*Cancer and Metastasis Reviews* **20:** 13–25, 2001.


# Multidrug resistance in brain tumors: Roles of the blood–brain barrier

Anthony Régina[1], Michel Demeule[1], Alain Laplante[1], Julie Jodoin[1], Claude Dagenais[1], France Berthelet[2], Albert Moghrabi[1] and Richard Béliveau[1]
[1]*Laboratoire de Médecine Moléculaire, Hôpital Sainte-Justine-Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville;* [2]*Département de Pathologie, Hôpital Notre-Dame, Montréal, Québec, Canada*

*Key words:* blood–brain barrier, brain tumors, multidrug resistance, P-glycoprotein, endothelial cells

**Abstract**

Malignant brain tumors and brain metastases present a formidable clinical challenge against which no significant advances have been made over the last decade. Multidrug resistance (MDR) is one of the main factors in the failure of chemotherapy against central nervous system tumors. The *MDR1* gene encoding P-glycoprotein (P-gp), a drug efflux pump which plays a significant role in modulating MDR in a wide variety of human cancers, is highly expressed in the blood–brain barrier (BBB). The BBB controls central nervous system exposure to many endogenous and exogenous substances. The exact molecular mechanisms by which the BBB is involved in the resistance of brain tumors to chemotherapy remain to be identified.

The purpose of this review is to summarize reports demonstrating that P-gp, one of the most phenotypically important markers of the BBB, is present in primary brain tumors and thus plays a crucial role in their clinical resistance to chemotherapy.

## 1. Introduction

The treatment of brain cancer is one of the most challenging areas of oncology. Brain cancer, including primary and metastatic brain tumors, account for over 120,000 new patients each year in the United States. Brain tumors, and particularly glioblastoma multiform, have retained their dismal prognoses despite advances in neurosurgical techniques and in radiation and drug therapies. One of the difficulties encountered is the anatomical localization of the tumor, exacerbated by the invasion of surrounding tissue. This often results in incomplete surgical resection of the tumor, with the tumor usually recurring within a few centimeters of the margins of the resection. Radiotherapy is limited by low brain tolerance as well as by the infiltration of tumor cells into healthy brain. Adjuvant chemotherapy is thus essential for the treatment of some types of brain tumors. However, another major problem which limits the efficacy of conventional brain tumor chemotherapy is the phenomenon of multidrug resistance (MDR). MDR is generally described as an acquired or intrinsic resistance to a variety of structurally unrelated synthetic and natural cytotoxins. Many primary brain tumors are intrinsically chemoresistant; limited penetration of cytotoxic drugs across the blood–brain barrier (BBB) may also contribute to the poor response of brain tumors to chemotherapy. Brain metastases respond poorly to chemotherapy, which may be due to differences in chemosensitivity between primary and metastatic tumors. One reason for this is that in metastases of previously treated tumors, clonal selection of chemoresistant tumor cells may already have taken place. Small-cell lung cancer (SCLC) provides an example of this, where the response rate of intracerebral metastases to cyclophosphamide, etoposide and vincristine without radiotherapy is 53% as compared to a 79% response rate in the primary tumor [1].

A growing body of evidence links P-glycoprotein (P-gp) expression in untreated tumors to a poor prognosis for chemotherapy with P-gp substrate drugs. Nevertheless, while the connection has been firmly established for several hematological cancers, it remains controversial for most solid tumors. This

review will focus on the possible role of the BBB and, more specifically, on the multidrug transporter P-gp in clinical resistance to chemotherapy against brain tumors and will discuss current and future perspectives in brain tumor therapeutics.

## 2. The BBB: cytoarchitecture and physicochemical properties

The BBB and the blood–cerebrospinal fluid barrier (BCSFB) are interfaces that separate the systemic circulation from the brain parenchyma and cerebrospinal fluid, respectively. These barriers help to maintain brain homeostasis not only by regulating the entry of blood-borne substances into the CNS, but also by restricting the access of potential toxins to brain parenchyma. As a result of the latter property, some drugs do not accumulate within the brain to a concentration which permits them to exert their desired pharmacological effects.

The surface area of the BBB is estimated to be 5000-fold larger than that of the BCSFB and therefore the BBB is considered to be the main route for the uptake of endogenous and exogenous ligands into the brain parenchyma [2]. Brain microvessel endothelial cells are responsible for the morphological and functional characteristics of the BBB. As compared to peripheral endothelial cells, brain endothelial cells are sealed by continuous tight junctions and no fenestrations are present. Furthermore, brain endothelial cells exhibit very low pinocytic activity. As a result, solutes must cross the luminal membrane, the cytoplasm and the abluminal membrane of brain endothelial cells to gain access to the brain. Hence, carrier systems are essential to shuttle nutrients (e.g., glucose, amino acids), vitamins, hormones, monocarboxylic acids and amines to the brain [3]. For large proteins with important physiological functions (e.g., insulin and transferrin), receptor-mediated endocytosis and transcytosis can mediate transport across the BBB [4].

