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Multidrug resistance gene (MDR1) expression in human brain tumors

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✓ Multidrug resistance for many types of cancer outside the central nervous system (CNS) has been found to be due to the overexpression of the multidrug resistance gene MDR1, of which the gene-product P-glycoprotein acts as a membrane-bound efflux pump for many anticancer drugs. To examine whether brain tumors overexpress the MDR1 gene, 25 brain-tumor specimens were subjected to Northern blot analysis: 10 gliomas, eight meningiomas, three schwannomas, one malignant lymphoma, and three tumors metastatic to the brain. Ten fresh-frozen autopsy specimens of various parts of normal brain were also analyzed. Blots were hybridized with ^{32}P-labeled Chinese hamster complementary deoxyribonucleic acid (cDNA) and ^{32}P-labeled human MDR1 cDNA. The MDR1 gene messenger ribonucleic acid (mRNA) was detected in two tumors using the Chinese hamster probe (one sphenoid wing meningioma and one metastatic prostate tumor) and in one CNS lymphoma using the human probe. Intact mRNA could not be extracted from the fresh-frozen autopsy specimens of normal brain. Seventeen tumors were examined for P-glycoprotein by immunohistochemical staining using murine monoclonal antibody C219: eight gliomas, eight meningiomas, and one craniopharyngioma. The neoplastic cells from two gliomas and three meningiomas and the blood vessels within six gliomas and two meningiomas stained positively for P-glycoprotein. Seven of 10 normal brain specimens stained positively for P-glycoprotein in blood vessels but no specimen demonstrated staining of parenchymal cells. This study demonstrates that the MDR1 gene can be detected in normal brain, and in malignant, benign, and metastatic lesions. P-glycoprotein can be present in tumor blood vessels even when it is not seen in neoplastic cells. Although the role of P-glycoprotein in tumor blood vessels needs to be further examined and more clearly defined, drug resistance in malignant primary brain tumors may result from characteristics not solely of neoplastic cells but also tumor vasculature.

KEY WORDS · **drug resistance** · **brain neoplasm** · **chemotherapy**

RESISTANCE to chemotherapy remains a major obstacle in the treatment of patients with primary brain tumors. Recently, various human carcinomas, including liver, kidney, adrenal, and colon tumors, have been shown to be simultaneously resistant to multiple different hydrophobic drugs of natural product origin including Adriamycin (doxorubicin), vincristine, colchicine, and etoposide.[2,5,9,10,12,19,21,22] Multidrug resistance for this broad category of agents was found to be associated with decreased intracellular drug accumulation and increased expression of a highly conserved gene that has been given the name "multidrug resistance (MDR) gene."[1] The MDR gene family in humans has two members, MDR1 and MDR2.[4] The MDR1 gene is transcribed into a 4.5-kilobase (kb) messenger ribonucleic acid (RNA) and the gene product is a membrane-bound glycoprotein of 170,000 D called "P-glycoprotein" which acts as an energy-dependent efflux pump for the anticancer drugs mentioned above. The function of the MDR2 gene is unknown.[6,25]

Multidrug resistance of cancers that exhibit overexpression of the MDR1 gene can be overcome when competitive inhibitors such as calcium channel blockers and calmodulin inhibitors block P-glycoprotein-mediated drug efflux. Clinical trials based on the rationale that P-glycoprotein inhibitors should be given along with chemotherapeutic agents have been initiated.[11] If malignant brain tumors overexpress the MDR1 gene, new possibilities could be opened for the treatment of these lesions, which currently result in a dismal patient outcome.

Using Northern blot analysis, we examined whether malignant, benign, and metastatic lesions to the brain overexpressed MDR1. Normal brain sections were also

analyzed. To complement these studies, immunohistochemical staining was utilized using a murine monoclonal antibody with high specificity for P-glycoprotein.

