Subcellular Localization and Distribution of the Breast Cancer Resistance Protein Transporter in Normal Human Tissues

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ABSTRACT
High expression of the Breast Cancer Resistance Protein (BCRP) gene has been shown to be involved in resistance to chemotherapeutic drugs. Knowledge of the localization of BCRP protein in normal tissues may help unravel the normal function of this protein. Therefore, we characterized the tissue distribution and cellular localization of BCRP in frozen sections of normal human tissues. For this purpose, we used the recently described monoclonal antibody BXP-34 and another independently developed monoclonal antibody directed against BCRP, BXP-21. Both monoclonal antibodies show specific BCRP plasma membrane staining on cytospins obtained from topotecan- or mitoxantrone-selected cell lines, as well as from BCRP-transfected cell lines. Immunoprecipitation experiments using either BXP-21 or BXP-34 yielded a clear M, 72,000 BCRP band from BCRP-overexpressing tumor cells. In the topotecan-selected T8 and mitoxantrone-selected MX3 tumor cell lines, BCRP turned out to be differentially glycosylated. In contrast to BXP-34, BXP-21 is able to detect the M, 72,000 BCRP protein on immunoblots and is reactive with BCRP in formalin-fixed, paraffin-embedded tissues. Using BXP-21 and BXP-34, prominent staining of BCRP was observed in placental spongiotrophoblasts, in the epithelium of the small intestine and colon, in the liver canalicular membrane, and in ducts and lobules of the breast. Furthermore, BCRP was present in veins and capillary endothelium, but not in arterial endothelium in all of the tissues investigated. In the tissues studied, the mRNA levels of BCRP were assessed using reverse transcription-PCR, and these corresponded with the levels of BCRP protein estimated from immunohistochemical staining. The presence of BCRP at the placental spongiotrophoblasts is consistent with the hypothesis of a protective role of BCRP for the fetus. The apical localization in the epithelium of the small intestine and colon indicates a possible role of BCRP in the regulation of the uptake of p.o. administered BCRP substrates by back-transport of substrate drugs entering from the gut lumen. Therefore, it may be useful to attempt to modulate the uptake of p.o. delivered BCRP substrates, e.g., topotecan or irinotecan, by using a BCRP inhibitor. Clinical trials testing this hypothesis have been initiated in our institute.

INTRODUCTION
Drug transporters other than P-gp or the MRP family may be important for resistance to anticancer drugs. Recently, elevated expression of the BCRP (ABCG2) gene, a member of the ATP-binding cassette transporter family, has been described in drug-resistant ovary, breast, colon, gastric cancer, and fibrosarcoma cell lines (1–5). BCRP has only one ATP-binding cassette and six putative transmembrane domains, suggesting that BCRP is a half-transporter, which may function as a homodimer or heterodimer. However, the identity of a putative partner protein is unknown as yet.

For many drug transporters, a normal physiological role is known. For instance, P-gp is highly expressed in the blood-brain barrier, the intestine, and the placenta and has a protective function for the brain and the fetus by extruding toxic agents (6). MRP1 also appears to function as an outward pump for xenobiotics (7) and contributes to the blood-cerebrospinal fluid barrier (8, 9). For BCRP, the normal physiological function has not been established as yet. In normal human tissues, high expression of BCRP mRNA has been noted in the placenta (1, 4). Furthermore, although results between previous studies (1, 4) differ somewhat, low expression of BCRP in liver, small intestine, colon, ovary, kidney, and heart was reported. This expression profile allows speculation on a role of BCRP, like P-gp, in protection of the fetus and in the regulation of transport of chemicals through the epithelium of the gastrointestinal tract. The mRNA expression of BCRP has been assessed in tissue extracts, but it has not yet been determined in which cell types BCRP is expressed and what the subcellular localization of BCRP is.

We have developed recently the Mab BXP-34 directed against human BCRP (10). In this study, we describe a newly developed Mab directed against BCRP, BXP-21. We have used the BXP-21 and BXP-34 Mabs to characterize the tissue distribution and subcellular localization of BCRP. Moreover, for a number of tissues we isolated mRNA from the same blocks for semiquantitative RT-PCR evaluation of the BCRP expression levels. Knowledge of the normal tissue distribution may add to the understanding of the normal function of BCRP and may be valuable for future clinical purposes.

