The multidrug resistance protein BMDP/ABCG2: A new and highly relevant efflux pump at the blood–brain barrier

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Abstract. Combinatorial chemistry is able to generate high numbers of compounds of pharmaceutical interest. An important aspect for a new drug is to know its availability in the nervous system, which means the ability to cross the barriers between blood and brain. Thus the permeability of thousands of compounds will have to be screened in the near future. This will not be possible in vivo, so that powerful in vitro models are demanded that closely mimic the in vivo system at least with respect to barrier properties. Two barrier systems are of main interest with respect to the passage of compounds into the brain: the blood–brain barrier (BBB) of the cerebral capillaries and the blood–liquor barrier (BLB) of the choroid plexus. In this paper main focus will be on the development of an in vitro model for the BBB. The brain capillary endothelial cells (BCEC) are isolated from freshly slaughtered pigs and cultivated on permeable filters to mimic the interfacial localization between blood and CNS. Cultivating the cells in serum-free medium improves their barrier properties. Hydrocortisone supplementation additionally reinforces the BBB properties. Under these conditions we observed an up-regulation of an efflux pump that has been found earlier in a breast cancer cell line, there named BCRP (Breast Cancer Resistance Protein). This efflux pump is also known as Mitoxantrone Resistance Protein (MXR) or Placenta-specific ABC Protein (ABCP). We identified this protein for the first time at the blood–brain barrier. mRNA expression studies of different brain cells showed that this efflux pump is mainly expressed in the brain capillary endothelial cells. With RT-PCR we showed a minor expression in pericytes as well as in epithelial cells from the choroid plexus and in spinal cord astrocytes. From cDNA sequence analysis we know that this efflux pump is a homologue to the ABCG2-transporter consisting of only one ATP-binding cassette and six membrane spanning helices. For this so-called half transporter, an oligomerization in the plasma membrane is discussed. Quantitative real-time...
PCR analysis indicates that this pump is highly relevant for the blood–brain barrier functionality since its expression level is about 3 fold higher compared to P-gp. Northern blot analysis yielded a 30 fold higher mRNA content. Immunocytochemistry revealed that in brain endothelial cells the protein is predominantly localized in the apical plasma membrane which compares to the blood side in vivo. The transport direction for known ABCG2 substrates consistently supports this finding. This paper will discuss the importance of this newly discovered ABC transporter at the blood–brain barrier in comparison to other members of ABC subfamilies. © 2005 Elsevier B.V. All rights reserved.

**Keywords:** Blood–brain barrier; Multidrug resistance; Brain capillary endothelial cells; Efflux pump; ABCG2

1. Introduction

The blood–brain barrier (BBB) controls the passage of endogeneous and xenobiotic substances between the blood and the interstitial fluid of the brain. The transfer of compounds is strictly regulated by brain capillary endothelial cells, which cover the inner wall of the cerebral blood vessels. Intercellular tight junctions seal the cleft between adjacent cells, thus preventing passive diffusion of hydrophilic compounds. Hydrophobic compounds, however, may easily diffuse through the cellular bilayer membrane, thus getting access to the brain. Their passage is controlled by so-called ABC transporters, which are located in the endothelial plasma membrane and mediate the efflux of molecules from the cytoplasm [1—4]. These transporters belong to the superfamily of ATP Binding Cassette (ABC) proteins that are present in almost all organisms from bacteria to human. These efflux pumps transport a wide variety of structurally diverse but mainly hydrophobic/amphiphilic chemical compounds as substrates. The different subfamilies, named ABCA to ABCG, have some specificities for lipids, cholesterol, bile salts but also peptides (ABCA, B and G), organic anions, anionic conjugates like glutathione, sulfate or glucoronyl residues (ABCC) for example. In general however they exhibit a strong overlap in their substrate spectrum [5]. ABC transporters utilize the energy of the ATP hydrolysis for their transport activity. Thus, they possess at least one ATP binding site and the “Walker” motif, which is the catalytic unit that hydrolyses ATP.

At the blood–brain barrier some of these transporters (ABCB1 or P-gp (P-glycoprotein) [6], ABCC1, C2 and C5 or MRP proteins [7]) play a crucial role as efflux pumps transporting compounds out of the CNS. Here we report the discovery of a new ABCG2 transporter expressed in porcine brain capillary endothelial cells.