Materials and Methods

Specimens for RNA Analysis

All specimens were obtained in accordance with the Johns Hopkins Joint Committee for Clinical Investigation guidelines. Fifty-seven sequential tumor biopsy specimens were obtained in the operating room, quick-frozen in liquid nitrogen, and stored at −70°C. After total RNA extraction and oligo-dT chromatography,[8] 25 of these 57 specimens yielded a sufficient amount of messenger RNA (mRNA) for analysis. The 25 specimens included four recurrent glioblastomas multiforme, three glioblastomas multiforme, one anaplastic astrocytoma, two low-grade astrocytomas, eight meningiomas, three schwannomas, one malignant lymphoma, and three metastatic tumors. Eight of these specimens were also analyzed by immunohistochemical staining for P-glycoprotein. Ten fresh-frozen autopsy specimens provided by the Johns Hopkins Hospital Brain Bank were also subjected to Northern blot analysis. These 10 biopsy specimens included two each from the cortex, white matter, amygdala, cerebellum, and hippocampus. The Chinese hamster ovary drug-resistant cell line CHRC5 (high MDR1 expressor) was used for the positive control, and the Chinese hamster ovary wild type cell line AuxB1 was used for the negative control (low MDR1 expressor).

RNA Preparation

Samples were homogenized in guanidine isothiocyanate and RNA was extracted by cesium chloride gradient centrifugation.[8] Total RNA concentrations were determined spectrophotometrically. Poly(A)+ RNA was isolated by oligo-dT chromatography.[8]

Northern Blot Analysis

Messenger RNA samples were size-fractionated on 0.8% formaldehyde-agarose gels and transferred onto nitrocellulose.[17] Blots were baked for 2 hours at 80°C and prehybridized for 4 hours at 42°C in 50% formamide, 5 × Denhardt's medium, 0.05 M NaH_2PO_4 (pH 7.4), and 0.015 mg/ml denatured herring sperm, using a method modified from that of Maniatis, *et al.*[18] Hybridization proceeded overnight in the same mixture with a ^{32}P-labeled Chinese hamster MDR1 complementary deoxyribonucleic acid (cDNA) probe, provided as a 600-base pair (bp) EcoR1 insert.*[21] The same blots were stripped and rehybridized to a human MDR1 cDNA probe provided as a 656-bp HaeIII fragment.† Hybridization with ^{32}P-labeled human β-actin cDNA verified intact mRNA samples and standardized quantities of mRNA.[13] After hybridization, filters were washed twice for 20 minutes each at 42°C in 50% formamide, 5 × SET (0.75 M NaCl, 5 mM ethylenediaminetetra-acetic acid, 50 mM Tris, pH 7.8), and 0.25% sodium dodecyl sulfate (SDS), with a final wash at 65°C in 0.5 × SET and 0.5% SDS (modified from the method of Maniatis, *et al.*[18]).

TABLE 1

*The MDR1 gene mRNA:β-actin signal ratio for the specimens studied**

Specimen No.	Specimen	MDR1 mRNA:β-Actin Ratio
control cell line		
	AuxB1	0.08
	CHRC5	0.79
tumor specimens		
8	glioblastoma multiforme, recurrent	0.25
2	glioblastoma multiforme, recurrent	0.12
1	glioblastoma multiforme, recurrent	0
11	glioblastoma multiforme, recurrent	0
17	glioblastoma multiforme	0
24	glioblastoma multiforme	0
47	glioblastoma multiforme	0
18	anaplastic astrocytoma	0
6	low-grade astrocytoma	0.50
45	low-grade astrocytoma	0
16	meningioma	0.41†
28	meningioma	0
30	meningioma	0
41	meningioma	0
42	meningioma	0
43	meningioma	0
46	meningioma	0
48	meningioma	0
5	schwannoma	0
13	schwannoma	0.28
26	schwannoma	0
9	malignant CNS lymphoma	0.06 (0.25)†,‡
7	metastatic adenocarcinoma	0.73†
10	metastatic adenocarcinoma	0.08
29	metastatic adenocarcinoma	0

* Ratio in optical density units of MDR1 mRNA:β-actin for AuxB1 (low MDR1 expressor), CHRC5 (high MDR1 expressor), and human brain-tumor specimens using the Chinese hamster cDNA probe. CNS = central nervous system. Intact mRNA could not be extracted from normal brain fresh-frozen autopsy specimens.