The determinants of passive drug entry into the CNS have been recently reviewed [5]. Due to the physical nature of cell membranes, lipid solubility is an important determinant of passive BBB permeability. The ability of a compound to partition into an organic solvent relative to a physiologic buffer (e.g., octanol/buffer partition coefficient) is a common measure of lipophilicity. Nevertheless, the overall hydrophilic/lipophilic balance of a molecule appears to be a better predictor of BBB permeability than the octanol/water partition coefficient. Steady-state brain distribution *in vivo* is not only a function of passive diffusion across the BBB, but depends also on the relative affinity for plasma proteins and brain tissue. Ionization at physiologic pH (pKa), the affinity and capacity of transport systems, potential BBB/cerebral metabolism and egress through the CSF route are also important factors. Furthermore, there are a number of active efflux transport mechanisms present in the CNS, located both in the BBB and in the choroid plexus [6]. The efflux of many endogenous substances and xenobiotics by transport systems reduces their effective penetration from blood to brain, which ultimately decreases brain exposure to these compounds. In this line of research, much attention has been focused on the multidrug transporters such as P-gp and multidrug-resistance associated protein (MRP) (Figure 1).

## 3. P-glycoprotein

### *3.1. Expression of P-gp in the BBB and other tissues*

P-gp is a membrane transporter present in the BBB that belongs to the ATP binding cassette (ABC) superfamily [7]. It was initially discovered due to its expression in different types of MDR tumors [8,9]. It was shown to confer the MDR phenotype [10], by which a cancer cell exposed to a single anticancer drug becomes simultaneously resistant both to that drug and to other drugs of unrelated structure or function. P-gp is the product of the *MDR1* and *MDR2* or *MDR3* genes in humans, as well as the *mdr1a*, *mdr2* and *mdr1b* genes in rodents. In humans, the MDR phenotype is caused by the *MDR1* gene, whereas it is caused by *mdr1a* and *mdr1b* in rodents [11,12].

P-gp expression was first detected in the human brain by *in situ* hybridization [13], and later by immunocytochemistry using different monoclonal antibodies. It was also established at that time that P-gp is present in human tissues such as liver, kidney, intestine, adrenal glands and blood–tissue barriers such as placenta, testis capillaries and brain capillaries [14,15].

### *3.2. Expression of P-gp in brain endothelial cells*

The P-gp isoforms expressed in mouse brain capillaries were determined by Western immunoblotting using antibodies specific to each isoform. The *mdr1a*

