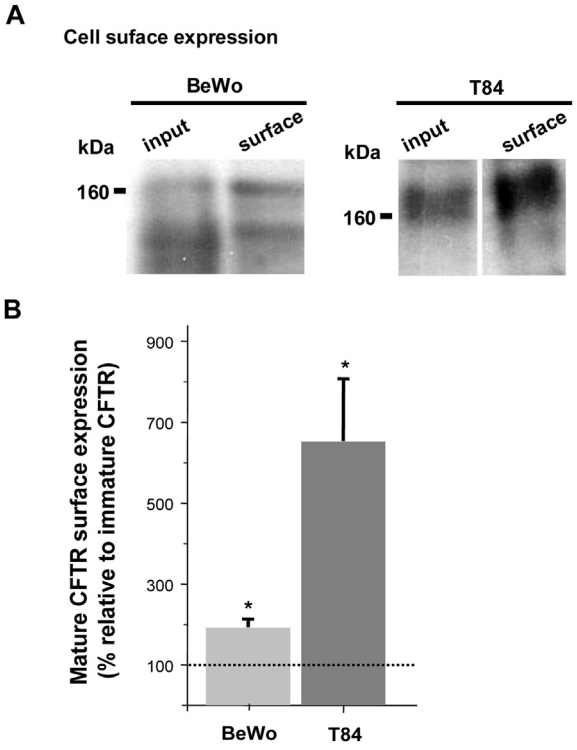

Fig. 2. Surface expression of CFTR in BeWo and T84 cells. A. Representative immunoblots demonstrate an increase in surface expression of mature CFTR in BeWo cells with respect to immature CFTR form. BeWo and T84 cells (used as positive control) were subjected to cell surface biotinylation prior to lysis (surface). As input controls, nonbiotinylated cell lysates proteins were used (input). Proteins were detected with CFTR H-182-Santa Cruz Biotechnology (Ca, USA) antibody. Protein loading was the same in all cases (75 μg). B. The CFTR expression at the cell surface was quantified by densitometric analysis. The intensities of the surface signals were normalized to the intensities of nonbiotinylated lysate signals (input). The calculated values for immature CFTR were set as 100%. The scale bars represents the mean percent change in the expression of CFTR at surface of BeWo or T84 compared with immature CFTR controls ± S.E. Mature CFTR increased about twice at the surface of BeWo cells ($p < 0.05$, $n = 5$) and was more abundant in the surface of T84 than in the trophoblastic cell line ($p < 0.05$, $n = 3$). The asterisk indicates a significant difference in the expression of the mature with respect to the immature form in each cell type.

control (Fig. 2A). In this condition, we observed that mature CFTR protein was more abundant that the immature in BeWo cell surface, relative to the non-biotinylated samples. Densitometric analysis of the signals indicates that the intensity of the biotinylated mature CFTR was higher (about twice) than the immature CFTR in Bewo cells ($p < 0.05$, $n = 5$) (Fig. 2B). Thus, CFTR was more abundant in the surface of T84 than in the trophoblastic cell line ($p < 0.05$, $n = 3$).

3.2. CFTR functional study

CFTR is normally activated in cells by cAMP signaling pathways and inactivated by phosphatases [23]. Forskolin (20 μM), an adenylate cyclase activator, was used to induce channel activation and 10 min of exposure was enough to achieve maximal effect. Fig. 3A shows currents before and after activation by forskolin and the forskolin activated current fraction obtained by subtracting control currents to treated currents. These currents activate very fast and show no time dependence. It is evident the nonlinear current–voltage (I–V) relationship in the forskolin-activated currents (Fig. 3B and C) with mean conductances (−80 to 100 mV) from 20.1 ± 0.8 pS in the negative voltage range to 32.5 ± 0.8 pS in the positive voltage range ($n = 4$), indicating the presence in these cells of an outward rectification, due to the activity of ORCC [14] besides the CFTR. The Er was −8.0 ± 0.5 mV ($n = 4$), a value close to the theoretical Nernst equilibrium potential for a Cl^- selective current with the Cl^- concentrations used in these experiments (−5.0 mV), suggesting a Cl^- movement through anionic channels.