2. Materials and methods

2.1. Cell culture

The preparation of porcine cell culture from different tissues was performed as described before for brain capillary endothelial cells (PBCEC) [8], porcine choroids plexus epithelial cells [9] and porcine pericytes [8]. The cell lines were cultivated as described before for rat astrocytes [10], porcine spinal cord astrocytes [11], human intestine epithelial Caco-2 cells [12], immortalized rat brain microvessel endothelial...
cell line RBE4 [13], rat brain endothelial cell line GPNT [14], human brain microvessel endothelial cells (HBMEC) [15], and human umbilical vein endothelial cells (HUVEC).

Isolation of total RNA and Northern blot analysis was performed as described [16].

2.2. Quantitative real-time PCR analysis

Gene expression analysis was performed using quantitative real-time polymerase chain reaction (qPCR) (GeneAmp 5700 Sequence Detection System; Applied Biosystems) in combination with the qPCR Core Kit for SYBR Green I (Eurogentec) according to the manufacturer’s recommendations. First-strand cDNA was synthesized using the Reverse Transcription core kit (Eurogentec). PCR was performed with the following primers:

BMDP-F 5’-GCCAAGGCCACGTGATTGT//BMDP-R 5’-ATGTACTGGCGCC-GAGTATT;
P-gp-F 5’-ACCCAAGGCAAGCCAGCAGAAGAAGAAG//P-gp-R 5’-CAGCTCTAGATCTCCTCAAAAGG;
β-Actin-F 5’-CAGCTTCAGATCTCCTCAAAAGG//β-Actin-R 5’-CGCACTTCATGACGCAGTTGA.

The thermocycling parameters were: 50 °C/2 min, 95 °C/10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Data analysis was performed with Sequence Detector Software SDS 2.0 (Applied Biosystems) as described [17]. Average readout $C_T$ values of triplicates were normalised to the endogeneous control, the housekeeping gene β-Actin and are thus presented by $\Delta C_T$ ($C_T$ of target minus $C_T$ of β-Actin). Final data are presented as $15-\Delta C_T$ values. In this presentation highly expressed targets have high values. $15-\Delta C_T$ values of 0 or below represent $C_T$ values of 30 or higher. Such signals are not significantly over the background signals.

2.3. Antibody generation

A polyclonal anti-MBP/BMDP antibody was raised in rabbits with the heterologously expressed BMDP fused to a maltose-binding protein (MBP) derived from the pMAL system. The designed fusion protein consists of 146 aa of BMDP taken from an intracellular loop near to the amino-terminus and the MBP. After immunisation of rabbits (Biogenes) the antisera were purified by affinity chromatography using MBP/BMDP coated dynabeads. The affinity purified antibody was named anti-MBP/BMDP.

2.4. Western blot analysis

Membrane vesicles or crude protein extracts were treated as described [18]. After SDS-PAGE, electro-transfer onto nitrocellulose membranes and blocking with non-fat dry milk, specific labelling was achieved with the polyclonal anti-MBP/BMDP antibody in dilution 1:1000. Immunoreactive bands were visualized by a POD-labelled secondary antibody and subsequent enhanced chemiluminescence detection. Equal protein loading of the gels was verified by protogold staining after membrane development.
2.5. Cross-linking assay

Amino-specific chemical cross-linking was performed with BMDP containing membrane vesicles shocked with hypoosmotic buffer and resuspended in non-reducing isotonic buffer. Vesicles (10 μg of protein) were incubated under argon atmosphere with 5 mM (Bis(sulfoisuccinimidyl)suberat) BS^3 for 30 min at RT. After SDS-PAGE proteins were analysed by Western blot.

2.6. Glycosylation analysis

BMDP containing membrane vesicles were incubated with PNGaseF (0.5 U/μg of protein) for 5 min at RT [19]. After SDS-PAGE proteins were analysed by Western blot.

2.7. Immunocytochemistry

PBCEC were grown on gelatin coated glass cover slips and fixed with methanol/ethanol (1:1) for 1 min at RT before incubation with 2% (v/v) NGS and 0.5% (v/v) Triton X-100. Immuno-labelling was achieved by sequential incubation with anti-MBP/BMDP (1:100) and Alexa fluor 488 goat anti-rabbit IgG.