† Positive MDR1 mRNA expressor.

‡ Detected using human MDR1 cDNA probe (with the human MDR1 cDNA probe, CHRC5 MDR1 mRNA:β-actin = 0.58).

Autoradiography and Data Analysis

Autoradiographs were exposed at −70°C on Kodak XAR film with special screens.‡ Densitometry imaging was performed on an LAI Automated Biological Medical Image Analysis System.§ The MDR1 mRNA:β-

* EcoR1 insert supplied by Dr. Victor Ling, The Ontario Cancer Institute, Toronto, Ontario, Canada.

† HaeIII fragment supplied by Dr. Antonio Fojo, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

‡ Cronex Lightning-Plus screens manufactured by Du Pont Co., Wilmington, Delaware.

§ LAI automated Biological Medical Image Analysis System manufactured by Loats Associates, Inc., Westminster, Maryland.

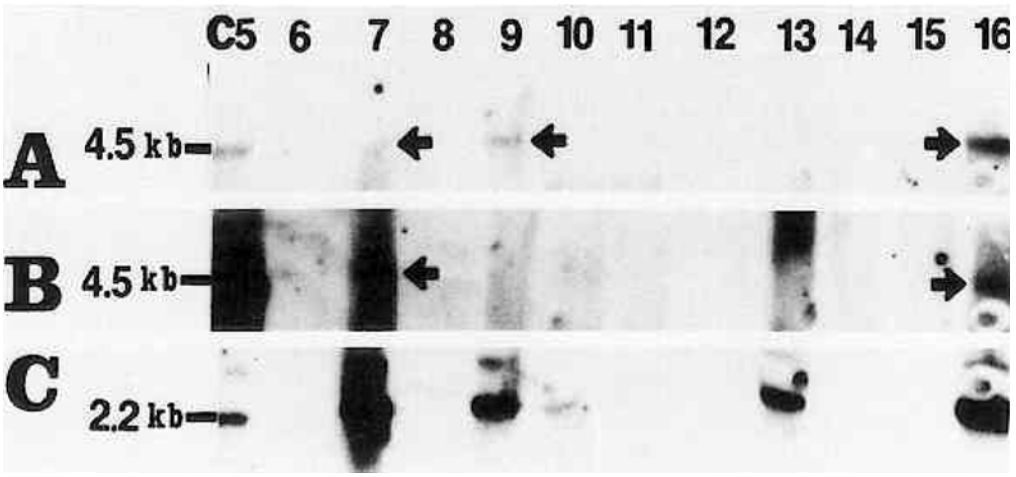

FIG. 1. Representative Northern blot findings of positive control and tumor specimens hybridized with human MDR1 complement deoxyribonucleic acid (cDNA) probe (A), Chinese hamster cDNA probe (B), and β-actin probe (C). Positive control CHRC5 is labeled C5; patient specimens are as follows: 8, 11 = recurrent glioblastoma multiforme; 12 = glioblastoma multiforme; 6 = low-grade astrocytoma; 14 = oligodendroglioma; 16 = meningioma; 13 = schwannoma; 9 = malignant lymphoma; and 7, 10, and 15 = metastatic tumors. Positive expressors are noted by *arrows* in Specimens 7, 9, and 16 using the human probe and Specimens 7 and 16 using the Chinese hamster probe.

actin signal ratio was calculated for each specimen. Expression of MDR1 was defined as positive in cases with a discrete band at 4.5 kb and an MDR1 mRNA:β-actin signal ratio above that of the low MDR1 expressor, AuxB1. Samples exhibiting smears of degraded RNA and no band at 4.5 kb were eliminated from further analysis.