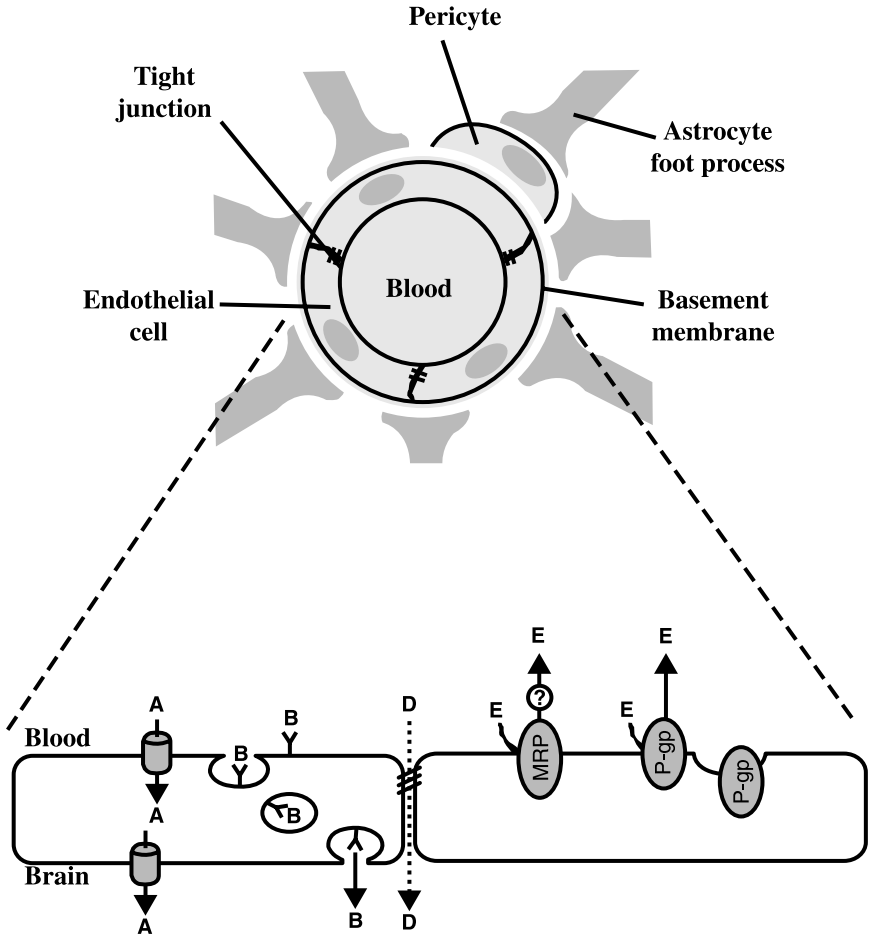


*Figure 1*. Schematic view of a brain capillary. Endothelial cells are sealed by continous tight junctions and surrounded by a basal lamina. Pericytes are present at the periphery of the vessel. Astrocyte foot processes are in close contact. Hydrophilic molecules cannot diffuse through the plasma membrane, so specific transporters present on both the luminal and the abluminal membranes and carrier-mediated endocytosis allow the uptake of nutrients and hormones (A,B). A very small amount of water-soluble molecules use the paracellular pathway by diffusing through the tight junctions (D). Efflux pumps (P-gp, MRPs) impede the brain penetration of lipophilic substrates (E).

encoding isoform is expressed in brain capillaries isolated from mouse, whereas the *mdr1b* and *mdr2* encoding isoforms are not observed there [16]. Analysis of *mdr* RNA by RT-PCR showed that *mdr1a* and *mdr1b* are both present in rat brain, where *mdr1a* was specifically detected in brain capillaries and *mdr1b* in brain parenchyma [17].

Recently published data from our laboratory showed that P-gp is predominantly expressed in brain endothelial cells [18]. Endothelial cells were isolated from brain and other tissues using magnetic microbeads cross-linked to an antibody directed against the platelet-endothelial cell adhesion molecule-1 (PECAM-1). The endothelial cells were immobilized in a cell separation column mounted onto a magnet, while the other cells were eluted (negative fraction). The enrichment of endothelial cells in the preparation was about 68-fold, assayed by immunodetection of endothelial nitric oxide synthase (eNOS) in the positive and negative fractions [18]. P-gp was strongly enriched (59-fold) in this endothelial cell fraction from brain and was absent from the negative fraction, in which the glial fibrillary acidic protein (GFAP), an astrocytic marker, was present. It was also shown in this study that the P-gp isoform found in brain endothelial cells is encoded by *mdr1a* [18].

### *3.3. Subcellular localization of P-gp in brain endothelial cells*

The data above are in agreement with a previous study that showed that P-gp was strongly enriched in the luminal membranes of rodent brain vascular endothelium [19]. This was done using a method that allows the isolation of luminal membranes of blood vessels. P-gp in luminal membranes from brain, and from other tissues, was immunodetected with monoclonal antibody C219 and polyclonal antibody Ab-1. This procedure provided a 17-fold enrichment of P-gp compared with isolated brain capillaries and a 400–500-fold enrichment compared with membranes from the whole brain. In these isolated endothelial cells, GFAP showed a very weak enrichment (1.4-fold over brain capillaries) indicating minimal contamination by astrocytes. Studies using other techniques such as immunoelectron and confocal microscopy also indicated that P-gp is localized at the luminal side of the BBB endothelium rather than the abluminal side [20,21]. Taken together, these results support the finding that brain endothelial cell P-gp is mainly located in the luminal membranes.

This model of luminal localization of P-gp in endothelial cells has been challenged [22] by an alternative model contending that immunoreactive P-gp is located on astrocyte foot processes. While this postulate is interesting, it contradicts the findings of many other studies demonstrating P-gp localization on the luminal side of human brain endothelium [13,20,21]. Clearly, further experiments are needed to confirm the cellular localization of P-gp at the human BBB. One possible explanation for these conflicting data could be a difference between species.

In addition to being found in the luminal membrane of brain endothelial cells, P-gp expression has been also reported in caveolae [23–25]. Caveolae are flask-shaped plasma membrane invaginations involved in many cellular events, such as signal transduction, lipid and protein sorting, endocytosis and potocytosis [26]. In capillary endothelial cells, caveolae play a role in the transport of macromolecules across the cells by transcytosis [27]. We have observed that P-gp is localized in the caveolae of rat brain capillaries. Caveolae were isolated by flotation of low-density microdomains on a sucrose gradient, producing a subcellular fraction where caveolin-1, the structural protein of caveolae, was enriched. P-gp contains a caveolin-binding motif that is present within proteins reported to interact with caveolin. The caveolin-binding motif mediates the association of caveolin-binding proteins with the scaffolding domain of caveolin [28]. We showed, by co-immunoprecipitation studies, that P-gp interacts with caveolin-1 [25]. Association of P-gp with caveolin-1 could enable P-gp to move between the plasma membrane and caveolae. The scaffolding domain of caveolin-1 is involved in interactions with numerous proteins and it has been reported to regulate the signaling molecules localized in caveolae such as eNOS, protein kinase C, epidermal growth factor receptor and the insulin receptor [29]. The effect of caveolin-1 on P-gp activity is still unknown and the function of P-gp in caveolae of endothelial cells of the BBB remains to be determined.