2.8. Transport assay

The transport assay was performed as described [8]. All ^3H-substrates: daunomycin (8.2 Ci/mmol); hydrocortisone (78 Ci/mmol); methotrexate (7.7 Ci/mmol); vinblastine (2.2 Ci/mmol) and vincristine (4.6 Ci/mmol) were used in a concentration of 0.2 μM. Samples were taken after 0.5, 6, 24, 30, 48, 54 h with the exception of vincristine—here samples were taken after 0.5, 6, 19, 24, 30, 48 h.

3. Expression of BMDP, a new multidrug resistance protein at the blood–brain barrier

3.1. Expression analysis in cerebral porcine endothelial cells under the effect of hydrocortisone

In a BBB-cell culture model based on porcine brain capillary endothelial cells (PBCEC) the expression of an ABC transporter, named BMDP (brain multidrug resistance protein), has recently been shown [8,16]. In a differential screening assay we found that the expression level is regulated by the glucocorticoid hydrocortisone (HC) which we used as medium supplement. The expression analysis is given in Fig. 1. Northern blot analysis (Fig. 1A), Western blot analysis (Fig. 1B) as well as quantitative real-time PCR (Fig. 1C) clearly demonstrate an up-regulation of the expression of this transporter on the mRNA as well as on the protein level. In the presence of physiological concentrations of HC the expression level is increased by 45% as deduced from real-time PCR readings. Analysis of the cDNA revealed a 656 amino acid sequence encoding a 72.4 kDa protein. A database search revealed 86% agreement in amino acid sequence with BCRP, the human breast cancer resistance protein. Analysis of the peptide sequence predicts six membrane-spanning α-helices and, in contrast to the typical structure of ABC transporters, only one ATP-binding site. Thus BMDP is classified as a so-called half-transporter.
-HC +HC

A

BMDP

GAPDH

B

-HC  +HC

BMDP

C

14

13.5

13

12.5

12

11.5

11

10.5

10

- HC

+ HC

12.9

13.46

15 - ΔCₚ values

3.2. Comparative expression studies in different tissues

By Northern blot analysis we addressed the in vivo expression of BMDP in different porcine organs and tissues (Fig. 2). BMDP expression is highest in brain, kidney and lung, whereas it shows lowest expression in heart, skeletal muscle, spleen, stomach and pancreas, while liver, colon and small intestine show only moderate expression (Fig. 2A).

Within the brain the increasing signal intensity from homogenized brain tissue to isolated capillaries confirms that BMDP expression is not artificially induced in cultured cells, and that BMDP is predominantly expressed in the capillary endothelial cells (Fig. 2B). In other brain cell types (Fig. 2C) like pericytes, choroid plexus epithelial cells and astrocytes there is only a rather low expression if at all. Taken together these expression studies suggest that BMDP is important for transport activities at the BBB, whereas BMDP expression in the choroid plexus and in brain parenchyma is only of minor importance. However, it should be noted that rat astrocytes were used for the Northern-blot studies, and species discrepancies may have to be considered, as rABCG2 was shown to be neither expressed in rat neurons nor in astrocytes, whereas the expression in pericytes is unclear [20]. In cell lines frequently used as BBB in vitro models, like RBE4, CaCo-2 and GPNT, the mRNA of BMDP could not be detected neither by Northern blot analysis (Fig. 2D) nor by RT-PCR (data not shown). The same is true for HBMEC and HUVECs.
Accordingly the suitability of these cell lines to study transport processes at the BBB is limited.

3.3. Comparison of the expression levels of BMDP and P-gp

P-gp is thought to have the highest impact in multidrug resistance at the BBB. P-gp (ABCB1) is well known for tissues with high transport activity, like liver, kidney and lung, and has also been identified in capillary endothelial cells [8]. In order to scrutinize the importance of the newly discovered BMBP we performed quantitative real-time PCR in endothelial cells, spinal cord astrocytes and pericytes in order to compare the mRNA expression level of BMDP and P-gp (Fig. 3). In porcine brain endothelial cells BMDP is roughly 3.2 times higher expressed than P-gp, whereas the latter one has a higher expression level in pericytes (2.4 times higher) and astrocytes (2.5 times higher) with respect to BMDP. This quantification relies on the assumption of similar primer annealing for both mRNA species. These differences underline earlier reported results [8] were a 30 fold increase was observed by Northern blot analysis. Our new finding support the earlier made suggestion that BMDP is a highly relevant multidrug resistance transporter.
Fig. 3. Comparison of the BMDP and P-gp RNA expression levels in brain cell types by means of quantitative real-time PCR. cDNA equivalents to 50 ng of total RNA were used for each real-time PCR assay with primers specific for BMDP and P-gp, respectively. Average values of triplicates were normalised to the endogeneous control β-Actin (∆C_T). Final data are presented as 15—ΔC_T values. In this presentation highly expressed targets have high values. The difference of one cycle corresponds to a doubled expression of mRNA.