To confirm that effectively CFTR presents activity in these cells, we stimulated with forskolin and subsequently inhibited with $CFTR_{inh}$-172 (2 min). This compound is a potent allosteric inhibitor that blocks specifically and reversibly disrupts CFTR channel gating. It is permeable to cells, and it is not inhibit other chloride channels [24–26]. The conductances in the presence of $CFTR_{inh}$-172 were significantly lower (16.0 ± 0.7 pS/pF and 32.6 ± 1.5 pS/pF) than in presence of only forskolin (29.7 ± 0.9 and 47.0 ± 2.0 pS/pF) ($p < 0.05$, $n = 4$) in the negative and positive voltage range, respectively. $CFTR_{inh}$-172 inhibited currents ($ICFTR_{inh}$-172) were obtained by subtracting the currents remaining after the blocking of those treated with only forskolin. The linear I–V relationship of $ICFTR_{inh}$-172 indicated a channel slope conductance of 14.9 ± 0.7 pS/pF, illustrating the nonrectifying properties of the CFTR (Fig. 4).

3.3. Participation of the CFTR in BeWo wound healing

A section of the cell layer was removed with the tip of a micropipette. This action left a clear space on the plastic for the cells to fill, and the wound border served as a migratory start line. This cleared space was subsequently visualized under the microscope and photographed to assess the ability of the cells to migrate and fill the wounded area (Fig. 5, left).

The cells were cultured at physiological pH (7.4) with 1% FBS. With cells exposed to 20 μM forskolin we observed a significant

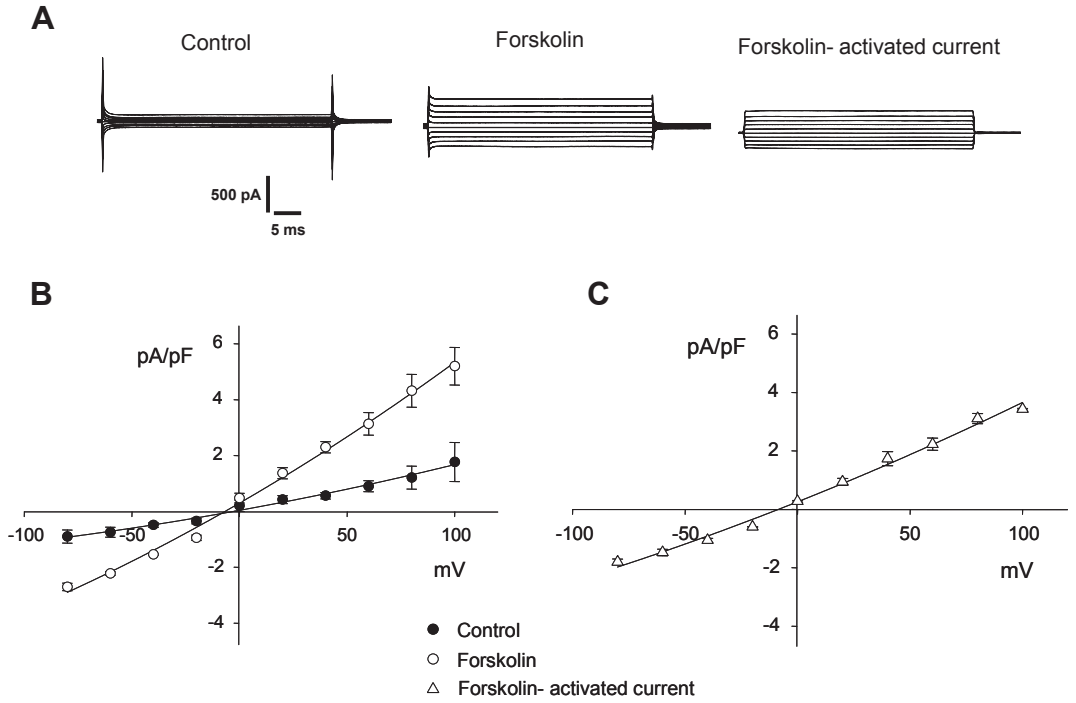

Fig. 3. A. Whole cell currents in a BeWo cell in the absence (control) and presence of 20 μM forskolin. The forskolin-activated current fraction was obtained by subtracting control currents to treated currents. B and C. Current densities (current per unit cell capacitance) vs. the voltage, obtained in the same conditions as described above. The difference between control and forskolin conditions was statistically different at all tested potentials ($p < 0.05$, $n = 4$).

increase in wound healing in comparison with the non-treated cells ($p < 0.01$, $n = 6$). The coadministration of $CFTR_{inh}$-172 (2 μM) reduced the healing behavior to levels similar to control (11.2 ± 0.4, 24.0 ± 1.7 and 13.9 ± 1.0% reinvasion of the wound when cells were incubated under control, forskolin and forskolin plus $CFTR_{inh}$-172 treatments, respectively). $CFTR_{inh}$-172 treatment alone did not affect wound healing when compared with control samples (9.2 ± 0.3% reinvasion of the wound) (Fig. 5, right).