MATERIALS AND METHODS
Cell Lines and Tissues. The following cell lines were used in this study: the human IGROV1 ovarian carcinoma cell line, the topotecan-selected subline T8, and the mitoxantrone-selected subline MX3. The T8 and MX3 cells are resistant to topotecan and mitoxantrone and concomitantly show overexpression of the BCRP gene (3). Furthermore, a partially revertant T8 cell line (3), the doxorubicin-resistant human GLC4/ADR small cell lung cancer cell line (11), the MCF-7 breast carcinoma cell line, the doxorubicin-selected subline MCF-7 Dox40, the mitoxantrone-selected subline MCF-7 MR (12), as well as the myeloma cell line 8226, the doxorubicin-selected subline 8226 Dox40, and the mitoxantrone-selected subline 8226 MR20 (13) were used. The ovarian carcinoma cell line 2008 and the MRPl- and MRPl2-transfected sublines were described by Kool et al. (14) and Scheffer et al. (15), and the monkey kidney CV-1 cells, transformed by an origin-defective mutant of SV40 that codes for wild-type T antigen COS7 cells, by Gluzman (16). For control purposes, a dog-kidney-derived MDR1 p-gp-transfected cell line, MDCKII/MDR1 (17), was used. All of the cell lines were cultured in RPMI 1640 or DMEM supplemented with 10% FCS, 25 mM HEPES, 110 IU/ml penicillin, and 100 µg/ml streptomycin. Cells were kept in continuous logarithmic growth at 37°C in a humidified atmosphere of 5% CO2 in air. Normal human tissues were obtained from the tissue cryobank of The Netherlands Cancer Institute or the Free University in Amsterdam. Normal human tissue was obtained from surgical or autopsy specimens. All of the tissues were snap frozen in liquid nitrogen and subsequently stored at −70°C. Blood cells were obtained from
healthy volunteers. Fresh bone-marrow progenitor cells were from a neuroblasto
toma patient and were obtained via the Central Laboratory of the Nether-
lands Red Cross Blood Transfusion Service (Amsterdam, the Netherlands).

**Immunization and Mab Production.** The development of the Mab BXP-34, directed against BCRP, has been described elsewhere (10). An independently developed Mab, BXP-21, was raised in Balb/C mice analo-
gous to described methods (10, 18) by injection with a fusion protein con-
sisting of the *Escherichia coli* maltose-binding protein and a 126 amino
acids part of the BCRP peptide [amino acids 271-396 of BCRP (GenBank
accession no. AF098951)]. This fusion protein was made using the plasmid
vector pMal-c (19). The fusion protein was produced in *Escherichia coli*
DH5α and purified by amylose resin affinity chromatography (19). The
mice were housed and treated according to current regulations and stand-
ards of the Animal Ethics Committee.

The mice were killed, and subsequently draining popliteal lymph nodes
were removed and used for fusion with mouse myeloma Sp2/0 cells, as
described previously (14). Hybridoma supernatants containing Mabs were first
screened on ELISA plates, coated with the above-mentioned fusion protein, or
coated with an irrelevant fusion protein as negative control. BCRP fusion
protein-positive cultures were screened on octo-spins containing eight cyto-
spins of a mixture of MCF-7 MR and MCF-7 parental tumor cells. Slides were
stained as described below. Hybrid cells that secreted antibodies of interest
were selected and subcloned three times by limiting dilution. The isotype was
determined using *IsoStrips* (Boehringer Mannheim).

**Western Blotting.** Cells were scraped and subsequently lysed in hypotonic
lysis buffer, consisting of 100 μM KCl, 2 μM MgCl₂, 100 μM Tris-Cl (pH 7.4),
1% SDS, supplemented with protease inhibitors (“Complete”; Roche
Diagnostics, Germany). Lysates were sonicated and stored at −80°C. Protein
levels were determined using the Lowry method. Proteins were separated on
a 7.5% polyacrylamide gel and subsequently transferred electrophoretically to
nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Proteins
were hybridized using BXP-21 (1:50) and HRP-conjugated goat antiam
IgG (1:1000; Dako, Glostrup, Denmark). For control purposes, P-gp, MRP1,
and Pglycoprotein were stained as described below. Hybrid cells that secreted antibodies of interest
were selected and subcloned three times by limiting dilution. The isotype was
determined using *IsoStrips* (Boehringer Mannheim).

