Multidrug Resistance Proteins in Gastrointestinal Stromal Tumors: Site-Dependent Expression and Initial Response to Imatinib

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Abstract Gastrointestinal stromal tumors (GIST) are the most frequent mesenchymal tumors of the digestive tract and respond poorly to chemotherapy. A tyrosine kinase inhibitor treatment, imatinib mesylate, was recently shown to have antitumor effects in metastatic patients. However, this drug is a substrate for multidrug resistance (MDR) proteins. Therefore, we investigated the expression of ABCB1 (P-glycoprotein), ABCC1 (MRP1), and ABCG2 (BCRP) by Western blotting in 21 GISTs and 3 leiomyosarcomas. All the GISTs were positive for either ABCB1 (86% of cases) or ABCC1 expression (62%), but negative for ABCG2. ABCB1 was expressed in all gastric GISTs, but in only 67% of nongastric GISTs. By contrast, ABCC1 expression was more common in nongastric tumors (78% versus 42%). The levels of these MDR proteins in gastric GISTs were higher for ABCB1 ($P = 0.007$) and lower for ABCC1 ($P = 0.004$) compared with nongastric GISTs. We found no correlation between MDR protein expression and the risk assessment. None of the six patients treated with imatinib was resistant, although all were positive for at least one MDR protein. These results confirm that gastric and nongastric GISTs have different biological characteristics and suggest that MDR proteins do not impair the initial response of the tumor to imatinib.

Gastrointestinal stromal tumors (GIST) are the most frequent mesenchymal tumors of the digestive tract (1). They may occur along the entire length of the gastrointestinal tract, from the esophagus to the anus, and sometimes in the omentum or the mesentery. GISTs are now considered as a distinct type of tumor in which diagnosis is based on histology and expression of the KIT tyrosine kinase membrane receptor (CD117, stem cell factor receptor; refs. 2–4). Several studies have investigated the factors determining the prognosis of GISTs (5, 6) and have identified tumor size and the mitotic index as the main factors. Some studies have reported that the presence of *KIT* mutations in GISTs were of prognostic value (7–9), whereas others failed to confirm this (9–11). GISTs are less responsive to cancer chemotherapy than other soft tissue sarcomas (12, 13). It was recently shown that treatment with a tyrosine kinase inhibitor, imatinib mesylate, had antitumor effects in metastatic patients (14, 15).

The expression of P-glycoprotein (ABCB1) and multiresistance protein 1 (MRP1 or ABCC1) is implicated in the multidrug resistance (MDR) of several soft tissue sarcoma (16–18). MDR proteins confer drug resistance by reducing intracellular drug accumulation due to an active efflux. ABCB1 and ABCC1 expel a similar spectrum of drugs, including anthracyclines, *Vinca* alkaloids, and epipodophyllotoxins (19). Recently, some studies have shown that ABCB1, ABCC1, and ABCG2 interact directly with several tyrosine kinase inhibitors *in vitro* and particularly with imatinib (20, 21).

Here, we investigated the expression of ABCB1, ABCC1, and ABCG2 in 21 GIST using Western blotting and correlated these results with clinicopathologic characteristics.

Patients and Methods

Patients. The study included 21 patients with GISTs and three patients with leiomyosarcomas. The GISTs originated from the stomach ($n = 12$), intestine ($n = 5$), colon ($n = 2$), peritoneum ($n = 1$), and mesentery ($n = 1$). The pathology of all cases was reviewed on sections from paraffin-embedded samples. Risk assessment was evaluated as recommended by the international workshop (4). Immunohistochemistry was done using anti-CD117 (KIT polyclonal rabbit, Dakopatts, Copenhagen, Denmark), anti-CD34 (clone QBEND mouse, Immunotech, Marseilles, France), anti-ABCC1 (MC-201, Syrinx-Diagnostika, Frankfurt, Germany) and anti-S100 protein (polyclonal rabbit, Dakopatts) antibodies. Clinical records were reviewed and no patients received chemotherapy or imatinib before surgical resection of the tumor. All molecular analyses were done on primary tumors. All the human samples were obtained between 1995 and 2003 by surgical resection done for therapeutic purposes according to local ethical guidelines.