Cell proliferation assessed with the reagent MTT was not changed in any of these treatments. Nontreated cells were

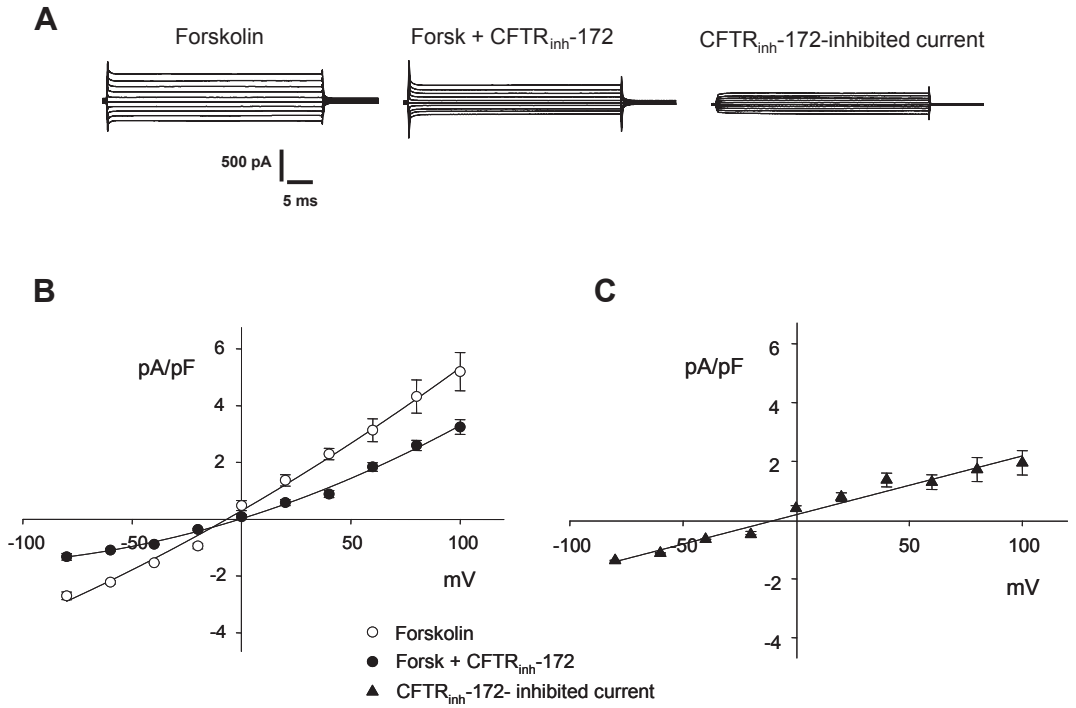

Fig. 4. A. Whole cell currents in a BeWo cell in the presence of 20 μM forskolin and the effect of $CFTR_{inh}$-172. The $CFTR_{inh}$-172-inhibited current fraction was obtained by subtracting forskolin-currents to $CFTR_{inh}$-172 treated currents. B and C. I–V plot of the experiment show in A. The data represent the average $CFTR_{inh}$-172-inhibited fraction obtained by subtracting the currents in the presence of forskolin to $CFTR_{inh}$-172 plus forskolin currents. The difference between the absence and presence of $CFTR_{inh}$-172 conditions was statistically different at all tested potentials ($p < 0.05$, $n = 4$).