Immunohistochemical Study

Seventeen brain-tumor biopsy specimens, eight of which were examined by Northern blot, were analyzed using immunohistochemistry. Specimens included three recurrent glioblastomas multiforme, three glioblastomas multiforme, one anaplastic astrocytoma, one oligodendroglioma, eight meningiomas, and one craniopharyngioma. Eleven fresh-frozen autopsy specimens were also examined using immunohistochemistry, including four hippocampus, two cerebellum, two amygdala, and three cortex biopsy specimens. To provide positive and negative controls, cultures of Chinese hamster ovary cell lines CHRC5 and AuxB1, respectively, were harvested by scraping, then were pelleted, frozen, and sectioned. Tissue sections 10 μ thick were made using a cryostat, then air-dried and immediately incubated with the primary murine monoclonal antibody C219‖ at a concentration of 5 μg/ml. Monoclonal antibody C219 recognizes an epitope of P-glycoprotein on the inner surface of the cell membrane. Therefore, C219 incubation medium contained 0.1% Triton × 100. Positive (CHRC5) and negative (AuxB1) controls were run concurrently. Normal horse serum, 1%, served as nonreactive control.[20] Slides were kept overnight in a moisture chamber at 4°C. Secondary antibodies were biotinylated horse anti-mouse immunoglobulin G (heavy + light-chain specific) affinity-purified antibodies followed by avidin-biotin-peroxidase complexes.[14] Following a wash in phosphate-buffered saline, pH 7.4, and 0.05 M Tris buffer, pH 7.7, the sections were incubated with 0.5% filtered 3,3-diaminobenzidine in 0.01% H_2O_2.[14] After a wash and counterstaining with hematoxylin, slides were mounted with Permount.[7] All slides were examined by three independent observers. Staining for P-glycoprotein was designated as strongly positive (++), weakly positive (+), or absent (−).

‖ Primary murine monoclonal antibody C219 manufactured by Centocor, Malvern, Pennsylvania.

Results

Northern Blot Analysis

The β-actin probe signal verified intact mRNA for 25 brain-tumor specimens. The MDR1 mRNA:β-actin signal ratio for each specimen is presented in Table 1. Expression of MDR1 mRNA was detected in three tumors. The Chinese hamster probe detected MDR1 mRNA expression in one sphenoid wing meningioma (Specimen 16) in a patient with an aggressive tumor that required repeated resections, and one metastatic prostate tumor (Specimen 7) in a patient with known prostate cancer and no prior chemotherapy. The Northern blot results for these cases can be seen in Fig. 1. In one additional case, a patient with a central nervous system lymphoma and a prior history of Hodgkin's disease, MDR1 mRNA expression was detected using the human probe (Specimen 9). This patient had received prior chemotherapy consisting of six cycles of the MOPP protocol (nitrogen mustard, vincristine, procarbazine, and prednisone) 2 years prior to her crani-

