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The blocking of aquaporin-3 (AQP3) impairs extravillous trophoblast cell migration

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ABSTRACT

Several aquaporins (AQPs) are expressed in extravillous (EVT) and villous trophoblast cells. Among them, AQP3 is the most abundant AQP expressed in chorionic villi samples from first trimester, followed by AQP1 and AQP9. Although AQP3 expression persists in term placentas, it is significantly decreased in placentas from preeclamptic pregnancies. AQP3 is involved in the migration of different cell types, however its role in human placenta is still unknown.

Here, we evaluated the role of AQP3 in the migration of EVT cells during early gestation.

Our results showed that Swan 71 cells expressed AQP1, AQP3 and AQP9 but only the blocking of AQP3 by $CuSO_4$ or the silencing of its expression by siRNA significantly attenuates EVT cell migration.

Our work provides evidence that AQP3 is required for EVT cell migration and suggests that an altered expression of placental AQP3 may produce failures in placentation such as in preeclampsia.

1. Introduction

Development of the human placenta and its different epithelial trophoblasts is critical to ensure the normal fetal growth and the maintenance of a healthy pregnancy. In this process, trophoblast cells differentiate along the villous or the extravillous trophoblast (EVT) pathways [1,2]. EVT cells proliferate, migrate and invade into the maternal decidua and the inner myometrium, remodeling the maternal spiral arteries. On the other hand, the villous trophoblasts form the outermost layer of the chorionic villi and play a central role in the regulation of feto-maternal exchange.

Failures of these events may lead to placental hypoperfusion, tissue injury, and trophoblast hypoxia and their consequences, including fetal death, growth restriction, and preeclampsia [2–6]. Preeclampsia is a gestational disorder associated with alterations in placental development and the differentiation of trophoblast cells, however, the underlying molecular mechanisms of this syndrome are still unknown [6].

Aquaporins (AQPs) are water channel proteins that allow the rapid movement of water across the plasma membrane in response to osmotic/hydrostatic pressure gradients [7,8]. The expression of several AQPs was found from blastocyst stages to term placenta and fetal membranes [9]. Escobar and coworkers have recently described that AQP3 was the most abundant AQP expressed in chorionic villi sample from first trimester, followed by AQP1 and AQP9 [10]. Thus, these proteins might be important in the normal fetal growth and homeostasis.

AQP3 is a member of the aquaporin family that can permeate water, urea and glycerol. It is expressed in trophoblast cells throughout gestation, but its physiological role is still unknown [10–12]. Apart from the classical functions in transcellular water transport, recent studies have revealed that AQPs may cooperate in different cellular processes, such as apoptosis, cell migration and proliferation [13–15].

Regarding the role of AQP3 in human placentas, we recently explored the participation of AQPs in placental programmed cell death and observed that the inhibition of AQP3 abrogates the trophoblast apoptosis [16]. Although the apoptosis of the trophoblast cells is a normal event that increases throughout gestation, it is exacerbated in placentas from pregnancies complicated by preeclampsia [2,3].

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In this context, we assumed that an increase in AQP3 might correlate with the increase in trophoblast apoptosis observed in preeclampsia, but in subsequent experiments, we found a reduced expression of AQP3 in these placentas [17].

Taken into account that preeclampsia is associated with defects in placental development during the early stages of pregnancy and the abnormal expression of AQP3 was found in the third trimester when the maternal syndrome is established, and the placenta is completely formed, we speculated that a normal AQP3 expression and function may be required for normal placentation.

Along with this idea, increasing evidence from both *in vitro* and *in vivo* experiments suggested that AQP3 could facilitate tumor cell migration [18]. Since EVT cells display a phenotype strikingly similar to that of cancer cells [19,20] we hypothesized that alterations in AQP3 since early stages of placenta development could be associated with pregnancy disorders. However, up to now, the role of AQP3 during early gestation was unexplored.

In the present study, we investigated the participation of AQP3 in EVT cell migration during placentation.

2. Methods

2.1. Cell culture

Swan 71 cell line (derived by telomerase-mediated transformation of a 7 week cytotrophoblast isolate) was kindly provided by Dr. Gil Mor [21]. Cells were cultured in DMEM-F12 (Life Technologies, Inc. BLR, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Laboratorio Natocor, Cordoba, Argentina), 5 mM L-Glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B in humidified air at 37 °C with 5% CO_2.