3.4. Expression of BMDP on protein level

The expression of BMDP on protein level was demonstrated by Western blot analysis after separating membrane vesicle fractions (MV-F) or total protein from PBCEC cell lysates on an SDS-PAGE. A polyclonal anti-MBP/BMDP antibody was raised in rabbits against a fusion protein containing a maltose binding protein (MBP) and a 146 aa long internal part of the BMDP, heterologously expressed in Escherichia coli BL21 pLys. The antiserum was purified by affinity chromatography. In Western blot analysis the antibody...
recognizes a 72 kDa protein in cultured PBCEC as well as in brain capillaries (Fig. 4A). In contrast, the expression of BMDP in other cell types of the CNS is marginal (Fig. 4B). No expression of BMDP was detected in astrocytes and choroid plexus epithelial cells, whereas the expression level of BMDP in pericytes is very small. Three specific signals for the MBP/BMDP antibody were detected in different porcine tissues with sizes of 72 kDa, 95 kDa and 66 kDa (Fig. 4C). The latter one corresponds to the non-glycosylated BMDP (Fig. 6B). These results give strong evidence for different isoforms of BMDP or differently glycosylated forms in different tissues like it has been shown earlier for rABCG2 [21].

4. Cellular localization and structural characterization

4.1. Cellular localization

It has been reported that ABCG2 immunoreactivity was detected in venous and capillary endothelial cells within human peripheral tissues, such as stomach, prostate and ovary, following immunohistochemical analysis [22]. However, the function of ABCG2 in these endothelial cells remains unclear. In rat brain capillaries immunoreactivity of rABCG2 was detected at the luminal side of the capillaries [21]. We were able to show for the first time the localization of BMDP at the apical membrane of a cellular monolayer of porcine brain capillary endothelial cells, which corresponds to the blood side in vivo (Fig. 5). This localization of this ABC transporter corresponds to the transport direction measured for ABCG2 substrates in PBCEC (Fig. 8A).

4.2. Structural characterization

Most of the ABC transporters have usually two nucleotide binding domains (NBD) and two transmembrane domains (TMD), presenting the common TMD-NBD-NBD organisation [23]. In contrast, ABCG2, like other members of the ABCG subfamily, has an organisation of only one NBD-TMD, with the NBD located at the

![Fig. 5. Fluorescence micrograph of the immunocytochemical localization of BMDP. PBCEC were fixed with methanol/ethanol and stained using a 1:100 dilution of the polyclonal anti-MBP/BMDP antibody and a 1:1000 dilution of the Alexa fluor 488 labelled secondary antibody. (A) Cross section in the (x, y)-plane (lateral distribution of the PBCEC). (B) Cross section in the (x, z)-plane (height profile of the PBCEC).]
amino terminus. Therefore ABCG2 was thought to function as a homodimer [24–26]. Furthermore, human ABCG2 has been functionally expressed in both insect and bacteria cells, suggesting that no other mammalian partner is needed for ABCG2 function [27], supported by immunoprecipitation experiments [20]. The human homologues of BMDP the ABCG5 and ABCG8 are reported to form heterodimers [28].

According to SDS-PAGE experiments under reducing or non-reducing conditions (+/− DTT) the BMDP monomer could be detected at 72 kDa. For non-reducing conditions BMDP was detected at about the anticipated dimeric size (data not shown). To investigate the oligomerization in detail we performed a cross-linking assay (Fig. 6) using BS3, an aminospecific cross-linker. Besides the signal for the BMDP monomer at 72 kDa, resulting from uncomplete cross-linking, we observed a 288 kDa band and the complete disappearance of the dimeric signal at about 170 kDa. These results indicate a dimerisation of BMDP—possibly the formation of a dimer of dimers (tetramer) which is consistent with the results obtained from hABCG2 extracted with non-reducing, non-denaturating conditions [20].

The glycosylation status of BMDP was addressed by means of a deglycosylation assay. After PNGasF treatment the glycosylated 72 kDa BMDP fraction disappears and a deglycosylated protein is observed at about 64 kDa (Fig. 6B).