### *3.4. Functions of the P-gp in the BBB*

In brain capillaries, P-gp plays an important role in preventing many hydrophobic molecules (drugs or other substrates) from crossing the BBB and reaching the CNS. An increase in the brain concentrations of P-gp substrates was measured in *mdr1a* knock-out mice, which lack P-gp in the BBB. These mice were 50–100 times more sensitive to the pesticide ivermectin than were wild-type mice. The accumulation of this neurotoxic drug in brains of *mdr1a(−/−)* mice was increased 80–100-fold compared to wild-type mice [30]. Similar results were obtained with *mdr1a* knock-out mice exposed to digoxin, ondansetron, loperamide, paclitaxel, vinblastine and doxorubicin.

## 4. Multidrug-resistance associated protein

A second protein expressed in many MDR tumors is MRP1. Like P-gp, MRP1 is a member of the ABC superfamily and is expressed in many MDR cell lines which do not express P-gp [31–33]. In humans, seven MRP homologues have been identified to date [34]. MRPs are distributed throughout most human tissues [35]. MRP1 is expressed and localized on the basal membrane of Sertoli cells in the testes and on the basolateral membrane of lung epithelial cells [36,37]. It has been demonstrated by Western blot analysis that MRP1 is expressed in the enriched membrane fraction of human and rat choroid plexus, and MRP1 has been localized to the basal membrane of primary cultured rat choroid plexus epithelial cells [38]. The expression of MRP1 at the BBB is still under debate. Western blot analysis and RT-PCR suggest that MRP1 is expressed in isolated rat brain capillaries, primary cultured rat, porcine and

bovine brain endothelial cells, and immortalized rat and mouse brain endothelial cells [17,39,40]. However, MRP1 expression is greater in rat and porcine brain homogenate and primary rat and porcine cultures than in isolated brain capillaries [17,41]. No expression of MRP1 was detected in isolated human brain capillaries by immunohistochemistry whereas expression of both P-gp and platelet-endothelial cell adhesion molecule-1 (PECAM-1, an endothelial marker) was clearly detected [42]. Recently, the expression of MRP1, 4, 5 and 6 was demonstrated in both primary cultured bovine brain endothelial cells and a capillary-enriched fraction. Low levels of MRP3 were detected in cultured cells but not in the capillary-enriched fraction [43].

MRPs are organic anion transporters which transport anionic drugs and neutral drugs conjugated to acidic ligands such as glutathione (GSH), glucuronate or sulfate. However, MRP1, MRP2 and MRP3 can also cause resistance to neutral organic drugs that are not known to be conjugated to acidic ligands, such as doxorubicin, daunorubicin, epirubicin, vincristine, vinblastine and etoposide, by transporting these drugs together with free GSH [44]. Recently, it has been demonstrated that MRP1, MRP2 and MRP3 are also involved in resistance to methotrexate, which is an important chemotherapeutic agent in the treatment of malignancies that disseminate to the brain [45,46].

## 5. The BBB in brain tumors

Brain tumors can be divided into three groups according to the development of their vasculature: (1) primary tumors that develop *in situ* and derive their blood supply from neural vessels; (2) secondary tumors that arise elsewhere in the body and metastasize to the brain and also derive their vasculature from neural vessels; and (3) meningiomas, which arise from extra-axial tissues within the cranium or spinal canal and which initially derive their vascular supply from the meninges.

### *5.1. Evidence for BBB disruption*

The primary evidence against a major role of the BBB in the low efficacy of chemotherapy is the increased microvascular permeability in gliomas that leads to brain edema. Indeed, in primary and metastatic brain tumors a number of alterations in the brain capillary ultrastructure have been described [47]. Interendothelial junctions in brain tumor vessels are abnormal. A relationship between suppression of the tight-junction associated protein claudin-1 and the alteration of tight-junction morphology in vessels of human glioblastomas has been shown recently [48]. Fenestrations have been described in primary non-glial tumor vessels and are common in most if not all metastatic tumor vessels. An increase in the number of pinocytic vesicles has also been reported [49].

### *5.2. Evidence for BBB integrity*

The most obvious evidence of BBB integrity is the low concentrations found for most chemotherapeutic agents in primary brain tumors and brain metastases. Some ultrastructural studies showed that, even in high-grade tumors, the neo-vasculature preserved partial BBB function at the cellular level. A recent study found that ZO-1, a protein associated with tight junctions, was present in the endothelial cells of microvessels in all astrocytic tumors studied [50]. Fenestrations are extremely rare in glial tumor vessels, even in glioblastoma multiforme [49]. Brain tumors grow by increasing tumor mass and by infiltrating surrounding normal brain. Changes in the ultrastructure and morphology of peritumoral capillaries have been investigated [51]. In this study, peritumoral capillaries presented structurally normal tight junctions. No differences were observed between normal and peritumoral capillaries with respect to diameter, wall thickness, endothelial thickness or endothelial vesicle density.

