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The Multidrug-Resistance Transporter ABCB5 is Expressed in Human Placenta

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Summary

ATP-binding cassette (ABC) transporters in placenta protectively transport drugs and xenobiotics. ABCB5 [subfamily B (MDR/TAP)] is a novel ABC multidrug-resistance transporter that also mediates cell fusion, stem cell function, and vasculogenic plasticity. Immunohistochemistry and double-labeling immunofluorescence staining for ABCB5 and ABCB5/CD200, respectively, was performed on formalin-fixed, paraffin-embedded placental tissue from 5 first trimester, 5 second trimester, and 5 term pregnancies as well as 5 partial moles, and 5 complete moles. In addition, tumor cells from 5 choriocarcinoma and 5 placental site trophoblastic tumor cases were examined. ABCB5 staining was observed in villous trophoblasts in 100% (5/5) of first trimester placentas (with progressive decrease in term placentas); 100% of partial moles (5/5); and 100% of complete moles (5/5). Notably, reactivity was discretely restricted to the inner trophoblast layer, with no staining of overlying syncytiotrophoblast. Antibody specificity and localization was confirmed further by *in situ* hybridization. ABCB5 expression was retained in 20% of choriocarcinomas (1/5) and 40% of placental site trophoblastic tumors (2/5). Prior studies have localized expression of multidrug-resistance-1, also known as ABCB1, within the syncytiotrophoblast of early placentas, where it serves a protective function as an efflux transporter. Our results show that ABCB5 is preferentially expressed in the cytotrophoblast layer of placental villi. The expression of this novel biomarker at the maternal-fetal interface raises questions on its role in placental structure and function as well as on its potential contribution to the protective efflux provided by other P-glycoprotein transporters.

Keywords

Stem cell; Trophoblast; Syncytiotrophoblast; Cytotrophoblast; MDR transporter; ABCB5

Placental development requires cytotrophoblast stem cell differentiation by 2 pathways (1,2): (i) cytotrophoblasts remaining in the fetal compartment give rise to floating villi and multinucleated syncytiotrophoblasts (1,2); and (ii) cytotrophoblasts derived from anchoring

villi form a column of extravillous trophoblast that invades the decidua and undergoes vasculogenic interaction with maternal vessels (1–3). Transfer of substances across trophoblast barriers involves passive diffusion and active/facilitated transport (4), the latter resulting in protective efflux of drugs and xenobiotics (5–8). Known members of ATP-binding cassette (ABC) multidrug-resistance (MDR) transporters expressed in placental tissue are proposed to minimize fetal exposure to drugs and other xenobiotics (4). The best characterized are P-glycoprotein (P-gp or MDR1, gene symbol *ABCB1*), the breast cancer resistant protein (BCRP, gene symbol *ABCG2*), and the multidrug-resistance proteins 1 to 3 and 5 (MRP1–3 and 5, gene symbol *ABCC1–3* and 5). They have all been described to be localized at the syncytiotrophoblast including the apical surface (P-gp, BCRP, MRP2), the basolateral surface (ABCC5), or both (ABCC1 and ABCC3) (8). Expression of these MDR transporters may vary over time, with P-gp and BCRP expression decreasing, and MRP2 expression increasing with advancing gestational age (9–12).

ABCB5, an ATPase-binding MDR transporter, is a biomarker for physiological and pathologic stem cells (13,14) and a mediator of cell fusion (13), vasculogenesis (15), and drug efflux (16). Given the multiplicity of potentially relevant functions, we reasoned that ABCB5 may be expressed by human placenta. Our aim is to evaluate this hypothesis through the use of antibodies and RNA probes. Our data for the first time document ABCB5 expression in non-neoplastic and neoplastic placental tissue and raise questions regarding its multifunctional potential in trophoblast health and disease.

MATERIALS AND METHODS

Paraffin-embedded sections (total: 35; 5 from each category) included first trimester placenta (6–10 wk), second trimester placenta, term placenta, choriocarcinoma, placental site trophoblastic tumor, partial mole, and complete mole. Normal first and second trimester placentas were obtained from elective terminations. Term placentas were obtained from vaginal deliveries of healthy babies. All samples of non-neoplastic placentas failed to show specific alterations upon macroscopic and histologic examination, although foci of hemorrhage, edema, and fibrosis were occasionally documented. Mouse anti-ABCB5 antibody and goat anti-CD200 antibody (R&D System, Minneapolis, MN) were employed. The ABCB5 antibody has been employed in numerous studies concerned with cell fusion, drug efflux, and stem cell function (13–16), and CD200 is a biomarker known to label the syncytiotrophoblast (17). Sections from all samples were deparaffinized and rehydrated. Heat-induced epitope retrieval was performed. Sections were then incubated with antibodies for ABCB5 or CD200 at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated horse anti-mouse IgG or anti-goat IgG secondary antibodies at room temperature for 30 min. NovaRed peroxidase substrate (Vector Laboratories, Burlingame, CA) was used for detecting secondary antibodies. Controls consisted of replacement of primary antibodies with isotype-specific mouse IgG or goat IgG. For immunofluorescence double labeling, sections were incubated with ABCB5 and CD200 antibody at 4°C overnight, then with Alexa Fluor 488 donkey anti-mouse IgG and Alexa Fluor 594 donkey anti-goat antibodies (Invitrogen, Carlsbad, CA) at room temperature for 1 hr.