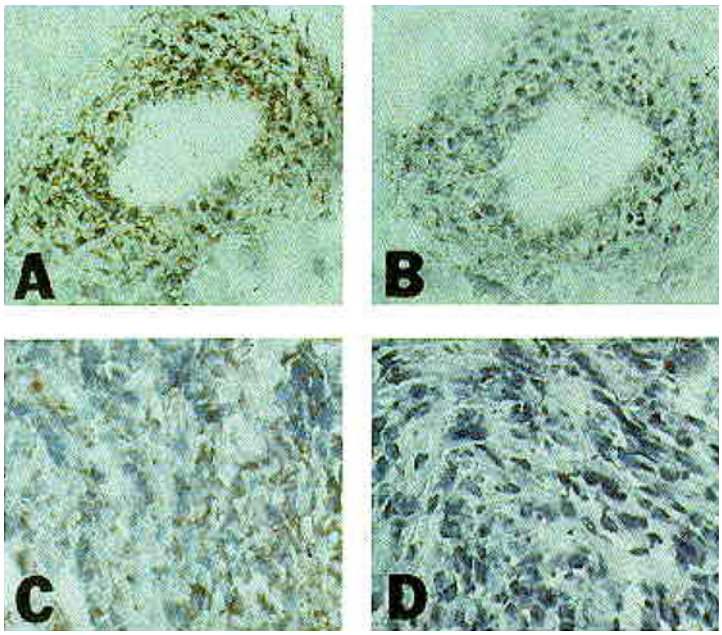

FIG. 2. Immunohistochemical staining of two tumor specimens (A and C) and two control specimens (B and D). A: A glioblastoma multiforme (Specimen 50) demonstrating strongly positive tumor vessel staining for P-glycoprotein outlining the endothelial cell membranes of a penetrating vessel. The section had numerous strongly staining vessels throughout the specimen and absent staining of the tumor cells. × 250. B: Control specimen. × 250. C: A meningioma (Specimen 51) demonstrating uniform weakly positive tumor cell staining for P-glycoprotein throughout the entire section. × 400. D: Control specimen. × 400.

otomy. Based on the criteria for MDR1 mRNA expression stated above, Specimens 2, 6, 8, and 13 had MDR1 mRNA:β-actin ratios greater than 0.08 but no discrete bands at 4.5 kb, and were therefore not considered MDR1 mRNA expressors. Specimens 6 and 8 were also noted to have low β-actin signals, which may have influenced detection of MDR1 mRNA expression, as did Specimens 11, 41, and 46. The Northern blot results together with clinical correlations are summarized in Table 2. Using the β-actin probe, we found that we were unable to obtain intact mRNA from any of the normal fresh-frozen autopsy specimens. This was not unexpected, considering the nature of the source of the specimens.

Immunohistochemical Findings

A summary of immunohistochemical results with clinical correlation is provided in Table 2. Two patterns of P-glycoprotein staining were observed: staining of penetrating tumor vessel endothelia (Fig. 2A and B) and/or staining of neoplastic cells (Fig. 2C and D). Staining of neoplastic cells occurred uniformly throughout the entire specimen or in isolated regions: one recurrent glioma and two meningiomas stained uniformly for P-glycoprotein, and one anaplastic oligodendroglioma and one meningioma stained in isolated regions. Neoplastic cells in two of eight gliomas and three of six meningiomas demonstrated P-glycoprotein: neoplastic cells in one glioblastoma multiforme and one meningioma stained very positively for P-glycoprotein, and neoplastic cells in one anaplastic astrocytoma and two meningiomas stained weakly positively for P-glycoprotein. Tumor vasculature stained positively in six of eight gliomas (seven of which were high-grade gliomas) and in four of six meningiomas. Penetrating blood vessels in four glioblastomas multiforme, one anaplastic astrocytoma, and one meningioma stained very positively for P-glycoprotein, and blood vessels in one glioblastoma multiforme and three meningiomas stained weakly positively for P-glycoprotein. Four of eight gliomas stained positively for P-glycoprotein within the tumor vasculature, with no staining of neoplastic cells (Fig. 2A and B), in contrast to one of six meningiomas. Two of eight gliomas demonstrated P-glycoprotein in both the tumor vasculature and neoplastic cells, as opposed to three of four meningiomas. In no case was staining present in neoplastic cells and not in the blood vessels. Due to nonspecific staining in the control specimens, two meningiomas were excluded from further analysis.