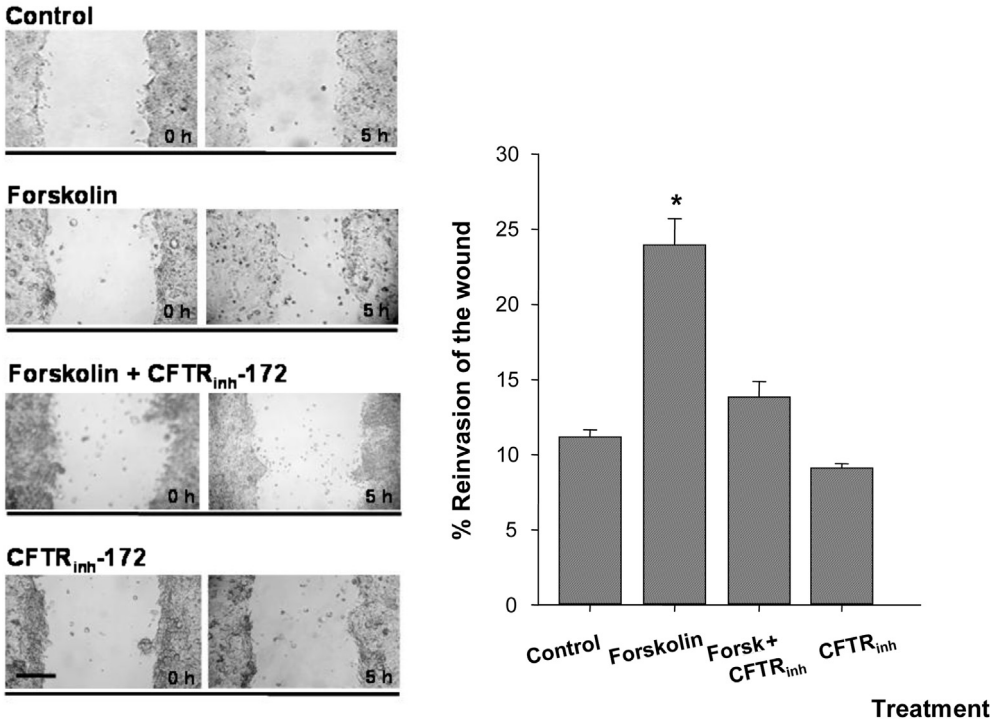

Fig. 5. Wound healing in the presence of forskolin and $CFTR_{inh}$-172. Left. Images of BeWo epithelial sheets cultured at pH 7.4 under different treatments: control conditions (vehicle), forskolin 20 μM, forskolin 20 μM plus $CFTR_{inh}$-172 2 μM, and $CFTR_{inh}$-172 2 μM. Pictures were taken when the sheets were wounded by scratching (time 0) and 5–6 h later. The bar represents 200 μm. Right. Mean values of wound healing in the presence of forskolin and $CFTR_{inh}$-172. Results were expressed as reinvasion percentage of the wound compared to time 0 (mean ± standard error). The asterisk indicates a significant difference in the % reinvasion of the wound in forskolin- treated cells with respect to the other treatments.

considered as 100%, and then 97.6 ± 2.9%, 97.0 ± 4.5% and 96.2 ± 5.3% MTT reductions were observed for the forskolin, forskolin plus $CFTR_{inh}$-172, and $CFTR_{inh}$-172 treatments, respectively ($p > 0.05$, $n = 5$).

4. Discussion

Our results indicate 1) the BeWo cell line expresses the CFTR; 2) CFTR is present in the cell surface and 3) that the channel is functional and play a role in the wound healing of these trophoblast cells increasing the cell migration process.

A 160-kDa band that corresponds to glycosylated form of CFTR was detected in BeWo cells using two different antibodies. However, the unglycosylated variant of CFTR was only evident with one of them. N-linked glycosylation sites are in the position 894 and 900, in extracellular loop 4 of CFTR [20] and have an intrinsic ability to enhance the nascent CFTR folding, trafficking, localization, stability and lifetime of CFTR. Their absence does not affect the protein function [20,27]. Our results (Fig. 1) allow us to speculate that an incomplete glycosylation of CFTR might alter the antigenic determinant for the Alomone antibody, thereby affecting the recognition of the immature form of the protein (unglycosylated CFTR).

The results with biotinylation experiments allowed us to say that the channel is also expressed on the cell membrane in BeWo cells where the mature form is more abundant. In addition, a band of a lower molecular weight was expressed, probably representing an immature, deglycosylated form of CFTR, in agreement with other authors [20,21,27]. The large unbiotinylated pool of maturely glycosylated CFTR in T84 cells, used as positive control, suggests that CFTR resides in an intracellular compartment well as being present at the cell surface in these cells [17].

We employed the patch clamp technique to characterize the ion channel in BeWo cells. CFTR is normally activated in cells by a cAMP-dependent PKA phosphorylation event which activates the channel, which in turn can be inactivated by phosphatases. An intracellular cAMP rise may be generated by different stimuli in human cells. In all the experiments we used forskolin, an adenylate cyclase activator to elicit currents through these channels. We demonstrated the presence of a current inhibited by $CFTR_{inh}$-172 (2 μM) that resembles the CFTR described in epithelial and non-epithelial cells [23]. This drug reversibly inhibits CFTR currents in a voltage independent manner with an IC_{50} between 1.16 and 5 μM according to some authors [28,29]; it did not inhibit non CFTR Cl^- channels (ORCC), ATP sensitive K channels and other transporters [24–26,28,29].