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Western blot. Frozen tumors were mechanically homogenized (Mixer Mill MM 300) in lysis buffer (20 mmol/L Tris, 150 mmol/L NaCl, 1 mmol/L sodium orthovanadate, 10 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, 0.5 μg/mL leupeptin, 1 μg/mL pepstatin, 10 KIU/mL aprotinin, and 1% Triton X-100). The lysates were shaken gently for 30 minutes at 4°C and then centrifuged to remove insoluble material. The protein concentration of the supernatants was determined using Bradford solution and was normalized. Total lysates were resuspended in Laemmli buffer and 10 μg of proteins were subjected to SDS-PAGE (8%) under reducing conditions in 25 mmol/L Tris-HCl (pH 8.3), including 0.1% SDS and 0.2 mol/L glycine at room temperature at 20 mA. For quantification and to compare results, two identical positive samples were included on each gel. The proteins were electroblotted onto nitrocellulose membranes (Amersham Biosciences, Chalfort St. Giles) for 15 hours at 4°C at 40 V in 25 mmol/L Tris-HCl (pH 8.3) containing 0.2 mol/L glycine, 20% methanol, and 0.1% SDS. Nonspecific binding sites were blocked by incubation with 10% skimmed milk in Tris buffer [20 mmol/L Tris-HCl (pH 7.5), 0.2 mol/L, 0.1% Tween 20] for 1 hour at room temperature. Western blots were carried out using anti-ABCB1 monoclonal antibodies (C219 1/100, DAKO, Copenhagen, Denmark), and anti-ABCC1 (1/500, Chemicon, Chandlers Ford, United Kingdom) and anti-ABCG2 (BXP-21 1/100, Chemicon, United Kingdom) antibodies. Immunoreactive bands were visualized using the appropriate secondary horseradish peroxidase–conjugated antibodies and enhanced chemiluminescence (Amersham Biosciences). ABCB1 and ABCC1 proteins were quantified

Table 1. Characteristics of the 21 patients with a GIST

Patients	Age/sex	Primary site	KIT/CD34	*KIT* or *PDGFRA* mutations	R.A.	Follow-up (mo)	Alive/dead	CR/disease	Treatment of relapse or metastases	Response
71103	46/m	Colon	++/+++	No	High	0.4	Alive	CR	None	
71105	73/m	Peritoneum	+++/+	No	High	12	Alive	Disease	Imatinib	PR, then progression
71176	78/m	Intestine	++/+++	No	Low	0.2	Dead	CR	None	
71181	64/f	Intestine	+++/+	*KIT*: E9: ins 502-503	Low	26.6	Alive	CR	None	
71186	66/f	Intestine	++/++	No	Very low	12.1	Alive	CR	None	
71187	60/m	Mesentery	++/+++	*KIT*: E11:ins 574-591	High	32.4	Dead	Disease	Imatinib	PR, then progression
71231	57/m	Intestine	++/+	*KIT*: E11:del 557-558 plus V559C	High	0.2	Alive	CR	None	
71233	55/f	Colon	+/0	No	High	31.9	Alive	CR	Surgical resection of hepatic metastasis	
71385	49/m	Intestine	+++/0	No	High	1.1	Alive	Disease	None	
71183	39/m	Stomach	++/++	No	Inter	11.8	Alive	CR	None	
71224	71/m	Stomach	++/+++	*PDGFRA*: E18: del 842-845, D846R, S847V	High	34.7	Alive	Disease	Imatinib	PR
71227	64/f	Stomach	+/+	PDGFRA: E18: del 843-846, S847I	Inter	13.9	Alive	CR	None	
71100	46/f	Stomach	+++/+++	*KIT*: E11:del 553-558	High	36	Alive	Disease	Imatinib	Stable disease
71101	77/m	Stomach	++/++	*KIT*: E11:del 576	Inter	0.6	Alive	CR	None	
71175	69/m	Stomach	++/+++	*KIT*: E11:W557R	High	36.7	Alive	CR	None	
71178	42/f	Stomach	++/+++	*KIT*: E11:V560D	Low	20	Alive	CR	None	
71185	56/f	Stomach	+++/++	*KIT*: E11: del 579	Inter	16.1	Alive	CR	None	
71225	57/f	Stomach	+++/++	*KIT*: E11:del 552-558 plus V559I	High	60.8	Alive	CR	None	
71229	75/f	Stomach	+++/++	*KIT*: E11:del 576	High	63.6	Alive	CR	None	
71237	67/m	Stomach	++/+++	*KIT*: E11:del 561-578	High	59.6	Alive	Disease	Imatinib	PR
71562	60/m	Stomach	+++/+++	*KIT*: E11: del 557-558	High	14	Alive	Disease	Imatinib	Stable disease

