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Survivin expression and its potential clinical significance in gastrointestinal stromal sarcoma

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ABSTRACT

This study was designed to determine the level of survivin expression and its clinical significance as a prognostic factor in gastrointestinal stromal sarcoma (GIST). Twenty patients (12 males and 8 females) ranging in age from 25 to 72, with a median age of 53 were evaluated. Failure of TKI treatment was higher in the survivin-positive group ($p = 0.06$). The rate of metastasis was significantly higher in the survivin positive group vs. the negative group (80% vs. 30%, $p = 0.18$). The median overall survival (OS) time was 114 (range 29–199) months, and the median disease-free survival (DFS) time was 88 (range 40–135) months. The median progression-free survival (PFS) time was 40 (range 24–55) months. Further, a comparison of patients with survivin positive versus negative tumors, revealed no significant difference for OS, DFS, and PFS ($p = 0.45$, $p = 0.19$, $p = 0.55$, respectively), number of mitoses in 50 HPF ($p = 0.14$), and tumor size ($p = 0.94$).
In conclusion, survivin was highly expressed in GISTs, although we found no correlation between survivin expression and PFS, DFS and OS, survivin may be a predictive marker in GISTs for disease progression. We believe that additional studies are warranted to determine the clinical significance of survivin expression as a prognostic or predictive marker in patients with GIST.

1. Introduction

Gastrointestinal stromal tumors (GISTs) are rare, responsible for only 0.13% of all gastrointestinal malignancies, and constitute 80% of the gastrointestinal mesenchymal tumors [1,2]. They are generally observed in middle to older age individuals with median age of 60 at diagnosis. There is also no significant difference in sex distributions. In nearly 95% of cases, the primary tumor site is the gastrointestinal tract including the stomach (50–60%), small intestine (20–30%), large intestine (10%), esophagus (5%), and the mesentery, omentum, and retroperitoneum in <10% of cases [3]. GISTs exhibit a wide spectrum of clinical attributes and outcomes. While some GIST lesions progress rapidly, with 15% to 50% being metastatic at the time of diagnosis, others are asymptomatic and remain stable for many years being diagnosed only incidentally [4,5]. In addition, despite surgical resection, 40% to 80% of GISTs recur locally, or metastasize to the peritoneum and/or liver. The 5-year overall survival (OS) rate is approximately 50% after radical resection, and the median survival time was estimated to be 9 to 20 months for unresectable or metastatic GISTs prior to imatinib therapy [7]. Regional lymph node metastasis and extra-abdominal metastases are rare [6]. Approximately 90% to 95% of GISTs express the Kit protein, a trans-membrane receptor for stem cell factor with the intracytoplasmic portion functioning as a tyrosine kinase [8,9]. GISTs are characterized by gain-of-function mutations in the Kit proto-oncogene, most commonly involving the exon 11, but in other cases involving exon 9, 13, or 17 [10]. Development of platelet-derived growth factor receptor (PDGFRa) mutations in GISTs without Kit mutations seems to be another alternative oncogenic mechanism [9]. Prior to the development of imatinib, the primary treatment for GIST was surgery, but in these patients recurrence was common and survival was poor [1,3]. Since the development of TKIs, such as imatinib, sunitinib, and nilotinib, outcomes have improved significantly, and studies are ongoing which may help to determine [3] the role of TKIs in adjuvant and neoadjuvant settings. It is hoped that these future studies will help in the development new agents for successful therapies against drug-resistant GISTs [1]. In recent years, research has helped us to understand the mechanisms of cancer development. One mechanism under study is apoptosis [10,11], which is regulated by numerous genes [12,13], including survivin, which is an anti-apoptotic protein that suppresses apoptosis by the inhibition of caspase-3 and caspase-7 activity. Survivin is

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also cell-cycle regulated and expressed in the G2/M phase of the cell cycle, interacting with mitotic spindle tubules and increased caspase-3 activity. In addition, over-expression of survivin has oncogenic potential as it may overcome the G2/M phase checkpoint to enforce cell progression through mitosis, and initiate development of neoplastic clones [14,15]. In most normal adult tissues, survivin is undetectable, but is detectable in fetal tissue from a variety of human cancer tissues [16]. In some studies, the over expression of survivin has been correlated with more aggressive clinical behavior, including liver, colorectal, breast, lung, esophageal, and gastric cancers [17-24]. Defects in apoptotic mechanisms also play a crucial role in resistance to chemotherapy and radiotherapy and it is here that survivin may serve as a diagnostic marker and potential drug target [18,25,26]. In this study, we determined the expression of survivin in tumor tissue from GIST patients and examined the prognostic and significance importance of this marker.

2. Materials and method

This study included a retrospective analysis of 20 patients (12 males and 8 females) who were diagnosed with GIST at Gazi University Medical Faculty Hospital, from June 1998, through January 2007. All patients were evaluated according to age, sex, clinical symptoms, tumor localization, presence of metastasis and region, risk groups according to tumor size, and number of mitoses. The project was approved by the hospital ethics committees.

