RESEARCH ARTICLE

Association between ABCB1 Immunohistochemical Expression and Overall Survival in Gastric Cancer Patients

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Abstract

Gastric cancer (GC) is one of the most common malignancies worldwide. The ABCB1 protein, a member of the ATP-binding cassette (ABC) transporter family, encoded by the ABCB1 gene, considerably influences the distribution of drugs across cell membranes as well as multidrug resistance (MDR) of antineoplastic drugs. In contrast to the extensive knowledge on the pharmacological action of ABCB1 protein, the correlation between the clinical-pathological data and ABCB1 protein expression in patients with GC remains unclear. The aim was to investigate association between ABCB1 expression and overall survival in GC patients. Human tumor fragments from 57 GC patients were examined by immunohistochemistry assay. We observed lower survival rate of patients with GC who were positive for ABCB1 expression (p=0.030). Based on these observations, we conclude that GC patients with positive ABCB1 protein immunohistochemical expression in their tumors suffer shorter overall survival.

Keywords: ABCB1 - immunohistochemistry - gastric cancer - survival

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Introduction

The gastric cancer (GC) is still the fourth most cancer and the second most common cause of cancer-related death in the world (Crew et al., 2006; Ferlay et al., 2010; Bray et al., 2012). Researchers in public health reported a decline in the GC mortality rate was observed during the 26 years, however the prognosis for affected patients is still poor due to the high aggressiveness of this neoplasm (Silva et al., 2011). Even with the advancement of human cancer therapy, mechanisms that have advanced in mammals to protect cells against cytotoxic drugs in the environment will continue to function as an important obstacle to successful cancer treatments (Gottesman, 2002; Kim and Tononock, 2005; Nobili et al., 2006). A recent retrospective analysis showed that adjuvant chemotherapy might improve outcomes in patients with potentially resectable GC (Zhang et al., 2013). Nevertheless, GC cells develop resistance to chemotherapy primarily by inactivating apoptotic factors and the effluxing of drugs mediated by overexpression of ATP-dependent drug-efflux pumps P-glycoprotein (Pgp), more recently known as ABCB1 protein (Zhu et al., 2013).

The ABCB1 protein plays an important role in the bioavailability of drugs, providing a barrier to the entry of xenobiotics, as well as monitoring its output in different tissues (Ueda et al. 1986). ABCB1 protein, a member of the ATP-binding cassette (ABC) transporter family, encoded by the ABCB1 gene, and more than 50 SNPs have been identified in this gene. Furthermore, these polymorphisms may have a direct effect on ABCB1 gene expression and function of the ABCB1 protein (Fromm et al., 2002; Ambudkar et al., 2006Salama et al, 2006; ; Dean, 2009). Nevertheless, in previous studies conducted by our group (Oliveira et al., 2012; Wu et al., 2014), described a lack of association of the MDR1 C3435T polymorphism with susceptibility to GC. Differently, in another field study by Ren et al (2012), suggested that the MDR1 gene polymorphism was strongly associated with increased susceptibility to hepatocellular carcinoma.

In contrast to the extensive knowledge on the pharmacological action of ABCB1 protein, this field still remains unclear about the correlation between the basic clinical- pathological data and ABCB1 protein expression status in patients with GC. Besides the prevalence and impact on GC patient survival, so it becomes necessary to understand the relationship between the expression of ABCB1 and impact on survival of patients affected by GC. The purpose of this study was to investigate the association between ABCB1 immunohistochemical expression and overall survival in GC patients, and to further to correlation the clinical-pathological data and ABCB1 protein expression status in these patients.

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Materials and Methods

We studied the expression of ABCB1 protein in patients with GC. Fifty-seven patients diagnosed with GC were treated in outpatient Oncology of the Discipline of Clinical Gastroenterology of UNIFESP-EPM. Surgical specimens was embedded in paraffin and stored in the Department of Pathology, UNIFESP-EPM. In this study were included patients with gastric adenocarcinoma and excluded smaller 18 years age. The study was approved by the Ethics Committee of the institution, and all patients signed an informed consent form. Data on sex, age, diagnosis of GC, histological type, grade of differentiation, tumor location, stage, realization of prior chemotherapy and survival (date of diagnosis and death) were collected from medical record review (paper and electronic).