NOTE: +, 5% to 20% of positive tumor cells; ++, 21% to 80% of positive tumor cells; +++, >80% of positive tumor cells.
Abbreviations: m, male; f, female; R.A., risk assessment; E11, exon 11; E9, exon 9; ins, insertion; del, deletion; PR, partial response.

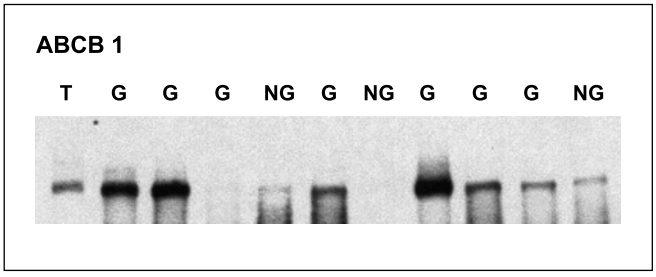

Fig. 1. Expression of ABCB1 in GISTs. Western blot analysis of whole tumor lysate with anti-ABCB1 antibody C219. In this first series of tumors, the levels of expression of ABCB1 were higher in gastric (*G*; 71224, 71227, 71183, 71229, 71100, and 71562) than nongastric (*NG*; 71103, 71105, 71385, and 71181) GISTs. Placenta sample was used as a positive control (*T*).

by densitometry using *Scion Image* program and analyzed using Excel software (Microsoft, Courtaboeuf, France). The ABCB1 and ABCC1 signals in the GIST samples were expressed relative to the signal measured in placenta extracts and ABCC1 colon extracts, respectively.

Mutations analysis. DNA was extracted from either paraffin-embedded tumors or frozen tumors after histologic control. *PDGFRA* (exons 12 and 18) and *KIT* (exons 9, 11, 13, and 17) mutations were detected by length analysis of PCR products and direct sequencing as previously described (22, 23).

Results

The mean age of the patients was 60.3 years (range 39-78) and the male-to-female ratio was 12:9 (Table 1). All GISTs were KIT positive. More than 10% of the tumor cells in 11 of 12 (92%) gastric GISTs and in 4 of 9 (44%) nongastric GISTs were CD34 positive. Except for one GIST, mutations were present in all gastric GISTs, and involved exon 11 of *KIT* in nine cases and exon 18 of *PDGFRA* in two cases. Only three of the nongastric GISTs exhibited mutations in exon 9 ($n = 1$) or exon 11 ($n = 2$) of *KIT*. After the initial surgery, six patients were treated with imatinib for tumor progression or metastasis.

Multidrug resistance protein expression. First, we analyzed a series of 11 patients with GISTs and found a higher expression of ABCB1 in gastric tumors (Fig. 1). We then analyzed all 21 patients and compared the results to a control placenta sample (Fig. 2).

We found ABCB1 expression in 18 of the 21 GISTs (86%). This larger series confirmed that ABCB1 was more frequently expressed in gastric tumors than nongastric tumors. Indeed, all 12 gastric GISTs were positive for ABCB1, where only six of the nine (67%) nongastric tumors were positive. In most gastric GISTs, the level of ABCB1 expression was higher than in the control placenta sample, whereas only one nongastric tumor had a higher expression than the control. Although this nongastric tumor was a 40 cm mesenteric tumor, retrospective analysis of the surgical report cannot exclude a primitive gastric origin. Quantification of ABCB1 expression in gastric and nongastric GISTs revealed an average level of expression of 2.56 and 0.73, respectively, compared with the control placenta sample ($P = 0.007$, two-sided nonparametric Wilcoxon test).