**Immunoprecipitation.** Cells were preincubated for 2 h with RPMI without
methionine and without FCS before labeling. Cells were labeled overnight with
[35S]methionine (Amersham Life Sciences) in RPMI without me-
thionine with 10% FCS. Cells were homogenized in lysis buffer (PBS, 1%
IgG (1:1000; Dako, Glostrup, Denmark). For control purposes, P-gp, MRP1,
and P-glycoprotein were stained as described below. Hybrid cells that secreted antibodies of interest
were selected and subcloned three times by limiting dilution. The isotype was
determined using *IsoStrips* (Boehringer Mannheim).

**Deglycosylation.** Half of a batch of cell lysates of [35S]methionine-labeled
T8 and MX3 tumor cells, as prepared for immunoprecipitation, were incubated
with 2 units of peptide-N-glycosidase F (Roche Diagnostics Nederland B.V.,
Almere, the Netherlands) at 37°C for 1 h, and the other half was mock-treated
under identical conditions. Subsequently, samples were treated as described
above for the immunoprecipitation, and gels were analyzed using the phos-
phorimager.

**Immunohistochemistry.** Cryostat sections (4 μm) were cut, dried overnight
at room temperature, and fixed in acetone for 8 min at room temperature.
Cytospin preparations of tumor cells were also fixed in acetone. Formalde-
hyde-fixed paraffin-embedded tissues were deparaffinized in xylene and reb-
drated. Endogenous peroxidase activity was blocked using 0.3% (v/v) H₂O₂ in
methanol for 20 min. Before staining, paraffin sections were pretreated with 10
mm citric acid (pH 6.0) for 20 min. The slides were first incubated with 5%
normal goat serum/PBS for 30 min. Subsequently, frozen sections and cyto-
sins were incubated for 60 min at room temperature with a 1:150 or 1:100
dilution of BXP-21 or BXP-34 hybridoma supernatant, respectively, whereas
paraffin sections were incubated with a 1:150 or 1:100 dilution of BXP-21
or BXP-34, respectively, at 4°C overnight. BXP-21 and BXP-34 were diluted in
PBS/BSA.

Two staining methods were applied. In the first method, biotinylated goat
antimouse IgG (Dako; 1:200) and HRP-conjugated streptavidin (both in 90%PBS/BSA + 10% normal human serum) were used as secondary reagents.
Color development was performed using 0.4 mg/ml AEC. The second method
used the tyramide/FITC amplification method, as described by De Vree et al.
(22). In this case, after incubation with the BXP-34 or BXP-21 Mabs, slides
were incubated for 60 min with HRP-conjugated goat antimouse IgG (Dako:
1:100 in 90% PBS/BSA + 10% normal human serum), subsequently incubated
for 10 min with 1:100 tyramide/FITC in amplification buffer (NEN Life
Science Products, Boston, Massachusetts), and finally incubated for 60 min
with HRP-conjugated rabbit anti-FITC (Dako; 1:100 in PBS/BSA). Color
development was achieved using AEC. After counterstaining with hematoxy-
lin, slides were mounted.

For each type of tissue, negative controls were included, i.e., by omission of
the primary Mab, by using the irrelevant IgG1 Mab MOPC 21 (ICN Pharma-
ceuticals, Aurora, Ohio) or the IgG2a Mab PI 17 (American Type Culture
Collection, Manassas, Virginia).

**BCRP Expression in Cell Lines and Normal Human Tissue.** Poly(A)⁺ RNA was isolated from cell lines or from 30 × 30-μm cryosections/tissue
sample using RNAzol, according to the manufacturer’s description. mRNA
 aliquots (3 µg) were used for semi-quantitative RT-PCR. The *PBGD
* (NM000190) gene was used as an internal standard. This housekeeping gene
was selected, because it is expressed independently of the cell cycle (23, 24).
The following primers were applied: 5'-ccactactgcttggcct-3' (forward, nt
1914-1933) and 5'-caaggccacgtgattcttcc-3' (reverse, nt 2118-2137) for BCRP
and 5'-ttgcttgagaacgactgggct-3' (forward, nt 31-50) and 5'-ccagggctat-
tcaAAGCT-3' (reverse, nt 264-283) for *PBGD*. For each RNA sample, 12
reactions were performed using 14, 16, and 18 to 36 cycles. Water was
amplified for a total of 36 cycles as a negative control. DNA was labeled using
[α-32P]dCTP, and products were separated electrophoretically on a 6% poly-
acrylamide gel. DNA bands were quantified using a phosphorimaging system.
Finally, the relative expression of *BCRP* was calculated as compared with that of *PBGD
*(BCRP/PB GD) was calculated using the method of de Lange et al. (25).
Briefly, at least four intensities of bands were measured in the log-linear part
of the amplification cycle versus signal intensity curves, and subsequently the
distance between the curves of the *BCRP* gene and that of the *PBGD*
gene was used to calculate the relative expression levels. The assay was performed in
duplicate. Poly(A)⁺ RNA from the human ovarian IGROV1 and the human
small cell lung cancer cell line GLC4-ADR (both having very low levels of
*BCRP*) and the *BCRP*-overexpressing cell line T8 (high level of *BCRP*; Ref. 3),
and the partial revertant T8-40 (intermediate levels of *BCRP*; Ref. 3) was used
as control.