In conclusion, the specific morphological characteristics of the BBB are maintained at the cellular level.

## 6. P-gp expression in brain tumors

### *6.1. Primary brain tumors*

#### *6.1.1. Whole tumors*

The exact mechanism by which brain tumors resist chemotherapy remains unknown. One of the main arguments contradicting involvement of the BBB in resistance to chemotherapy is the lack of correlation observed between the expression level of P-gp and the resistance levels of some tumors [52]. However, a recent report shows that even low levels of P-gp and MRP1 expression, which may be difficult to detect in tumors, can significantly affect their sensitivity to a wide range of clinically important P-gp substrate drugs [53]. This suggests that P-gp expression, even at a low

level, may have an impact on the availability and the distribution of drugs in brain tumors. Primary tumors derived from colon, kidney, liver and pancreas usually have high expression levels of P-gp, a reflection of the high levels in the normal cells from which the tumor arose. Expression of the *MDR1* gene is sometimes high in leukemias, lymphomas and some other cancers derived from tissues that do not normally express the protein. Normal astrocytes do not seem to express appreciable levels of P-gp. However, P-gp and MRP1 expression have been shown in cultured astrocytes [54]. Malignant astrocytomas are clinically resistant to most types of cytotoxic drugs, including those associated with the MDR phenotype. Consequently, it might be expected that these tumors would express high levels of P-gp. Different techniques have been used to detect P-gp in astrocytic tumors. Most of the strategies have focused on detecting either the presence of MDR1 mRNA using Northern blotting or the expression of P-gp using immunohistochemistry.

Immunohistochemical studies have provided conflicting data about the expression of P-gp in these tumors with some investigators finding expression in neoplastic cells of malignant gliomas [55–58], while others found little or no expression [59,60]. Systematic comparison of antibodies by Western blot analysis had shown that the levels of P-gp detection varied among antibodies [61]. One recent study examined the issue of poor specificity of the antibodies used to detect P-gp in immunohistochemistry studies [52]. Antibodies which have been widely used in studies aimed at correlating P-gp expression with drug response or with clinical outcome following chemotherapy have been shown to have significant cross-reactivities with proteins unrelated to drug resistance. We compared the expression of P-gp in various human brain tumors, including low and high grade gliomas, to its expression in normal brain [62]. Astrocytic tumors are the most common human brain tumors. They originate from astrocytic cell lineages and are classified, according to the World Health Organization guidelines, into four categories of increasing malignancies: pilocytic astrocytomas (grade I), astrocytomas (grade II), anaplastic astrocytomas (grade III) and glioblastomas (grade IV). P-gp was detected in brain tumors on Western blots using mAb anti-MDR1 Ab-2 which recognizes only human MDR1 and not MDR2. This method of analysis allows the quantification of the expression of a specific protein in a tissue and the discrimination of cross-reactive proteins, reducing the number of false positive results. Our results show that the level of MDR1 P-gp was similar in almost all samples from various malignant brain tumors as well as in normal brain (Figure 2A). These results are in agreement with previous studies which reported the presence of P-gp, by immunohistochemistry using mAb C219, both in resistant and in partially chemosensitive glioblastomas [55–58,63]. These results suggest that the low response of tumors to many anticancer drugs may be related to the intrinsic presence of P-gp in cell populations of primary brain tumors and that P-gp may be seen as a negative factor in the prognosis for patients with this pathology. In addition, our results suggest that the phenotype related to MDR1 expression is maintained in both low- and high-grade gliomas. It had been shown in an earlier study [64] that the percentage of P-gp immunoreactive tumor cells, as well as the number of P-gp-positive tumors, increased significantly with increasing grade of anaplasia. In our study there was also a trend towards increased expression of P-gp with advanced tumor grade, but it was not statistically significant. Among brain tumors, the highest level of P-gp expression was found in meningiomas, where its expression was 25-fold higher than in normal brain (Figure 2B). Since none of the patient with meningiomas had received chemotherapy or radiotherapy prior to surgery, the high expression of P-gp was intrinsic. Since meningiomas are highly vascularized tumors, the high P-gp expression observed in our study may well reflect the level of vascular supply.

*6.1.2. Tumor blood vessels*

P-gp is strongly expressed in almost all endothelial cells within primary brain tumors [55,58]. One study also suggested that there may be less expression of P-gp in the vasculature of malignant gliomas than in healthy brain vasculature [55]. However, another study has shown that endothelial cells of tumor blood vessels do express P-gp at the same level as do their normal counterparts [59]. In this study, immunohistochemistry showed that P-gp was expressed not only in glioma tumor cells but also in the endothelial cells of newly formed capillaries in the tumors, at the same level as found both in the tumor–brain border and in the brain further from the tumor. It was also shown that 86% of the gliomas analyzed and 50% of the brain metastatic tumors expressed P-gp in their vasculature. P-gp was undetected in the vasculature of non-brain malignant tumors. A more recent study also demonstrated P-gp expression in endothelial cells of gliomas and metastatic carcinomas [65].