We detected ABCC1 expression in 13 of 21 GISTs (62%), which was less than for ABCB1 expression. In contrast to ABCB1 expression, ABCC1 was less frequently expressed in gastric tumors than in nongastric tumors, 5 of 12 (42%) versus 7 of 9 (78%), respectively (Fig. 3). Moreover, the level of multiresistance protein expression was lower in gastric GISTs compared with nongastric GISTs ($P = 0.004$, two-sided nonparametric Wilcoxon test). Immunohistochemistry confirmed the variable expression of ABCB1 by GIST cells (Fig. 4).

Both ABCB1 and MRP1 was expressed in 9 of 21 (43%) GISTs, and no GIST was negative for both ABCB1 and ABCC1.

We then compared the level of expression of ABCB1 in gastric and nongastric GIST to their levels of expression in leiomyosarcomas (Fig. 5). We found that the level of expression of ABCB1 in gastric GIST was far higher than in leiomyosarcomas.

Lastly, neither gastric nor nongastric GIST expressed ABCG2 (Fig. 6).

Correlation of multidrug resistance protein expression with other patient characteristics. Three patients tested negative for ABCB1 and positive for ABCC1. All these patients had an nongastric GIST without *KIT* or *PDGFRA* mutations (Table 2).

Ten patients tested positive for both ABCB1 and ABCC1. Of these patients, half had gastric GISTs with either *KIT* or *PDGFRA* mutations. The five other cases were nongastric GISTs, of which three had *KIT* mutations (Table 2).

Eight patients tested positive for ABCB1 and negative for ABCC1. All patients except one were men, and all but one had gastric GISTs, with either *KIT* or *PDGFRA* mutations (Table 2).

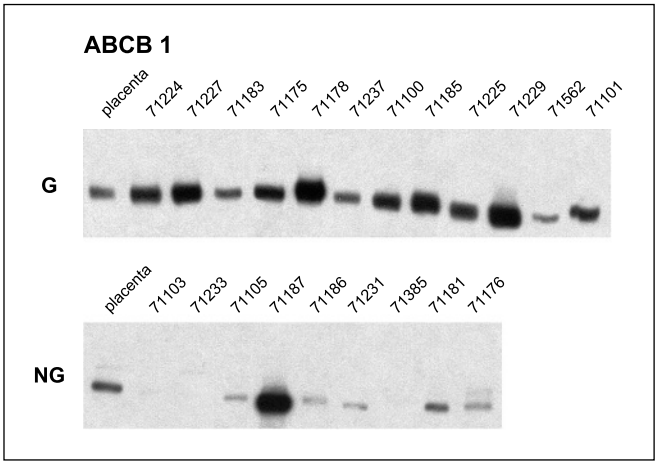

Fig. 2. Expression of ABCB1 in gastric and nongastric GISTs. Top gel contains gastric GISTs, all of which had high levels of expression of ABCB1. The levels of expression of ABCB1 were usually lower in nongastric GISTs (*bottom gel*). Placenta sample was used as positive control.

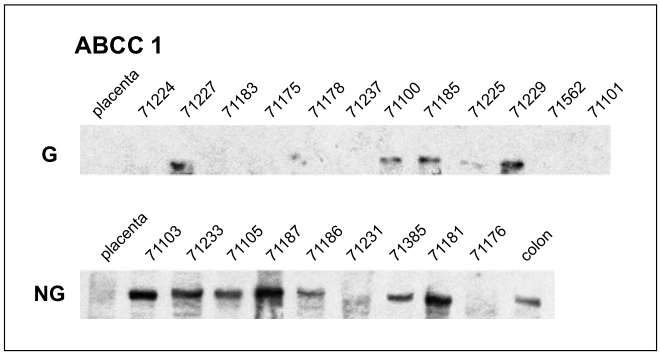

Fig. 3. Expression of ABCC1 in gastric and nongastric GISTs. ABCC1 expression was weak or absent in gastric GISTs (*top*), in contrast to its higher level of expression in nongastric GISTs (*bottom*). Colon sample was used as positive control.