Immunohistochemistry

Immunostaining was performed on sections of 3 μm mounted on 3-aminopropylotrimetoxy-silane (Sigma-Aldrich, St. Louis, MO, USA) coated slides. Briefly, sections were deparaffinized in xylene, rehydrated through graded ethanols, followed by blocking of endogenous peroxidase activity in 3% hydrogen peroxide for 20 minutes at room temperature. Antibody-binding epitopes were retrieved by pressure-cooking for 2.5 minutes in 10 mM sodium citrate buffer (pH 6.0). Sections were then incubated with rabbit polyclonal anti-ABCB1 dilution 1:200 (H-241) obtained from Santa Cruz Biotechnology, Inc. (USA), overnight at 4ºC in humid chamber. After washing twice with phosphate-buffered saline pH 7.4 (PBS), slides were incubated with biotinylated second-stage antibody for 30 minutes, followed by incubation with streptavidin-biotin-peroxidase complex (LSAB, Dako, Carpinteria, California, USA) for further 30 minutes, at room temperature. Staining was carried out using a solution 3-3'diaminobenzidine tetrahydrocloride (DAB- Dako, Carpinteria, California, USA). Washes with PBS were performed between each step. The ABCB1 was diluted in PBS containing 5% (wt/vol) bovine serum albumin. Nuclei were counterstained with Harris hematoxylin before mounting slides in Entellan (Sigma-Aldrich, St. Louis, MO, USA). Negative and positive controls were included in each batch of immunohistochemistry. Section of colon cancer known to express high levels of ABCB1 was included as positive control, while in negative control the primary antibody was omitted.

Analysis of immunostaining was performed by two investigators. The positive pattern was cytoplasmic and was graded semi-quantitatively according to the percentage of stained cells and their staining intensity. A numerical scoring system with two categories was used to assess the observed expression of the proteins. Category A documented the number of immunoreactive cells as 0 or negative (no immunoreactive cells), 1 (<10% immunoreactive cells), 2 (10-50% immunoreactive cells) and 3 (>50% immunoreactive cells). Category B documented the intensity of the immunostaining as (negative or no immunoreactive cells), 1 (weak immunostaining), 2 (moderate) and 3 (strong immunostaining). The values for categories A and B were multiplied to give the "immunoreactivity score", which could range from 0 to 9. Zero until 3 were considered negative, 4 until 9 were considered positive.

Statistical analysis

The Statistical Package for the Social Sciences v19.0 was used for statistical analysis. Age was compared between groups by the Student's t-test. The Chi-square test was used to determine differences between the two groups. Survival was estimated by the Kaplan-Meier method and survival curves were compared by the log-rank test.

Results

Expression pattern of the ABCB1 was predominantly localized in the cytoplasm, as illustrated in the figures below (Figure 1A, 1B, 1C and 1D). There were no significant differences between positive expression of ABCB1 according to the location of the tumor and normal adjacent mucosa (p=0.891) as is demonstrated in Table 1. We did not find significant differences when we compare the expression of ABCB1 and clinical-pathological data, as anatomical location (p=1.000), stage (I+II vs. III+IV) (p=0.526), Lauren´s classification (p=0.763) or histological grades (p=1.000) as showed in Table 2. We observed reduced survival of patients with GC who were persistently positive for ABCB1 protein

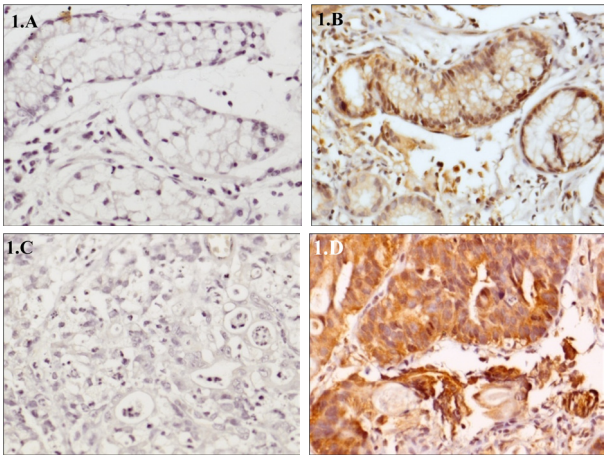

Figure 1. The Positive Pattern of ABCB1 was Predominantly The Cytoplasmic Type. 1.A Normal adjacent mucosa to the tumor with negative expression of ABCB1, 100X; 1.B Normal adjacent mucosa to the tumor with positive expression of ABCB1, 100X; 1.C Adenocarcinoma of diffuse type with negative expression of ABCB1, 100X; 1.D Adenocarcinoma of intestinal type with positive expression of ABCB1, 400X

Table 1. Expression of ABCB1 in The Tumor and Normal Adjacent Mucosa to The Tumor

Location	Positive n (%)	Negative n (%)	p
Tumour (55)	25 (45.5)	30 (54.5)	0.891
Normal adjacent mucosa (47)	22 (46.8)	25 (54.2)	

Categorical variables are expressed as n (%), #p for Pearson chi-square (x2)