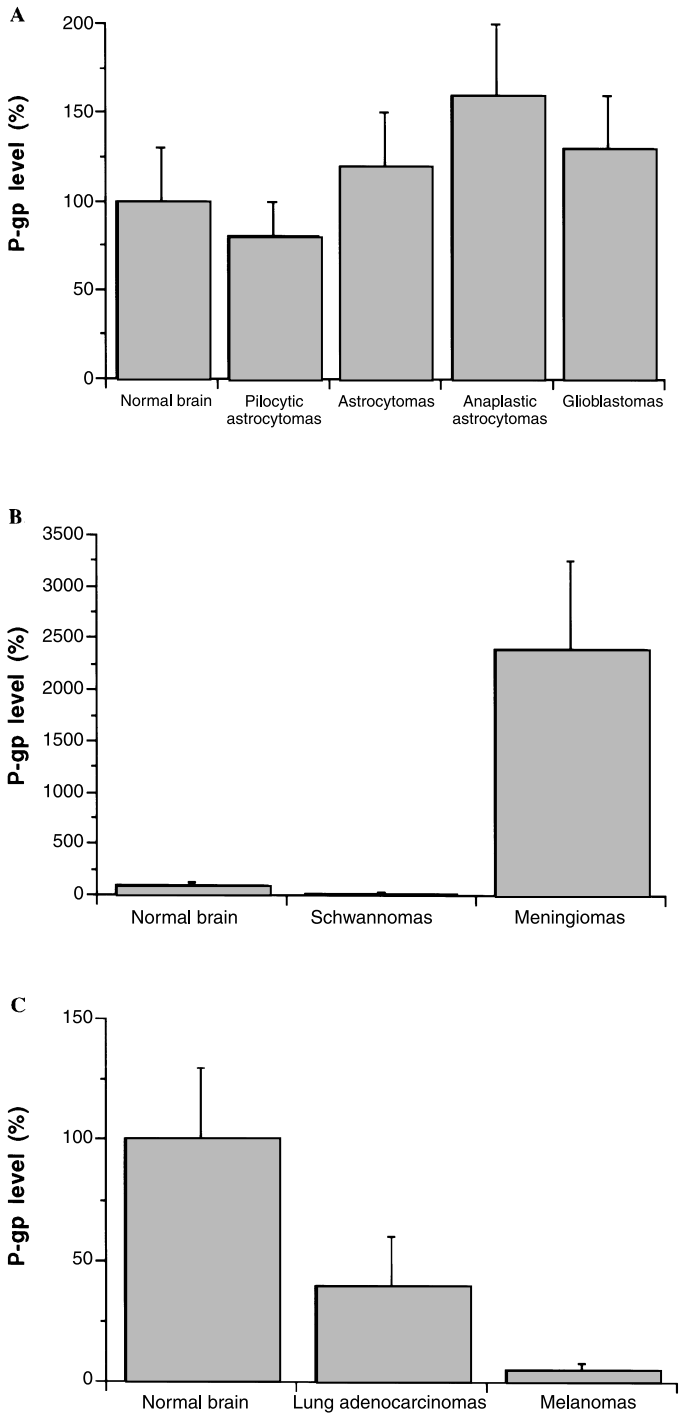


*Figure 2*. P-gp levels in brain tumors and in normal brain were estimated by laser densitometry from immunodetection experiments using MDR Ab-2. From these evaluations, P-gp levels in more than 50 brain tumors were compared to P-gp levels averaged from 4 normal brains. Results are expressed as percentage of P-gp expression in normal brain: (A) Malignant brain tumors, (B) Benign brain tumors and (C) Brain metastases.

Taken together, these results indicate that primary brain tumors of all grades express as much, if not more, P-gp than does normal brain. Since P-gp appears to be one of the best phenotypic markers of the BBB, these results indicate that brain tumors present an intact BBB at the molecular level. This barrier may well restrict the brain distribution of most chemotherapeutic agents which are P-gp substrates.

### *6.2. Brain metastasis*

We investigated the expression of P-gp in brain metastases from melanomas and from lung carcinomas (Figure 2C). Strikingly, melanoma metastases express only 5% of the P-gp level found in normal brain. Lung carcinoma metastases express 40% of the P-gp level found in normal brain. Metastatic malignant melanomas are well-known for their poor response to chemotherapy, whereas some response to chemotherapy has been observed with lung carcinomas. The lower P-gp expression observed in brain metastases from lung adenocarcinomas and melanomas suggests that another MDR mechanism could be involved in their weak response to chemotherapy. The lack of P-gp expression in primary lung tumors and in their corresponding brain metastases also indicates that these metastases did not acquire the same level of P-gp expression during their development than did primary tumors originating in the brain.