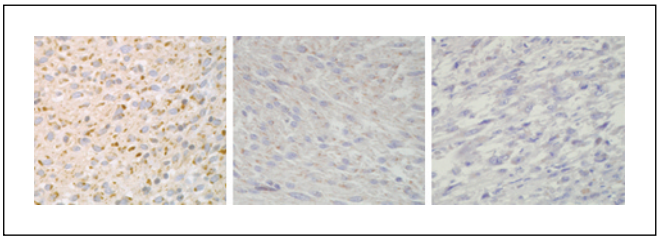

Fig. 4. Immunohistochemical detection of ABCC1 in GIST. Expression of ABCC1 was detected by immunohistochemistry (*brown staining*) within tumor cells of some GISTs. As observed with Western blot, ABCC1 was abundant (*left*), moderately abundant (*middle*), or absent (*right*) in the GISTs cells. Original magnification, ×400.

Neither ABCB1, ABCC1, nor their coexpression were correlated with the risk assessment.

After the initial resection, six patients were treated with 400 to 800 mg/d of imatinib for metastatic malignant GISTs. A partial response to treatment was seen in four patients, with the other two having a stable disease during the first 6 months of treatment. After an initial partial response, two patients suffered a disease progression.

Discussion

The expression of MDR proteins in soft tissue sarcoma has been correlated with a worse survival rate (24) or with a higher histologic grade (25). Leiomyosarcomas have been shown to have a weaker response to chemotherapy compared with other histologic types of sarcoma (26). However, until recently, GISTs were frequently misdiagnosed as leiomyosarcomas (1). Recent studies have shown that GISTs are more resistant to chemotherapies than leiomyosarcomas (12, 13). Therefore, we investigated the expression of MDR proteins in GISTs.

We investigated MDR protein expression by Western blot on proteins extracted from frozen tumors, including 21 KIT-positive GISTs. We showed that the level of ABCB1 expression was higher in GISTs than in leiomyosarcomas. Plaat et al. (27) showed similar results using a different technique; however, only 15 of the 22 GISTs in this study were KIT positive. We found that GISTs were positive for ABCB1 and ABCC1 expression in 86% and 62% of cases, respectively, whereas both proteins were expressed in 68% of GISTs in the study of Plaat et al. (27). In our study, we found that no GIST was negative for both ABCB1 and ABCC1, whereas all GISTs were negative for ABCG2. The more frequent and higher levels of expression of MDR proteins in GISTs compared with leiomyosarcomas may contribute to the different responses to chemotherapy (12) and may be related to the resistance of GISTs to some cytotoxic chemotherapy (28).

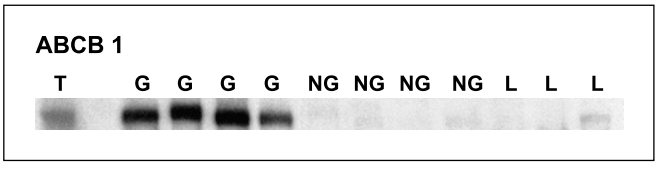

Fig. 5. Expression of ABCB1 in GISTs and leiomyosarcomas. ABCB1 expression was stronger in gastric GISTs compared with nongastric GISTs and to leiomyosarcomas (*L*). Placenta sample was used as positive control.

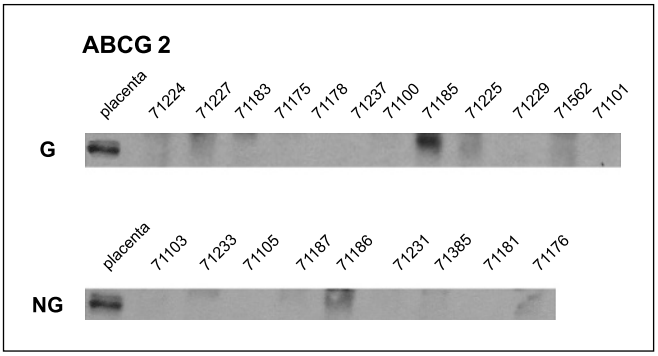

Fig. 6. Expression of ABCG2 in gastric and nongastric GISTs. No expression of ABCG2 in all GISTs (gastric or nongastric). Placenta sample was used as positive control.