**RESULTS**

The development of the BXP-34 Mab directed against human
BCRP was described elsewhere (10). The antibody is capable of
staining cell membranes of *BCRP*-overexpressing cell lines, e.g.,
MCF-7 MR (12), 8226 MR20 (26), T8, and MX3 cells (3). BXP-21
was developed using fusion proteins containing aa 271-396 of BCRP.
BXP-21 was shown to be of IgG2a subclass. Incubation of cyto-
sins of *BCRP*-overexpressing cells with BXP-21 yielded a clear plasma
membrane staining, as shown for the topotecan-selected T8 cell line in
Fig. 1. Specificity of the antibody was further demonstrated using
*BCRP*-transfected monkey kidney COS7 cells, as described previ-
ously for BXP-34 (10; data not shown). BXP-34 was shown before to
not be suitable for Western blotting of BCRP (10). However, by using
BXP-21, a band of approximately Mᵦ 72,000, corresponding to the
predicted molecular weight of BCRP, was detected in Western
blots of *BCRP*-overexpressing tumor cell lines (Fig. 2). Relative levels of
BCRP correlated with known mRNA expression levels of BCRP in the cell lines studied (2, 3). BXP-21 did not cross-react with the known MDR transporters P-gp, MRP1, and MRP2 in cytospin preparations (data not shown) and in Western blots (Fig. 3). Immunoprecipitation experiments using BXP-21 or BXP-34 Mabs resulted in bands of approximately Mr 72,000, whereas no bands were detected at this position using an isotype-matched irrelevant antibody (Fig. 4A). Interestingly, the molecular weight of the bands in the T8 and MX3 differed slightly in both Western blots and immunoprecipitation experiments. Treatment with peptide-N-glycosidase F yielded bands with an equal, slightly lower, molecular weight of approximately 62,000 in both cell lines, showing that this difference was caused by different levels of glycosylation in these cell lines (Fig. 4B).

In this study, we assessed the subcellular localization and distribution of BCRP in normal human tissues using BXP-21 and BXP-34 Mabs. BXP-21 Mab is suitable for staining paraffin-embedded, formalin-fixed tissue for BCRP, but BXP-34 did not yield specific staining (data not shown). In this study, therefore, we used frozen sections of normal human tissues to allow comparative analysis using the two different Mabs. The BXP-34 Mab was used for all of the stainings, and a number of tissues was also stained using BXP-21. As controls for staining, BCRP-negative IGROV1 and BCRP-overexpressing T8 cell cytospin preparations were used. Pronounced staining with antibodies to BCRP was observed in the topotecan-selected cell line T8, moderate staining was observed in the partial revertant T8, and no staining was observed in the IGROV1 and GLC4/ADR cells (Table 1; Fig. 1). In several normal human tissues, strong immunoreactivity was observed (Table 2). Placental tissue showed apical staining of the syncytiotrophoblasts (Fig. 5A), whereas liver was strongly stained at the bile canalicular membrane (Fig. 5B). Prominent staining was observed in the gastrointestinal tract, with strong apical staining of the epithelium of the small intestine and colon (Fig. 5, C and D, respectively), and at the apical side in a proportion (but not all) of the ducts and lobules of the breast (Table 2). Furthermore, in almost all of the tissues tested, staining of the venous and capillary endothelial cells was observed (e.g., Fig. 5, E and F). In contrast, only sporadic staining for BCRP was observed in arterial endothelium (Fig. 5, G and H). No significant staining was observed in blood cells, i.e., erythrocytes, leukocytes, and platelets.