5. Transport studies

It is one of our objectives to evaluate the functional relevance of BMDP at the blood–brain barrier in comparison to other well-known drug transporters like ABCB1 (P-gp) and e.g. ABCC1 (MRP1). Therefore a substrate and inhibitor spectrum for ABCB1 (P-gp), some ABCCs (MRPs) and ABCG2 (BCRP) was derived from the literature databases (Fig. 7) [7,26].
Based on these specificities a set of appropriate substrates has been selected and used in transport studies with PBCEC in vitro. We used PBCEC monolayers grown on permeable filter inserts with the apical chamber corresponding to the blood and the basolateral chamber corresponding to the brain side in vivo. The integrity of the PBCEC monolayer was initially checked by measuring the transendothelial electrical resistance (TEER) [29] and during the experiment via the $^{14}$C-sucrose-permeability. The $^3$H-substrates were added in equal concentrations on both sides of the filter and substrate concentration was measured in both compartments after different times. Fig. 8A shows the change in $^3$H-daunorubicin-counts in both, the apical and the basolateral compartment along a time course of 54 h. The substrate concentration increases on the apical side and correspondingly decreases at the basolateral side indicating CNS to blood transport in vivo.

These observed concentration changes imply an active transport towards the apical side. The daunorubicin transport direction corresponds to the proposed mechanism that it diffuses into the cell at the basolateral membrane and is actively transported out at the apical side [8].

The active daunorubicin transport was also measured in the presence of a set of inhibitors: Cyclosporin A, GF 120918, PSC 833 and Verapamil (see Fig. 8B). The cells

![Fig. 8. Transport studies with daunorubicin. (A) Change in $^3$H-daunorubicin-counts/1000 versus time [h]. 0.2μM $^3$H-daunorubicin was added at both sides of a filter. Samples were taken from each compartment after 0.5, 6, 24, 30, 48, 54 h. (B) Active transport of $^3$H-daunorubicin from basolateral to apical side affected by different inhibitors measured after 72 h.](image)
were preincubated with 1 μM inhibitor—the control adequately with medium—1 h before the transport assay was started. Transport was measured after 72 h.

The individual inhibitors show a graded inhibitory effect. Whereas GF 120918 strongly inhibits substrate transport down to 15%, PSC 833 and Cyclosporin A decrease the transport activity moderately leading to 33% or 36% of the initial value. Verapamil inhibits only weakly with a remaining value of 73%. By these experiments we were able to show that BMDP contributes to the daunorubicin transport since (i) substrate transport was strongly inhibited by GF 120918, which is a P-gp and highly potent BCRP inhibitor and (ii) inhibition was less efficient with PSC 833, a specific P-gp inhibitor [8].

As shown in Fig. 9A daunorubicin is transported from the basolateral to the apical side leading to a 3 fold increased substrate concentration (300%) in the apical compartment. Even stronger active transport is observed for vinblastine (750%) but less for vincristine (145%) and only weak transport was measured with hydrocortisone (125%) and methotrexate (115%) (see also Fig. 9B).

It has been unexpected that vinblastine is transported with such high efficiency because it is well known as a P-gp specific substrate and all other P-gp substrates are consistently transported at much smaller rates. Either the affinity of vinblastine to P-gp is significantly different compared to the other substrates or it is also accepted by BMDP and/or other multidrug resistance proteins. Furthermore we expected methotrexate to be transported to a higher extent because it is a BCRP and MRP1,3,4 substrate. It seems that it is not or only a low affinity BMDP substrate in particular since the role of the MRPs at the BBB is doubtful. The slightly different substrate specificity of BMDP compared to ABCG2 may be due to the amino acid at position 482. It is discussed that a mutation at this position may change the substrate specificity [30,31]. In BMDP an arginine residue is found at this position. In mutants of BCRP the arginine is replaced by threonine or glycine. According to the results of Mitomo and Robey daunorubicin would not, but methotrexate should be transported by BMDP. The observed differences in substrate specificity may be based on different expression systems. ABCG2 may form different heterodimers and this may change the substrate

![Fig. 9. Active transport of 3H-substrates versus time [h]. (A) All substrates: Daun.=daunorubicin; HC=hydrocortisone; Methotr.=methotrexate; Vinbl.=vinblastine; Vincr.=vincristine. (B) Focus on the substrates hydrocortisone, methotrexate and vincristine (larger scale).](image-url)
specificity [21]. Mitomo and Robey used HEK-cell lines whereas we analyse an endogenous system.