Forskolin increased the total current baseline, indicating the presence of Cl^- channels activated by cAMP. Accordingly, the observed increase in the total current could be due to the presence of CFTR and ORCC in BeWo cells, present in these cells [14]. ORCC could also be activated by forskolin with a clear outwardly rectification [30]. When we inhibited with $CFTR_{inh}$-172, the resulting current is mainly due to CFTR, with a perfectly linear relationship, typical of CFTR, but a residual current by ORCC may be present or perhaps a chloride channel which has not been characterized yet. According with Ma et al. [24] the Ki of $CFTR_{inh}$-172 is 0.3 μM, and in CFTR current studies the concentration range used of the inhibitor was 0.2–5 μM [24]. In other studies higher inhibitor concentrations were used, for example, 20 μM [31]. We used a concentration of 2 μM.

We tested the effects of forskolin and $CFTR_{inh}$-172 on wound healing and cell migration. The scratch wound assay studies the migration of a sheet of cells in two dimensions in response to formation of a wound. This initiates migration from a standing start in

a defined direction. Cell proliferation could mask the contribution of cell migration to wound healing so we decided to test wound healing at 5–6 h, when mitosis is still scarce and to use culture medium with 1% FBS, to minimize proliferation. Thus, cell proliferation assessed with the reagent MTT was not changed in any of these treatments, suggesting that cellular migration is the main factor for the reinvasion of the wound healing. This result is in agreement with other reports showing the lack of effect of inhibition of CFTR with $CFTR_{inh}$-172 in cell proliferation in these conditions [1].

The reinvasion of the wound was about 12% of its initial value and forskolin enhanced cell migration by a factor of 2, most likely through its effects upon the CFTR channels because the reinvasion of the wound with the CFTR blocker was similar to basal values. Long-term treatment with forskolin (100 μM, >12 h) enhances the tight junction formation in BeWo cells and promotes differentiation into multinucleated cells [32,33], which is not this case.

How might CFTR modulate BeWo migration? Cell migration is involved in neuronal targeting, immunity, inflammation, wound healing, angiogenesis and metastatic spread. Movement of cells requires a sequence of cell protrusions and retractions that depends on the actin cytoskeleton and volume changes. Membrane receptors, ion channels and transporters actively participate in this process [34–37]. Under these circumstances, the ions moving the cells through the CFTR could be an intracellular signal before cells start to migrate. Sun et al. [38] showed that CFTR was involved in the generation of wound current that serves as a critical guidance for cell migration. Other authors have implied that CFTR anions transport and the consequent water movement favor the cell swelling required for lamellipodium expansion [1,8]. In addition, the consequent changes in the membrane potential generated by CFTR may stimulate cytoskeletal reorganization needed for migration [35]. In this regard, there are evidences that CFTR may function as a mechanosensor with implications in ion transport and cell volume regulation [39]. In all these cases, the inhibitory effect of $CFTR_{inh}$-172 on BeWo cell migration that we observed can be explained.

Schiller et al. [1] proposed another mechanism for the participation of CFTR upon migration of airway cells by regulating the extracellular pH through the transport of HCO_3^- [3]. The consequent alkalinization of the extracellular space would expect to reduce adhesion and increasing migration.

Although the role of CFTR in placental tissues is still poorly understood, its diminished expression in preeclamptic placentas [10] may be relevant to this condition. Preeclampsia is one of the leading causes for maternal and neonatal mortality and morbidity. Its etiology is not clear; it is characterized by defective placentation and endothelial dysfunction, and is associated with impaired peripheral use of oxygen [see Refs. [40,41] for references]. One hypothesis for the pathogenesis of preeclampsia is an impaired arterial remodeling which normally leads to the formation of maternal lacunae compromises placental and foetal blood flow. This impairment implies that trophoblast cells migrate and invade in a lesser rate. The consequent hypoxia generated may affect the whole placental physiology, as it was observed in other tissues exposed to hypoxic conditions where ion and fluid transport was compromised. Thus, the lower expression of CFTR as well as other channels such as ENaC [10,11] could have consequences in many areas of placental function, including ion transport, placental migration and proliferation.

Conflict of interest

None.

Acknowledgments

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