Immunohistochemical results of normal brain fresh-frozen autopsy specimens is presented in Table 3. Normal brain specimens demonstrated weakly and strongly positive staining of the vasculature only, with no staining in parenchymal cells. Six regions demonstrated strongly positive staining of the penetrating vasculature: one specimen from the hippocampus, two from the cerebellum, one from the amygdala, and two from the cortex. One region of the hippocampus showed weakly positive staining for penetrating vessels. One specimen from the hippocampus, one from the cerebellum, and one from the amygdala stained negatively for both vessels and cells. The remaining hippocampal specimen was excluded from analysis due to nonspecific staining secondary to autolytic changes.

Discussion

By Northern blot analysis, three of 25 brain-tumor specimens demonstrated detectable MDR1 mRNA expression. The human probe identified one case not

TABLE 2

*Clinical profile of Northern blot and immunohistochemical findings in tumor specimens**

Specimen No.	Tumor	MDR1 mRNA Expressor	Staining Pattern† (Blood Vessels/ Tumor Cells)	Patient Profile: Age (yrs), Sex	Patient Profile: Prior Chemotherapy‡
1	GBM, recurrent	no	+/−	41, M	BCNU (1 yr)§
8	GBM, recurrent	no	++/++	60, F	no§
2	GBM, recurrent	no	ND	51, M	BCNU + cis-platinum (10 mos)§
11	GBM, recurrent	no	ND	56, F	no§
53	GBM, recurrent	ND	−/−	30, F	no§
24	GBM	no	++/−	66, F	no
17	GBM	no	ND	37, M	no
47	GBM	no	ND	74, F	no
12	GBM	ND	++/−	30, M	no
50	GBM (prior breast ca)	ND	++/−	72, F	NA (20 yrs)
18	anaplastic astrocytoma	no	ND	30, M	no
45	low-grade astrocytoma	no	ND	7, M	no
6	low-grade astrocytoma	no	ND	46, M	no
54	anaplastic oligo	ND	++/+	45, M	no
14	oligo	ND	−/−	45, F	no
42	meningioma	no	+/−	43, F	no
28	meningioma	no	−/−	59, F	no
43	meningioma	no	−/−	39, F	no
41	meningioma	no	NSS	53, F	no
48	meningioma	no	NSS	65, F	no
16	meningioma	yes	ND	48, M	no
30	meningioma	no	ND	58, M	no
46	meningioma	no	ND	68, F	no
52	meningioma	ND	++/++	19, M	no
33	meningioma	ND	+/+	60, F	no
51	meningioma	ND	+/+	47, M	no
5	schwannoma	no	ND	33, M	no
13	schwannoma	no	ND	47, M	no
26	schwannoma	no	ND	47, M	no
9	malignant lymphoma‖	yes	ND	39, F	MOPP (2 yrs)
7	met adeno	yes	ND	67, M	no
10	met adeno	no	ND	46, M	no
29	met adeno	no	ND	49, M	no
55	craniopharyn	ND	−/−	5, M	no

* GBM = glioblastoma multiforme; ca = carcinoma; oligo = oligodendroglioma; met adeno = metastatic adenocarcinoma; ND = not done; NA = data not available; craniopharyn = craniopharyngioma.

† Immunohistochemical staining pattern: ++ = strongly positive; + = weakly positive; − = absent; NSS = excluded for nonspecific stain in specimen controls.

‡ Where relevant, the interval between chemotherapy and craniotomy is given in parentheses. BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea; MOPP = nitrogen mustard, vincristine, procarbazine, and prednisolone.

§ Patient received prior radiation therapy.

‖ Patient had previously had Hodgkin's disease.