6. Conclusion

We have identified a new ABC transport protein (BMDP) expressed in porcine brain capillary endothelial cells that is closely related to the drug transporter BCRP/MXR/ABCP. It is localized at the apical membrane of cultured endothelial cells, consistent with the transport direction of typical substrates. Comparison of expression levels revealed that its expression on RNA level in PBCEC is much higher than the one of P-gp, which has been considered up to now as the most important ABC transporter at the BBB. To summarise, BMDP is suggested to play an important or even the major role in protecting the brain from cytotoxic drugs besides P-gp. Current investigations should clarify the specific physiological role of BMDP in comparison to other multidrug resistance proteins.

References


Discussion

Fricker

Do you know to what extent is BCRP expressed in brain tumours? We talk about resistance of brain tumours to chemotherapy and failure of effects by blocking PGP, and when I see this overlapping substrate specificity of BCRP and MRP and PGP, it is no wonder that many inhibitors have no effect.

Galla

We have not investigated to what extent BCRP is expressed in brain tumours. If you say that the expression of this transporter is 56%, and that of this other transporter is 43% that gives us an idea of the relationship. What is important is that nature would never rely on one system.

Abbott

BCRP is also found in non-brain endothelial cells, where the tight junctions are leakier, so you wouldn’t expect that protection of the tissue is such a feature of those endothelia. Does that indicate that BCRP is more likely to be a housekeeping transporter for endothelial cells? Or is it, in a non-brain endothelial cell, still protecting its particular
tissue in the same way that the brain endothelial cell does? Why would a non-brain endothelial cell need this kind of protection?

_Galla_

We haven’t looked at that, we just looked at the tissue in total, but not in detail, because we just wanted to see if it is especially expressed in the brain or in other tissues as well. As you already recognize, I hesitate to call it BCRP. It’s similar, it has a homology, but it’s not identical. Also, it is known that in different tissues it may be different.

_Abbott_

Pgp, for example, is not present in non-brain endothelial cells, or at least not at the same level as in brain endothelium, so that helps us to say that this is a barrier property.

_Galla_

Obviously in the UVEX it is not present. This, again, is a cell line.

_Sugiyama_

Of course, it’s not so easy to find out the specific substrate for BCRP or some other transporters and also it is not so easy to find out specific inhibitors. Regarding the specific substrate, I’d like to recommend you to try some sulfate conjugate of the xenobiotics and also of steroid hormones. As far as we checked, they are not good substrates for PGP or for MRP1 and 2, so they could be good substrates for BCRP. However, recent studies have found that these sulfate conjugates are very good substrates for MRP4. If you find out some positive result in the function, you can just wonder if it is either BCRP or MRP4.

_Galla_

I absolutely agree. The combinatory approaches do need a lot of work: that is why we thought that maybe we could use this very new technique of the RNAi. We thought this was exactly the technique, though at least in our system it was not. We ended up with transfection rates below 5% if we were lucky, sometimes we had 1% and that was not enough.

_Stanimirovic_

We looked at ABCG2 expression in dissected microvessels from human glioblastoma tumours and we found an up-regulation in both tumour vessels and parenchyma. Hence, there is some indication that ABCG2 might be up-regulated in tumours. You said that 150 clones were differentially expressed. Have you looked at other potential ABC transporters that might be co-expressed with ABCG2 among these clones?

_Galla_

We have to look again. We were not really looking for transporters. We were looking for tight junction proteins. I can state that none of the tight junction proteins is up-regulated, neither at the RNA level nor at the protein level. The only thing that we see under hydrocortisone is a rearrangement, so we can see the lines in the immunofluorescence are much sharper, more concentrated in the membrane. So there is some sort of rearrangement, that’s way these cytoskeleton proteins are also interesting because that might bring the whole thing together.

_Lage_

Are the tissue expression patterns of human ABCG2 and the porcine protein ABCG2 identical or are there differences?
Galla
We don’t have human endothelial cells.

Stanimirovic
ABCG2 is highly expressed in human brain endothelial cells. We also looked at human astrocytes, where the expression was lower than in brain endothelial cells. As I already mentioned, we also demonstrated the expression in glioblastoma vessels in situ.