In situ hybridization was performed on a selected case to confirm colocalization of ABCB5 protein with mRNA using ABCB5 probes that were prepared from templates synthesized by introducing the T7 promoter into the anti-sense strand and the SP6 promoter into the sense strand. The primer pair (5'-TAATACGACTCACTATAGGGATGTCTGGCTTTTCCCTTCTTGAC-3' and 5'-GATTTAGGTGACACTATAGAAATTCAAGCTGGACGAATGACCCA-3') was used to generate the DNA template for anti-sense and sense RNA probes spanning 200 bp of human ABCB5 cDNA.

RESULTS

ABCB5 was exclusively localized to the cytotrophoblast layer of first trimester placentas, whereas syncytiotrophoblasts, defined as variably multinucleated CD200-positive cells, restricted to the fetal-maternal interface were negative for ABCB5 (17) (Figs. 1A–J). Perinuclear and membranous/cytoplasmic ABCB5 staining was observed in villous trophoblasts in 100% (5/5) of first trimester placentas, with progressive loss resulting in markedly decreased expression in term placentas (Figs. 1K–M). The reduction in staining for ABCB5 with gestational age was seen in all cases and was diffusely reflected in all villi. In addition, ABCB5 was present in extravillous trophoblast of cell columns as well as in cells consistent with intermediate trophoblast invading into decidualized endometrium (Figs. 1N–P) where positive cells were intimately associated with walls of endothelial-lined maternal vessels, consistent with vasculogenic plasticity inherent to extravillous trophoblasts (Fig. 1Q) (1–3). Positivity for ABCB5 was also observed in scattered stromal cells within the villi (Fig. 1C). Specificity of ABCB5 localization to the cytotrophoblast layer was confirmed further by *in situ* hybridization (Figs. 2A–D). Although the mRNA expression could generally be localized to the cytotrophoblast layer (Figs. 2A, C), occasional cuts did not permit such spatial discrimination (eg, Fig. 2B). Finally, trophoblast ABCB5 expression was focally retained in partial moles (5/5) and complete moles (5/5), as well as in a subset of choriocarcinomas (1/5), and placental site trophoblastic tumors (2/5) (Fig. 3). In general, expression of ABCB5 in partial and complete moles did not involve all villi and was variable within villi. Also, possible expression of ABCB5 was observed in the lacy syncytiotrophoblast component, as well as in the cytotrophoblast compartment, of a complete mole case. Expression in placental neoplasms was similarly focal. Hence, although expression was preliminary documented in a small number of defined pathologic processes, it did not appear to show the uniformity of expression encountered in non-neoplastic tissue.

DISCUSSION

ABCB5 was first cloned in 2003 when it was shown to regulate progenitor cell fusion by altering the membrane potential in normal melanocytes that expressed the stem cell marker CD133 (13). Subsequently, ABCB5 was found to regulate drug efflux that confers chemoresistance to human melanoma cells (16). ABCB5 dysfunction induced by antibody blockade or silencing by siRNA increased sensitivity of normally refractory melanoma cells to multiple chemotherapeutic agents, including doxorubicin (16). Most recently, ABCB5 has been shown to identify not only human progenitor cells (13), but also melanoma cells that express stem cell markers and that are capable of self-renewal, tumorigenic growth, and differentiation plasticity (14). These cells also display immunomodulators such as B7-2 and PD-1 (18) and their stem-like function is driven in part by expression of the VEGFR-1 receptor (15). Accordingly, in human progenitor and certain cancer stem-like cells, ABCB5 has characteristics of a multifunctional biomarker involved in cell protection from deleterious immunogenic and xenobiotic factors (19).

Cytotrophoblast stem cells are precursors of differentiated cells in the placenta and are believed to be most abundant during early stages when trophoblast growth is most robust (20). The adult stem cell phenotype is characterized by the ability to form other cell types during appropriate pathophysiological circumstances. Moreover, presumably due to their key importance in tissue maintenance and function, stem cells have evolved mechanisms for xenobiotic efflux, immunoprotection, and apoptosis prevention (19). In the placenta, these functions are critical to fetal protection, and the syncytiotrophoblast layer is a key interface in this process.

Although the cytotrophoblast layer was uniformly and continuously stained in most early term villi, the possibility of heterogeneity in ABCB5 expression by trophoblast subpopulations could not be excluded. Furthermore, the decrease in ABCB5 staining in cytotrophoblast with advancing gestational age may reflect a combination of decreased expression as well as decreased density of this cell population. Although the ABCB5 immunoreactivity observed in this study appeared to be localized to the cytoplasm, membrane and perinuclear areas of all specimens; further characterization of its precise distribution would require more detailed multiplex immunohistochemical or immunoultrastructural approaches.