TABLE 3

Immunohistochemical results of fresh-frozen biopsy specimens of normal brain obtained at autopsy

Specimen No.	Anatomical Region	Staining Pattern* (Blood Vessels/ Parenchymal Cells)
499	hippocampus	++/−
629	hippocampus	+/−
430	hippocampus	−/−
487	hippocampus	NSS
560	cerebellum	++/−
460	cerebellum	++/−
487	amygdala	++/−
417	amygdala	−/−
515	cortex: occipital	++/−
399	cortex: medial frontal gyrus	++/−
560	cortex: medial frontal gyrus	−/−

* Immunohistochemical staining pattern: ++ = strongly positive; + = weakly positive; − = absent; NSS = nonspecific staining in specimen control with autolysis.

picked up by the Chinese hamster cDNA probe. With immunohistochemical techniques, 10 of 15 tumors demonstrated P-glycoprotein in tumor vessels and/or neoplastic cells by both strongly and weakly positive staining patterns. Immunohistochemistry may be a more sensitive technique to detect MDR1 expression in brain due to the often isolated distribution of P-glycoprotein. Messenger RNA constitutes merely 5% of the total RNA prepared from heterogeneous tissue samples, whereas only a small percentage of tissue such as the penetrating tumor capillaries or an isolated pocket of tumor cells may express MDR1. Therefore, it is not surprising that some positive specimens are not detected by Northern blot analysis. It is also not surprising that autopsy specimens, collected at various times postmortem, do not represent sources of intact mRNA of the same quality as operating room specimens, fresh-frozen at the time of surgical excision.

The MDR gene is highly conserved and retained throughout the evolution spectrum from bacteria through mammals.[3] The MDR1 gene product, P-glycoprotein, has been found to act as an efflux pump and play an integral role in the removal of wastes and toxic substances from the cell.[5] Expression of MDR1 in normal tissue has been demonstrated in liver, kidney, and colon.[5,23] Consistent with the results of this study, P-glycoprotein has been found by other investigators in normal brain within the endothelial cells of capillaries in the cortex, hippocampus, and cerebellum.[7,24] The role of P-glycoprotein has been hypothetically related to the blood-brain barrier mechanism.[7,24] Its presence within tumor vasculature may also be related to the removal of toxic substances. P-glycoprotein within the vessels of primary malignant brain tumors could pump chemotherapeutic agents back into the vasculature system before the drugs reach the neoplastic cells. On the other hand, P-glycoprotein may play a role in the enhanced removal of these agents from the interstitial space of neoplastic tissue.

In the majority of cases of high-grade gliomas, P-glycoprotein was present in tumor vessels even when it was not present in neoplastic cells. Although the exact part that this glycoprotein plays in tumor and/or normal vasculature needs to be further examined and more

clearly defined, drug resistance in primary malignant brain tumors may result from the characteristics not solely of neoplastic cells but also of the tumor vasculature. Tumors in these situations would manifest drug resistance *in vivo* and may be drug-sensitive *in vitro*. This might partially explain why the results of *in vitro* chemotherapy testing have not correlated well with patient outcome.[16] In our series, one-half of the cases that demonstrated P-glycoprotein exclusively in tumor vessels had previously received chemotherapy, which suggests that chemotherapeutic agents may induce P-glycoprotein in tumor vasculature without inducing it in neoplastic cells.

Expression of the MDR1 gene and P-glycoprotein was detected in primary malignant, benign, and tumors metastatic to the brain. P-glycoprotein was evident in neoplastic cells but was more widely apparent in penetrating tumor vasculature of the glioma specimens, where it may play a unique role in drug resistance. With widespread use of natural-product hydrophobic drugs in pediatric and adult brain-tumor chemotherapy protocols,[15,16] the presence of MDR1 gene expression in malignant primary brain tumors must be further examined, and its overall importance more clearly defined.