These results also suggest that the tissue origin of tumor cells may determine the phenotype of the tumor vascular beds. Glioma tumors originating from astrocytes remain capable of inducing or maintaining specific phenotype of the BBB such as the expression of P-gp in the primary brain tumor vasculature. On the other hand, the tumoral cells in brain metastases of different histological origin are unable to induce or maintain the BBB phenotype in the developing vasculature.

## 7. MRP expression in brain tumors

In some cases, including glioblastoma cell lines, P-gp overexpression does not explain the resistance to agents such as etoposide, adriamycin and vincristine [66]. Thus, it is important to know if other MDR-related genes could be involved in the drug resistance of human gliomas. MRP mRNA levels appear to be closely associated with cellular levels of resistance to etoposide, adriamycin and vincristine in human glioma cell lines [67]. Furthermore, MRP1 mRNA and protein levels were increased in some chemo-resistant glioblastoma cell lines. Recently, MRP1 mRNA was detected by RT-PCR and MRP1 protein expression was demonstrated by immunohistochemistry in 90% of the glioblastomas analyzed [68]. A recent study compared mRNA levels of *MDR1* and *MRP1-4* genes in normal brain and malignant gliomas [69]. Whereas no difference was seen in MDR1 and MRP2 expression between normal brain and tumor cells, MRP1 and MRP3 expression increased along with tumor grade. These results suggest that some MRPs may also be involved in the intrinsic MDR in some human gliomas. In these studies no difference was made between expression in the tumor vasculature or in the tumor cells themselves.

## 8. Novel therapeutic approaches for brain tumors

Brain tumors comprise a broad spectrum of biological and clinical entities. A single therapeutic approach is highly unlikely to be universally applicable. Although cure or long-term remission of malignant brain tumors seems unlikely in the near future, extension of lifespan for months or even years would be a great benefit. To overcome the MDR phenotype and the relative impermeability of the BBB to cytotoxic drugs, several strategies to enhance brain delivery by altering BBB function have been described.

### *8.1. P-glycoprotein inhibitors*

It has been firmly established by studies in P-gp knockout mice (mdr1a−/−) that, in the absence of P-gp, higher brain concentrations of P-gp substrates occur [30]. This leads to the hypothesis that in humans, modulation of P-gp might also lead to higher brain uptake of P-gp substrates. Several compounds that interact with P-gp and block drug efflux have been reported to reverse the MDR phenotype. The first generation modulators included calcium channel blockers, calmodulin inhibitors, hormonal/steroidal derivates, antibiotics, cardiovascular drugs and cyclosporins. However, the majority of these compounds have been associated with toxicity when used at the concentrations required to inhibit P-gp. Thus a second generation of more selective and more potent resistance modulators have been developed such as the non-immunosuppressive cyclosporin D analogue

SDZ-PSC 833 [70], VX-710 [71], the acridone carboxamide derivate GF120918 [72] and the substituted dibenzosuberane molecule LY335979 [73]. Clinical trials with some of these second-generation modulators are currently in progress, and initial studies have demonstrated some clinical benefit from the use of modulators such as SDZ-PSC 833 [74].

### *8.2. BBB opening agents*

The first described method for osmotic opening of the BBB by administration of hypertonic solutions (e.g., mannitol) resulted only in a slight increase in survival of patients with metastatic or primary brain tumors [75]. Direct modulation of the tight junctions has been made feasible by the paracrine peptide bradykinin and its synthetic analogue RPM-7. Following a number of experiments using preclinical models, it was proposed that co-administration of RPM-7 (Cereport) could increase the effectiveness of carboplatin (a highly polar drug which does not easily cross plasma membranes of any type) in the treatment of brain tumors. In support of this, uptake of carboplatin into rat glial tumors increase following intracarotid or intravenous administration of RPM-7 [76,77]. Based on this evidence, phase I and II clinical trials of Cereport and carboplatin were performed on patients with glioblastoma multiforme or anaplastic astrocytoma [78]. These studies demonstrated that carboplatin can be safely administered with Cereport at doses that are consistent with the intended therapeutic effects. Larger, randomized studies are still required to establish any benefit from RPM-7.

### *8.3. Drug delivery strategies*

Other means of overcoming the BBB and P-gp at the luminal side of endothelial cells are currently under investigation. Strategies utilizing specific transport mechanisms at the BBB to deliver a drug into the brain compartment at a therapeutic concentration are being developed. There are several transport systems at the BBB for nutrients and endogenous compounds. The use of these BBB transport systems is expected to provide a basis for enhancing the delivery of drugs into the brain. The more advanced delivery systems use the receptor-mediated endocytosis pathway. The OX-26 antibody against the rat transferrin receptor has been shown to be an effective drug delivery vehicle [4]. The utilization of antibodies against the insulin receptor at the BBB is also effective in primates and is currently under investigation [79].