To compare mRNA expression levels with protein levels of BCRP, semiquantitative RT-PCR was used to determine mRNA levels of BCRP in various types of tissue. The BCRP/PBGD ratios determined

Fig. 1. Staining of cytospins (×100) of the parental IGROV1 ovarian tumor cell line (A) and the drug-selected BCRP-overexpressing subline T8 (B) with BXP-21. Color development was with AEC.

Fig. 2. Western blot analysis of BCRP in total cell lysates from parental (IGROV1, MCF-7, and 8226), BCRP-overexpressing resistant (T8, T8 revertant, MX3, MCF-7 MR, and 8226 MR20), and BCRP-negative resistant tumor cell lines (MCF-7 Dox40 and 8226 Dox40). BCRP was demonstrated using BXP-21 (1:150) as primary and goat antimouse IgG (1:1000) as secondary reagent. Bands were visualized using ECL. The Mr 200,000 bands are most likely the result of aggregation attributable to the denaturation step. Upon longer exposure times, this band was also visible in all of the other BCRP-expressing cell lines.

Fig. 3. Western blot analysis of BCRP, MRP1, MRP2, and MDR P-gp in total cell lysates from parental IGROV1 and BCRP-overexpressing tumor cell lines. The BCRP-overexpressing T8 tumor cells were used, as well as MRP1, MRP2, and MDR1 P-gp-transfected cell lines, 2008/MDP1, 2000/MDP2, and MDCKII/MDR1, respectively. Blots were hybridized using BXP-21, MRP1, MRP3-6, and C219 as primary antibodies. BXP-21 did not cross-react with MRP1, MRP2, or MDR1 P-gp. Bands were visualized using ECL. Notably, the BXP-21-hybridized lanes were cut from the same blot with identical exposure of the film for all of the lanes. BCRP levels in the 2008 parental tumor cell line are lower than those observed in the IGROV parental tumor cell line.
INTRACELLULAR LOCALIZATION OF BCRP IN NORMAL HUMAN TISSUES

We have analyzed the normal human tissue distribution of BCRP using a recently developed Mab BXP34 (10) and a newly developed Mab BXP-21. Both Mabs show pronounced staining at the plasma membrane of BCRP-overexpressing tumor cell lines. In contrast to BXP-34, BXP-21 is also reactive on immunoblots, yielding a protein band of approximately M, 72,000, the expected size of BCRP. BXP-21 did not cross-react with other MDR transporters like P-gp, MRP1, and MRP2. In the Western blotting and immunoprecipitation experiments, BCRP was found to be differentially glycosylated in T8 and MX3 tumor cells. Whether this difference in glycosylation reflects differences in BCRP functioning remains to be investigated.

From previously reported mRNA data (1, 4), BCRP is known to be highly expressed in the placenta and at lower levels in the liver, small intestine, colon, and ovary. In one study (4), low expression of BCRP

Table 1 Immunohistochemical detection of BCRP using the BXP-21 and BXP-34 Mabs and BCRP/PBGD mRNA expression ratios in human tumor cell lines

<table>
<thead>
<tr>
<th>Tumor cell lines</th>
<th>BXP-21</th>
<th>BXP-34</th>
<th>BCRP/PBGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGROV1</td>
<td>○</td>
<td>○</td>
<td>0.04</td>
</tr>
<tr>
<td>T8</td>
<td>●●●</td>
<td>●</td>
<td>5.00</td>
</tr>
<tr>
<td>T8-revertant</td>
<td>○/○</td>
<td>○/○</td>
<td>1.00</td>
</tr>
<tr>
<td>GLC4-ADR</td>
<td>○</td>
<td>○</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* ○, no staining; ●●●, strongly positive staining on plasma membrane; ○/○, heterogeneous staining, i.e., approximately 15% of the cells positive on plasma membrane, all of the other cells negative.

Table 2 Immunohistochemical detection of BCRP in frozen sections of normal human tissue using the BXP-21 and BXP-34 Mabs and BCRP/PBGD mRNA expression ratios in these tissues