The mechanism of cytotrophoblast fusion to form syncytiotrophoblast is not completely understood. ABCB5 expression is known to regulate cellular membrane potential and resultant cell fusion in experimental (*in vivo*) systems (13), raising questions regarding its role in formation of syncytiotrophoblast in both placental health and disease. While the *in situ* hybridization performed in this study served to corroborate the localization of specific ABCB5 protein immunoreactivity to the cytotrophoblast layer, the attenuated nature of overlying syncytiotrophoblast cytoplasm as well as the extent of multinucleation centrally within the cells complicated definitive exclusion of cytoplasmic signal for ABCB5 mRNA in this cell type.

Recent data implicating the VEGF-VEGFR-1 pathway in mediating vasculogenic plasticity in ABCB5-positive melanoma stem cells is of potential relevance to trophoblasts that are also known to express VEGFR pathways within their placentomes (21). In addition to physiological implications, expression of ABCB5 in placental neoplasms now requires additional study to determine whether it defines cells that confer chemoresistance, immunoprotection, and/or tumorigenic growth.

The finding of ABCB5 positivity in isolated cells within villous stroma is in keeping with the known expression of other ABC transporters by Hofbauer cells (4). Alternatively, Abumaree et al. (22) have recently characterized the phenotypic and functional characteristics of mesenchymal stem cells derived from placental chorionic villi, revealing their intrinsic differentiation plasticity along osteogenic, chondrogenic, and adipogenic pathways. Whether ABCB5-positive cells within the mesenchymal cores of placental villi have similar differentiation plasticity *in vitro*, and how they may relate to Hofbauer cells, remains to be elucidated. In addition, we observed condensation of ABCB5-positive stromal cells around maternal vessels as well as individual ABCB5-positive cells within decidual tissue. Although these cells are consistent with invasive trophoblast we cannot definitely exclude other cell lineages including endothelial derivation. Moreover, the sample size did not permit evaluation of how ubiquitous this phenomenon was within the decidualized endometrium.

Although the retention of ABCB5 expression in partial and complete moles as well as in a subset of trophoblastic tumors is in keeping with some degree of placental cytodifferentiation, we cannot exclude the possibility that some ABCB5-expressing cells may have potential for self-renewal and tumorigenic growth, as has been demonstrated in malignant melanoma (14). Moreover, the expression of a functional MDR transporter must be considered in assessing potential mechanisms for chemoresistance of placenta-derived tumors (16,23). Finally, because complete moles appear to show a minor component in ABCB5 expression in the syncytiotrophoblast component (Fig. 3B), it is possible that with malignant transformation, dysregulation of ABCB5 synthesis occurs, resulting in more widespread expression of this biomarker.

In summary, we have described the expression of the stem cell-associated MDR transporter, ABCB5, selectively within the placental trophoblast and trophoblast-derived neoplasms. Because of the multifunctional nature of ABCB5 and its potential role in known trophoblast function and tumorigenesis, further studies are now indicated to define the biologic and translational relevance of this observation.

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FIG. 1.

Human placental ATP-binding cassette subfamily B5 (ABCB5) expression. (A–J), ABCB5 is localized to the cytotrophoblast layer of first trimester placentas in contrast to syncytiotrophoblast CD200 localization. (A and C), Progressive magnification of first trimester chorionic villi stained for ABCB5 (red chromogen), as compared to CD200 syncytiotrophoblast staining (B and D; brown chromogen). (E and H), Immunofluorescence for ABCB5 (green), (F and I), CD200 (red), and (G and J), merged. (K–M), ABCB5 reactivity dissipates from first (K) to second (L) to third (M) trimester placental stages. (N–Q), CD200 [(N), brown] delineates the outermost syncytiotrophoblast layer of a villus and ABCB5 [(O), red] labels the extravillous trophoblast. The ABCB5-expressing extravillous trophoblast form columns associated with invasion of the decidualized endometrium. ABCB5-positive columns form close associations with maternal vessels containing erythrocytes [(O), enclosed and (P), arrow] and ABCB5-positive cells also are intimately associated with walls of maternal vessels. Scattered individual cells migrating within the decidua are also positive (Q). EVT indicates extravillous trophoblast; MAT RBC, maternal vessel containing red blood cells; V, villus.

FIG. 2.

In situ hybridization for ATP-binding cassette subfamily B5 (ABCB5). (A–D), In (A–C), the anti-sense digoxigenin label is restricted to the cytotrophoblast cell layer, with no distinct labeling [(D): sense control] of syncytiotrophoblasts [arrows in (A) and (C), show lack of ABCB5 RNA expression in multinucleated syncytiotrophoblasts]. Occasional cells within the villous stroma are also positive.

FIG. 3. ABCB5 expression in human placental neoplasms. ATP-binding cassette subfamily B5 (ABCB5) expression is focally retained in partial moles (A), complete moles (B), in a subset of choriocarcinomas (C), placental site trophoblastic tumors (D). Possible expression is seen in the lacy syncytiotrophoblast component, as well as in the cytotrophoblast compartment of the complete mole (B).