We found that ABCB1 was expressed in all gastric GISTs, but in only 67% of nongastric tumors, whereas ABCC1 was more often expressed in nongastric tumors than gastric GISTs (78% versus 42%). Although not underlined in the study of Plaat et al. (27), the expression of ABCB1 was also more common in gastric GISTs (83% versus 54%). Other differences between gastric and nongastric GISTs have also been observed. For example, CD34 was shown to be more frequently expressed in gastric GISTs (2, 11). Some mutations, including internal tandem duplication of *KIT* exon 11 and *PDGFRA* mutations, are more specific of gastric GISTs (29–31), whereas *KIT* exon 9 mutations are closely associated with intestinal GISTs (11, 32, 33). Gastric and nongastric GISTs

Table 2. Quantification of MDR proteins

Patients	Primary site	Mutation	ABCB1	ABCC1
71103	Colon	No	0	3.87
71233	Colon	No	0	3.90
71385	Intestine	No	0	1.82
71231	Intestine	KIT	0.24	1.13
71105	Peritoneum	No	0.28	2.78
71186	Intestine	No	0.37	2.05
71181	Intestine	No	0.73	4.26
71225	Stomach	KIT	2.07	0.37
71100	Stomach	KIT	2.30	0.87
71227	Stomach	PDGFRA	3.35	1.49
71185	Stomach	KIT	3.39	1.29
71187	Mesentery	KIT	4.38	5.52
71229	Stomach	KIT	4.82	2.05
71176	Intestine	No	0.55	0
71562	Stomach	KIT	0.69	0
71237	Stomach	KIT	1.08	0
71183	Stomach	No	1.15	0
71101	Stomach	KIT	2.21	0
71175	Stomach	KIT	2.57	0
71224	Stomach	PDGFRA	2.66	0
71178	Stomach	KIT	4.44	0

NOTE: MDR protein quantification is expressed as the ratio of fluorescence intensity of ABCB1 and ABCC1 to control placenta and colon extract, respectively.

have also been distinguished by their gene expression profile (34). Although the anti-MDR treatment, VX-710, failed to reverse the resistance of GISTs to anthracycline (13), clinical prospective or retrospective studies should assess the different responses of GISTs to anti-MDR treatments according to the initial site of the GIST. Some studies have shown a correlation of MDR protein expression with histologic grade of soft tissue sarcoma (18, 22, 35–37). MDR protein expression was also correlated with tumor response (38) and survival (24). By contrast, Plaat et al. (27) found no correlation between MDR protein expression and histologic grade in GISTs, and we also found no correlation with the risk assessment.

The role of these three MDR proteins toward imatinib has been widely debated. Imatinib is an actively transported substrate of the human ABCB1 glycoprotein (20). Overexpression of ABCB1 has been shown to induce resistance of K562 cells to imatinib (39–43), although other studies failed to confirm these results (44). Imatinib is not a substrate of ABCC1 (20, 39), but the low expression of this MDR protein has been shown to be an independent predictor of response to imatinib in myeloid blast crisis of chronic myelogenous leukemia (45). Imatinib has been considered an actively transported substrate of the human ABCG2 (21, 42, 43), although other studies have suggested that imatinib is a potent inhibitor of ABCG2 (46).

We provide the first data of MDR protein expression in GISTs from patients treated with imatinib. The six patients of our series tested positive for ABCB1, and three also tested positive for ABCC1. None of the six patients presented a primary resistance to imatinib treatment. Although further studies are needed, these results suggest that MDR proteins have little effect on initial response of the tumor to imatinib. The biological role of the differential and high levels of expression of MDR proteins still remains to be investigated with respect to the anatomic origin of GISTs.

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