<table>
<thead>
<tr>
<th>Human tissues</th>
<th>BXP-21</th>
<th>BXP-34</th>
<th>BCRP/PBGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive system</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Esophagus Epithelium</td>
<td>n.d.</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Stomach Epithelium</td>
<td>○</td>
<td>○</td>
<td>0.45</td>
</tr>
<tr>
<td>Small intestine Epithelium</td>
<td>●</td>
<td>●</td>
<td>3.85</td>
</tr>
<tr>
<td>Colon Epithelium</td>
<td>○</td>
<td>●●●</td>
<td>1.60</td>
</tr>
<tr>
<td>Muscular layers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganglion cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Hepatocytes</td>
<td>●</td>
<td>●</td>
<td>2.25</td>
</tr>
<tr>
<td>Bile canaliculi</td>
<td>●</td>
<td>○</td>
<td>0.85</td>
</tr>
<tr>
<td>Bile ductules</td>
<td>○</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pancreas Acini</td>
<td>○</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ducts n.d.</td>
<td>○</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inlet of Langerhans</td>
<td>○</td>
<td></td>
<td>n.d.</td>
</tr>
<tr>
<td>Excretory system</td>
<td></td>
<td></td>
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<tr>
<td>Kidney Glomeruli</td>
<td>○</td>
<td>○</td>
<td>1.65</td>
</tr>
<tr>
<td>Tubules ○</td>
<td>○</td>
<td></td>
<td></td>
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<td>Urinary bladder Epithelium</td>
<td>n.d.</td>
<td>○</td>
<td>1.00</td>
</tr>
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<td>Muscularis n.d.</td>
<td>○</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male reproductive system</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Prostate gland Epithelium</td>
<td>n.d.</td>
<td>○</td>
<td>n.d.</td>
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<tr>
<td>Fibromuscular stroma</td>
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<tr>
<td>Testis Leydig cells</td>
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<td>○</td>
<td>2.20</td>
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<td>Seminiferous tubules</td>
<td>n.d.</td>
<td>○</td>
<td></td>
</tr>
<tr>
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<tr>
<td>Spermatogenic cells</td>
<td>n.d.</td>
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<tr>
<td>Female reproductive system</td>
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<tr>
<td>Mammary gland (breast)</td>
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<tr>
<td>Lobules ●●●</td>
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<tr>
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</tr>
<tr>
<td>Cervix Epithelium</td>
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<td>●</td>
<td>3.25</td>
</tr>
<tr>
<td>Cervical glands</td>
<td>○</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Ovary Follicular cells</td>
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<td></td>
</tr>
<tr>
<td>Germinal cells</td>
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<td></td>
<td></td>
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<tr>
<td>Luteinized stromal cells</td>
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<tr>
<td>Placenta Cytotrophoblast</td>
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<tr>
<td>Syncytiotrophoblast</td>
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<tr>
<td>Lymphatic cells</td>
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<tr>
<td>Spleen White pulp</td>
<td>n.d.</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Red pulp n.d.</td>
<td>○</td>
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<td></td>
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<tr>
<td>Nervous system</td>
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<tr>
<td>Brain Parenchyma</td>
<td>n.d.</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Choroid plexus Epithelium</td>
<td>○</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>Periferal nerve</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Endocrine system</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal Cortex</td>
<td>n.d.</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Medulla n.d.</td>
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<tr>
<td>Cardiovascular system</td>
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<tr>
<td>Heart Myocardium</td>
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<td>○</td>
<td>0.26</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Arteries Endothelium</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tunica media</td>
<td>○</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veins Endothelium</td>
<td>●</td>
<td>●</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tunica media</td>
<td>●</td>
<td>○</td>
<td>n.d.</td>
</tr>
<tr>
<td>Capillaries</td>
<td></td>
<td></td>
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<tr>
<td>Blood cells</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Erythrocytes ○</td>
<td>○</td>
<td></td>
<td>n.d.</td>
</tr>
<tr>
<td>Leukocytes ○</td>
<td>○</td>
<td></td>
<td>n.d.</td>
</tr>
<tr>
<td>Platelets n.d.</td>
<td>○</td>
<td></td>
<td>n.d.</td>
</tr>
<tr>
<td>Progenitor cells</td>
<td>n.d.</td>
<td>○</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

"n.d., not determined; ○, no staining; ●●●, strongly positive staining; ○/○, heterogeneous staining, i.e., some cells positive, most cells negative. Notably, staining in the endothelium of veins and capillaries in respective tissues is not included in the tissue-staining results. Poly A RNA was collected from the whole tissue section, as indicated with a bar, when appropriate.