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References

1. Beck WT: The cell biology of multiple drug resistance. **Biochem Pharmacol 36:**2879–2887, 1987
2. Chan HSL, Thorner PS, Haddad G, et al: Immunohistochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. **J Clin Oncol 8:** 689–704, 1990
3. Chen C, Chin JE, Ueda K, et al: Internal duplication and homology with bacterial transport proteins in the *mdr*1 (P-glycoprotein) gene from multidrug-resistant human cells. **Cell 47:**381–389, 1986
4. Chen C, Clark D, Ueda K, et al: Genomic organization of the human multidrug resistance (MDR1) gene and origin of P-glycoproteins. **J Biol Chem 265:**506–514, 1990
5. Chen YN, Mickley LA, Schwartz AM, et al: Characterization of adriamycin-resistant human breast cancer cells which display overexpression of a novel resistance-related membrane protein. **J Biol Chem 265:**10073–10080, 1990
6. Chin JE, Soffir R, Noonan KE, et al: Structure and expression of the human *MDR* (P-glycoprotein) gene family. **Mol Cell Biol 9:**3808–3820, 1989
7. Cordon-Cardo C, O'Brien JP, Casals D, et al: Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. **Proc Natl Acad Sci USA 86:**695–698, 1989
8. Davis LG, Dibner MD, Battey JF: **Basic Methods in Molecular Biology.** New York: Elsevier, 1986, pp 129–156
9. Fojo AT, Ueda K, Slamon DJ, et al: Expression of a multidrug-resistance gene in human tumors and tissues. **Proc Natl Acad Sci USA 84:**265–269, 1987
10. Goldstein LJ, Fojo AT, Ueda K, et al: Expression of the multidrug resistance, *MDR*1, gene in neuroblastoma. **J Clin Oncol 8:**128–136, 1990
11. Gottesman MM, Pastan I: Clinical trials of agents that reverse multidrug-resistance. **J Clin Oncol 7:**409–411, 1989
12. Gottesman MM, Pastan I: The multidrug transporter, a double-edged sword. **J Biol Chem 263:**12163–12166, 1988
13. Gunning P, Ponte P, Okayama H, et al: Isolation and characterization of full-length cDNA clones for human α-, β-, and γ-actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that subsequently removed. **Mol Cell Biol 3:**787–795, 1983
14. Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. A comparison between ABC and unlabeled antibody (PAP) procedures. **J Histochem Cytochem 29:** 577–580, 1981
15. Levin VA, Silver P, Hannigan J, et al: Superiority of post-radiotherapy adjuvant chemotherapy with CCNU, procarbazine, and vincristine (PCV) over BCNU for anaplastic gliomas: NCOG 6G61 final report. **Int J Radiat Oncol Biol Phys 18:**321–324, 1990
16. Kornblith PL, Walker M: Chemotherapy for malignant gliomas. **J Neurosurg 68:**1–17, 1988
17. Maniatis T, Fritsch EF, Sambrook J: **Molecular Cloning. A Laboratory Manual.** Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982, pp 187–210
19. Moscow JA, Fairchild CR, Madden MJ, et al: Expression of anionic glutathione-*S*-transferase and P-glycoprotein genes in human tissues and tumors. **Cancer Res 49:** 1422–1428, 1989
20. Reisner HM, Wick MR: Theoretical and technical considerations for the use of monoclonal antibodies in diagnostic immunohistochemistry, in Wick MR, Siegel GP (eds): **Monoclonal Antibodies in Diagnostic Immunohistochemistry.** New York: Marcel Dekker, 1988, pp 1–49
21. Riordan JR, Deuchars K, Kartner N, et al: Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. **Nature 316:**817–819, 1985
22. Sato H, Gottesman MM, Goldstein L, et al: Expression of the multidrug resistance gene in myeloid leukemias. **Leukemia Res 14:**11–22, 1990
23. Thiebaut F, Tsuruo T, Hamada H, et al: Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. **Proc Natl Acad Sci USA 84:**7735–7738, 1987
24. Thiebaut F, Tsuruo T, Hamada H, et al: Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and crossreactivity of one antibody with a muscle protein. **J Histochem Cytochem 37:**159–164, 1989
25. Ueda K, Clark DP, Chen C, et al: The human multidrug resistance (*mdr*1) gene. cDNA cloning and transcription initiation. **J Biol Chem 262:**505–508, 1987

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