2.1. Immunohistochemistry

Immunohistochemical (IHC) staining was performed on 4 μm sections of the parafine-embedded tissue blocks. The antigen was retrieved with 0.01 M citrate buffer (pH 6.0) by heating the sample in a microwave oven at a controlled final temperature of 600 C for 20 min. Endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide. The sections were then incubated for 2 h and 20 min at room temperature with primary antibody survivin Ab-5 (rabbit polyclonal antibody-Ig G, Lab Vision) and then secondary antibody (Multi-species Ultra Streptavidin detection system-HRP; Signet, Massachusetts, USA), respectively. Bound antibodies were visualized using a streptavidinbiotin-peroxidase complex-binding technique and AEC (3 Amino-9 Ethylcarbazol) was used as the chromogen counterstained with hematoxylin. As positive controls, immunostaining was performed on normal gastric mucosa tissue. Cells were considered positive for survivin when immunoreactivity was clearly observed in their cytoplasm or nuclei. The cases with positive cells less than 5% were considered as negative and those with positive cells ≥ 5% were considered as positive.

2.2. Statistical analysis

Statistical analyses were performed using SPSS 15.0 software. The study variants were analyzed on the basis of the One-Sample Kolmogorov–Smirnow test according to normal distribution. The data conforming to normal distribution are given with their arithmetic means and standard deviations, and the nonconforming data are provided with their median values. The X^2, Fischer–Exact, and Mann–Whitney U tests were used in the statistical evaluation. The impact of survivin status on survival was investigated by log rank test. Kaplan–Meier curves were drawn for survival estimated. $P<0.05$ was statistically accepted as significant.

3. Results

Among the 20 GIST patients in this series, symptoms and findings included abdominal pain (n = 10, 50%), melena (n = 8, 40%), and drowsiness (n = 2, 10%). Tumor sites included the small intestine (n = 11, 55%), stomach (n = 7, 35%), and the mesentery (n = 2, 10%). At the time of diagnosis, 5 patients had metastatic disease, and 5 patients who did not exhibit metastatic disease developed liver metastases by the time of follow-up. The most common site of metastasis was the liver (90%). Only one patient had peritoneal metastasis. All patients were treated surgically, 18 (85%) of whom had their tumors resected with curative intent and 2 of whom received debulking surgery. Four patients (20%) had positive surgical margins and during surgery, 8 patients (40%) had lymph nodes removed, although only one lymph node from single patient was positive. The histological subtypes, included spindle-cell GISTs in 15 patients, and mixed-cell GISTs in 5 patients. There were no epithelioid GISTs observed. The median tumor size was 9.96 cm (range 4–30 cm) that had a median 6.2 (range 0–22) mitosis per 50 high-power microscopic fields (HPF). A risk assessment was undertaken based on tumor size and the number of mitosis, with 9 patients (45%) in the middle risk group, 8 (40%) in the high risk group, and 3 (15%) in the low risk group. The tumors in all patients were positive for the stem cell factor receptor (CD 117) and cytoplasmic, but not nuclear expression of survivin observed in the tumors of 10 patients, (Fig. 1). The clinical and pathological characteristics of survivin-positive and negative cases are shown in Table 1.

After surgical intervention, 11 patients received imatinib due to relapse and/or progressive disease, 5 patients who developed progressive disease following imatinib treatment were given sunitinib, and 2 patients were given nilotinib because of progressive disease following sunitinib treatment. Five patients who had progressive disease despite treatment with TKIs were all survivin positive. The TKI treatment (imatinib) rate of failure was higher in the survivin-positive group ($p = 0.06$), although this was not a statistically significant observation (Fig. 2). Five patients developed metastasis at follow-up who did not have metastasis at diagnosis, and all but one were survivin positive. Seven of the 10 patients with metastatic disease were survivin positive, and the rate of metastasis was higher in the survivin positive group compared to the survivin negative group (80% vs. 30%, $p = 0.18$) (Fig. 3).

Four patients died due to disease progression, and 1 patient was lost during the follow-up phase. The median OS time was 114 (%95 CI, 29–199) months, the median DFS time was 88 (%95 CI, 40.0–135) months, and the median PFS was 62 (%95 CI, 30.9–93) months. There was no significant difference for OS (Fig. 4), DFS (Fig. 5), PFS (Fig. 6) ($p = 0.45$, $p = 0.26$, $p = 0.19$, respectively), number of mitoses in 50 HPFs ($p = 0.14$), and tumor size ($p = 0.94$) between survivin positive and negative patients, likely due to the small patient population.

4. Discussion

GISTs are the most common type of sarcoma in the gastrointestinal tract, and surgery is the primary treatment, although, many patients still develop recurrences and/or metastasis [27]. While some lesions progress rapidly, and 15% to 50% of GISTs have metastases at the time of diagnosis, others are asymptomatic remaining stable for many years and are diagnosed only incidentally [4,5]. Treatment with the TKIs imatinib and sunitinib has greatly enhanced patient OS rates; however, the emergence of drug-resistant tumors has limited the long-term benefit of these drugs in most patients [27,28].

Survivin is a well-known apoptosis inhibitor and is a bi-functional protein that suppresses apoptosis and regulates cell division. Over-expression of survivin has oncogenic potential [14,15] and it is widely expressed in fetal tissues and human cancers, but generally not in normal adult tissues except in placentae and thymi. It is also expressed in cancer cell lines, including some lung, colon, gastric, breast, prostate, and pancreatic lines, as well as in neuroblastoma and lymphoma. As a tumor marker, survivin has been a focus for many scientists [29]; however, data within the literature concerning the prognostic significance of survivin is controversial. Some of the literature reports that an increase in nuclear survivin expression