Table 2. Frequency of Patients with GC with Positive Expression of ABCB1 in The Adjacent Mucosa and Tumour According to The Clinical-Pathological Data

	Adjacent mucosa n (%)	Tumour n (%)	p
Anatomic location			
Cardia/Fundus	1 (4.6)	1 (4.0)	1.000#
Body/Antrum	21 (95.4)	24 (96)	
Stage			
I+II	9 (40.9)	8 (32.0)	0.526*
III+IV	13 (59.1)	17 (68.0)	
Lauren´s classification			
Intestinal	14 (66.7)	17 (70.9)	0.763*
Diffuse	7 (33.3)	7 (29.2)	
Histologic grade			
Well differentiated	5 (22.7)	5 (20.0)	1.000*
Moderatelydifferentiated	9 (40.9)	10 (40.0)	
Poorly differentiated	7 (31.8)	9 (36.0)	
Undifferentiated	1 (4.6)	1 (4.0)	4

Categorical variables are expressed as n (%), #p for Pearson chi-square (x2) and *p for Fisher's Exact Test

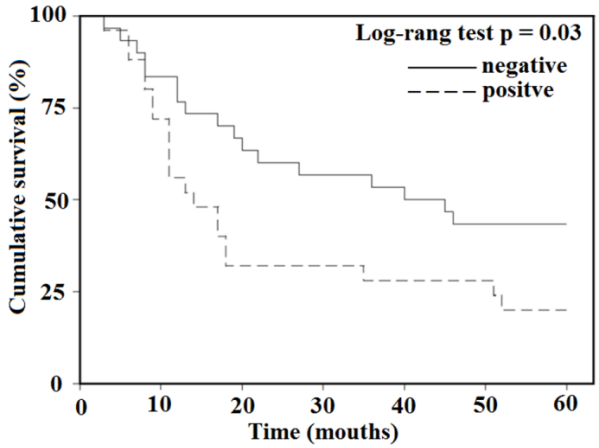

Figure 2. Kaplan-Meier Overall Survival Curves for ABCB1-Positive and Negative Patients with Cancer Gastric

expression (p=0.030), as plotted in Figure 2. Survival analysis of patients with stage III and IV GC showed no relationship between the clinical stage and the expression of ABCB1 (p=0.125). There was no difference in survival of individuals performing chemotherapy, regardless of the expression of ABCB1 in normal adjacent mucosa (p=0.601) or tumor (p=0.578). Additionally, we observed in these patients higher frequency of use of fluorouracil (28.9%) followed by folinic acid (26.7%).

Discussion

Most patients in the advanced stages of GC require chemotherapy but, one of the greatest obstacles to effective chemotherapy is the development of drug resistance. The ABCB1 protein is directly related to the control of the bioavailability of various substrates actively working in both excretion and elimination of drugs and toxic substances (Ueda et al., 1986).

In accordance with Baldissera et al (2012) that observed increased expression of ABCB1 protein in tissues of hepatocellular carcinoma compared to non-tumor tissues we found higher expression of ABCB1 protein in GC tissues compared to the normal adjacent mucosa. Choi et al (2002) found higher expression of ABCB1 protein in moderately differentiated tumors and especially in intestinal-type GC of Lauren's classification. Similarly, we found increased expression of ABCB1 protein in moderately differentiated tumors and intestinal-type of Lauren. In this study, patients with clinical stage III+IV presented higher expression of ABCB1 protein in relation to stages I+II.

Studies show that the level of expression of ABCB1 protein is related to membrane permeability, intracellular drug concentration and drug resistance. The positivity of ABCB1 protein between 25% and 71% of GC cells was associated with poor prognosis (Lacueva et al., 2000; Xie et al., 2004; Wei et al., 2005). Similarly, this study showed that patients with GC and ABCB1 protein positive had poor prognosis (p=0.03). Although patients with GC have been performing chemotherapy ABCB1 protein may cause a resistance to treatment. However, (Song et al., 2012) supposed that the downregulation of ABCB1 which led to intracellular drug accumulation, which led to reduction of cell detoxification ability.

One explanation for the lack of association between the survival of patients undergoing chemotherapy and expression of ABCB1 protein may be because most of our patients use drugs that are not known to be substrates for ABCB1 protein. Comparing our results with the literature, we observed differences in some variables and these can be associated with different ethnic groups as well as the number of patients included in the study.

In order to confirm all assays that immunohistochemical ABCB1 protein expression, we performed several analyses to ensure that analytical results were acceptably reproducible. Based on these observations, we conclude that gastric cancer patients with positive ABCB1 protein immunohistochemical expression in the tumor region had shorter overall survival. Additionally, we found no correlation between the clinical-pathological data and ABCB1 protein expression status.

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