For the general blocking of AQPs, 50 μM $HgCl_2$ (Sigma–Aldrich, St. Louis, MO, USA), a nonselective inhibitor of AQPs, was added to the cultured medium [22,23]. Hg^{2+} is a nonselective inhibitor of AQPs by binding to the cysteine residue in the transmembrane domain of AQPs. For specific blocking of AQP3, 100 μM $CuSO_4$ (Sigma–Aldrich, St. Louis, MO, USA), was added to the culture medium [22,24]. $CuSO_4$ is a potent inhibitor of AQP3 which binds to three extracellular amino acid residues of AQP3 (Trp128, Ser152, and His241). Other AQPs were also blocked using 100 μM tetraethylammonium chloride (TEA; Sigma–Aldrich, St. Louis, MO, USA) and 100 μM phloretin (TEA; Sigma–Aldrich, St. Louis, MO, USA). TEA inhibits AQP1 by binging to the Tyr186 located at the extracellular part of the protein [22,25] while phloretin blocks AQP9 by electrostatic interactions with the Asg216, the Asp69 and the His151 of the protein [22,26].

2.2. Transfection with AQP3 siRNA

Cells were also transfected with human specific siRNA pools of 2–5 target specific 19–25 nt pools (AQP3-1 sc-sc-29713; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) with siRNA Transfection

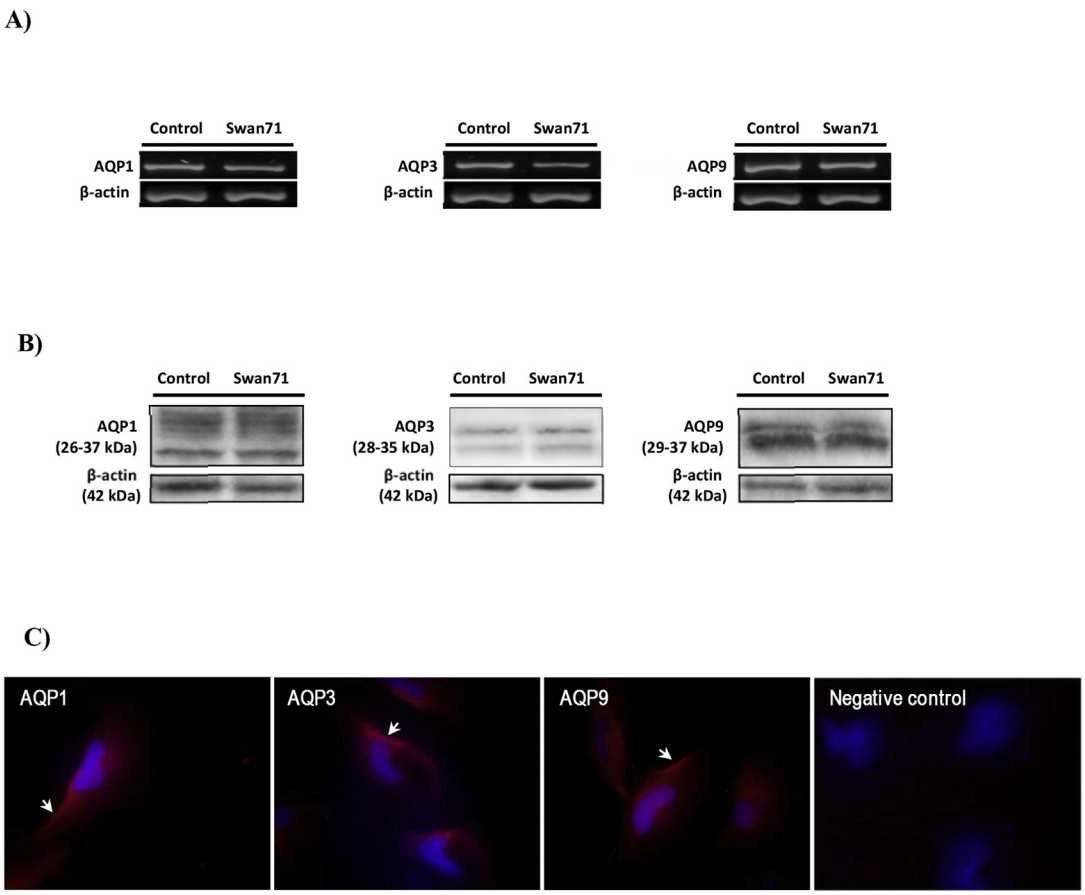

Fig. 1. Expression of AQP3 in Swan 71 cells.
A) Detection of AQP1, AQP3 and AQP9 mRNA in Swan 71 cells. Control: Term placenta.
B) Western blot of AQP1, AQP3 and AQP9 in Swan 71 cells. In all cases, two bands corresponding to the glycosylated and the non-glycosylated forms of AQPs were observed in Swan 71 cells. Control: Term placenta.
C) Immunofluorescence showing AQP1, AQP3 and AQP9 expression (white arrows) in plasma membrane of Swan 71 cells. *Magnification x1000.*

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