### *8.4. Anti-angiogenic therapies*

Numerous single-agent and multi-agent chemotherapy approaches have been evaluated for the treatment of primary and metastatic brain tumors. However, the survival benefit from chemotherapy has been negligible for most agents and only modest for those with some efficacy. This emphasizes the need for novel therapeutic agents with improved activity against brain tumors. Investigators are exploring the use of novel, often cytostatic, therapeutic strategies. Among the targets, angiogenesis has captured much attention since tumor neovascularization is a key feature in the growth and progression of malignant gliomas. Unlike cytotoxic agents, angiogenesis inhibitors are expected to have a cytostatic effect resulting in stabilization of the tumor without a decrease in its size. One approach to controlling angiogenesis is to employ endothelial cell inhibitors, of which numerous agents are in preclinical development for primary brain tumors and other malignancies. Some of the more promising agents include TNP-470, thalidomide, endostatin, squalamine, combretastatin, SU-6668 and retinoids. Another approach uses matrix metalloproteinase inhibitors (MMPI). Extracellular matrix breakdown is essential for angiogenesis. Marimastat, a synthetic low molecular weight hydroxamic acid analogue, is a broad spectrum MMPI that has demonstrated activity in various clinical trials [80]. Neovastat, an extract from shark cartilage, is a multifunctional anti-angiogenic compound that is currently undergoing phase III clinical trials for the treatment of refractory renal cell carcinoma and non small cell lung cancer, as well as a phase II trial for the treatment of multiple myeloma. Neovastat inhibits matrix metalloproteinases, serine elastases, the VEGF-mediated signaling pathway and induces specific endothelial cell apoptosis [81], making it a pleotropic anti-angiogenic compound. Neovastat was recently shown to inhibit the growth of brain tumors [82]. The fact that tumor endothelium and normal endothelium may differ at the molecular level for some markers may have significant implications for the development of new therapies. This has already been demonstrated for endothelial cells derived from blood vessels of normal and malignant colorectal tissues [83]. This research area, called angiomics, may be of particular interest for brain tumors.

*Table 1.* The blood–brain barrier plays a role in brain tumor resistance

| For | Against |
|---|---|
| • Clinical resistance to chemotherapeutic agents<br>• Increased brain penetration of drugs in P-gp knock-out models<br>• Low brain distribution of cytotoxic drugs to brain tumors<br>• Expression of tight-junction related protein in brain tumor vascular endothelium<br>• Intact BBB in peritumoral brain<br>• Persistent gene expression of BBB-specific gene LU glycoprotein in brain tumor vascular endothelium<br>• High expression of P-gp in brain tumor vascular endothelium | • Altered tight-junction morphology of brain tumor vascular endothelium<br>• Brain edema<br>• Down-regulation of BBB-specific gene GLUT-1 in brain tumor vascular endothelium |

### *8.5. Nutraceutics*

In light of the poor prognosis for patients with malignant brain tumors and the relative failure of therapies over the past few years, the objective of converting the tumor into a controlled, quiescent, chronic disease seems more feasible than complete eradication of the tumor. In the search for such cytostatic therapeutic strategies, there has been growing interest in nutraceutics. Substances present in vegetables and fruits may not only be used to prevent but also to cure certain disorders. Green tea polyphenols are a good example of such compounds. Tea preparations have been shown to inhibit tumorigenesis at the initiation, promotion and progression stages in different animal models. Recently, an inhibitory effect was reported for epigallocatechin-gallate (EGCg), the main constituent of green tea polyphenols, on brain tumor cell lines *in vitro* [84]. We have shown that green tea polyphenols and especially EGCg have a pleiotropic activity on several actors of tumoral progression. Green tea polyphenols are potent inhibitors of MMP activity and activation [85] interact with P-gp [86] and inhibit vascular endothelial growth factor receptor (VEGFR-2) phosphorylation [87].

## 9. Conclusion

The role of the BBB and P-gp in producing drug resistance in brain tumors appears important. Arguments in favor and against a significant role for the BBB in chemo-resistance are summarized in Table 1. Ultrastructural alterations of the endothelial cell tight-junction network, increased permeability leading to characteristic brain edema associated with brain tumors and down-regulation of BBB-specific genes such as the glucose transporter GLUT-1 [88] are arguments in favor of a breakdown of the BBB in brain tumors. Opposed to this, the strong clinical resistance to chemotherapy, evidence of persistence of the tight-junction proteins and the continued expression of other BBB-specific genes such as Lutheran glycoprotein [89] and P-gp [59], indicate an intact barrier at the molecular level. Thus, one of the characteristics of brain tumors that appears specific is the fact that, in addition to intrinsic mechanisms of resistance in the tumor cells themselves, resistance to chemotherapy is also provided by the blood vessels.

## Acknowledgments

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada to Richard Béliveau. Anthony Régina is a recipient from the Fonds de la Recherche en Santé du Québec. We thank The Fondation Charles Bruneau for its financial support.

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*Address for offprints:* Richard Béliveau, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montréal, Canada; *Tel:* 514 987 3000 (ext 8551); *Fax:* 514 987 0246; *E-mail:* oncomol@nobel.si.uqam.ca