Fig. 4. A, immunoprecipitation of BCRP from the IGROV1, T8, and MX3 cells. Cells were labeled using [35S]methionine, as described in “Materials and Methods.” BCRP was precipitated using the BXP-21 or BXP-34 Mab. As control for precipitation, mouse anti-human CD68 antibody was used. Gels were analyzed using a phosphorimaging system. B, immunoprecipitation of BCRP from the T8 and MX3 cells after treatment with 2 units of peptide-N-glycosidase F (PNGase F) at 37°C for 1 h (+) or mock-treated (−). Before this treatment, cells were labeled using [35S]methionine, as described in “Materials and Methods.” BCRP was precipitated using the BXP-21 Mab. Gels were analyzed using a phosphorimaging system.
was observed in the kidney and heart as well. Using semiquantitative RT-PCR, we confirmed the expression of BCRP in these tissues, although the relative levels differed. These differences may be caused by the sections of the respective tissues that were used in these studies. Notably, in contrast to the study of Allikmets (4), the expression level of BCRP in the heart, as determined by our semiquantitative RT-PCR assay, was one of the lowest of all of the tissues tested, in line with our staining results in the heart (Table 2). Furthermore, our RT-PCR data showed that low to moderate levels of BCRP mRNA are present in every tissue tested, in line with our staining results in the heart (Table 2). Furthermore, the expression level of BCRP in the heart, as determined by our semiquantitative RT-PCR assay, was one of the lowest of all of the tissues tested, in line with our staining results in the heart (Table 2).

Nonendothelial staining for BCRP was observed in only a limited number of tissues, i.e., the placenta, small intestine, colon, liver, and breast. The presence of BCRP in placental syncytiotrophoblastic cells indicates that BCRP may have a protective function for the fetus. Interestingly, both in wild-type and in mdr1a/1b(−/−) mice, inhibition of mouse Bcrp1 by GF120918, a recently described inhibitor of human BCRP and mouse Bcrp1 (27, 28), resulted in at least 2-fold increased uptake of p.o. administered BCRP substrate topotecan in the fetus (29). Notably, relative mRNA levels of mouse Bcrp1 in the placenta are lower than that observed in humans; therefore, the protective effect in humans may well be stronger than that observed in mice.

Furthermore, prominent apical staining of BCRP was observed in the epithelium of both the small intestine and the colon, as well as in the canalicular membranes of the liver. The staining in the liver canalicular membrane indicates that BCRP may be involved in excretion processes in the liver, similar to many other ABC transporters.

Fig. 5. BCRP staining of cryosections of human tissue using the BXP-21 (D and H) or BXP-34 (A–C, E–G) Mab. A, placenta (×100); B, liver (×100); C, small intestine (×100); D, colon (×100); E, prostate (×100); F, ovary (×100); G, vein (white arrow) and artery (black arrow) in the submucosa of the stomach (×33); H, choroid plexus (×100). Color development was with AEC.
e.g., P-gp and MRP2 (30). The presence of BCRP in the small intestine and colon suggests that BCRP is involved in the regulation of uptake of substrates from the gastrointestinal tract by back-transport of substrates entering from the gut lumen. This hypothesis is strengthened by the significantly increased plasma levels of p.o. administered topotecan in wild-type or mdr1a/1b (−/−) mice in the presence of the BCRP inhibitor GF120918 (29). Staining in the breast was observed at the apical side of some ductal epithelial cells. Staining for MRP1 has also been observed in this cell type (31). The function of these transporters at this location remains subject to additional investigations.

Finally, BCRP was present in the endothelial layer of veins and capillaries in all of the tissues. At this moment, it is not known if BCRP contributes to transport across the endothelium. The endothelium is known to be quite permeable for several substances, which are known to pass through the endothelial layer between loosely connected endothelial cells. The endothelium in the brain is different in that the endothelial cells form tight junctions, creating the blood-brain barrier. Therefore, expression of transporter proteins at the blood-brain barrier may contribute to protection of the brain. For p.o., such a specific role in the blood-brain barrier is well established (6). Studies investigating the influence of BCRP in the blood-brain barrier in mice are currently ongoing in our laboratory.

The knowledge of the normal tissue distribution of BCRP, as described in this report, allows attempts to increase exposure of certain tissues. One clinical implication of our findings is that p.o. administration of BCRP substrates, e.g., topotecan and irinotecan (3), may be more efficient when combined with an inhibitor of BCRP.

This concept has been proven in mice and patients for paclitaxel, certain tissues. One clinical implication of our findings is that p.o. administration of BCRP substrates, e.g., topotecan and irinotecan (3), may be more efficient when combined with an inhibitor of BCRP.

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Subcellular Localization and Distribution of the Breast Cancer Resistance Protein Transporter in Normal Human Tissues

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