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The high stromal SPARC expression is independently associated with poor survival of patients with resected pancreatic ductal adenocarcinoma treated with adjuvant gemcitabine in combination with S-1 or adjuvant gemcitabine alone

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ABSTRACT

Background: Although postoperative adjuvant chemotherapy for pancreatic ductal adenocarcinoma (PDAC) improves survival, its efficacy varies among individuals. Identification of biomarkers that can predict the efficacy of adjuvant chemotherapy for PDAC is essential.
Objectives: To investigate the predictive value of secreted protein acidic and rich in cysteine (SPARC) expression in patients with PDAC treated with adjuvant gemcitabine in combination with S-1 (adjuvant GS) or adjuvant gemcitabine alone (adjuvant G alone).
Methods: Stromal SPARC and cytoplasmic SPARC were examined immunohistochemically in 211 PDAC patients treated with adjuvant GS or G alone after resection. The association of SPARC expression with clinicopathological factors, disease-free survival (DFS) and overall survival (OS) were analyzed.
Results: In multivariate analysis, borderline resectable with arterial contact (BR-A) ($P = .002$), higher preoperative CA 19-9 level (≥91 U/ml) ($P = .005$), moderately or poorly ($P = .003$), presence of lymph node metastasis ($P = .012$) and high stromal SPARC expression ($P = .013$) were independent predictors of poor DFS. Moreover, BR-A ($P = .003$), higher preoperative CA 19-9 level (≥91 U/ml) ($P = .007$) and high stromal SPARC expression ($P < .001$) were identified as independent predictors of poor OS. In contrast, cytoplasmic SPARC expression did not affect DFS and OS.
Conclusions: High stromal SPARC expression was an independent predictor of poor DFS and OS in patients treated with adjuvant GS or G alone. Stromal SPARC expression could be a relevant biomarker for prediction of prognosis in PDAC patients after resection treated with adjuvant GS or G alone.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and devastating oncological diseases with an overall 5-year survival rate of less than 6% for patients with resectable and unresectable disease [1]. Multidisciplinary therapeutic strategies combined with surgical resection have recently been shown to result in improved prognosis in patients with resectable PDAC, leading to increased 5-year survival rates of 20–27% [2,3]. Adjuvant gemcitabine in combination with S-1 (adjuvant GS) or adjuvant gemcitabine alone (adjuvant G alone) has become the standard regimen of adjuvant chemotherapy for PDAC after the survival benefit of this approach was proven in large-scale randomized controlled trials [4–7]. However, the efficacy of adjuvant GS or G alone among individuals varies; therefore, it is important to identify biomarkers that can predict prognosis and chemosensitivity.

Recently, researchers have shown increasing interest in the role of the peritumoral stroma and tumor-stroma interactions in PDAC [8]. The dense desmoplastic stroma is considered a potential barrier against anticancer drugs. Secreted protein acidic and rich in cysteine (SPARC) is a calcium-binding protein that interacts with the extracellular matrix and has been reported to influence cell migration, proliferation, angiogenesis, matrix cell adhesion, and

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tissue remodeling [9–13]. SPARC has been reported as being overexpression in PDAC compared with normal tissue and to be associated with aggressive tumor behavior including tumor growth, metastatic spread and resistance to chemoradiotherapy [14,15]. Some reports have shown that stromal and/or cytoplasmic SPARC expression is associated with prognosis after surgical resection of PDAC [16–18]. However, the predictive value of SPARC expression for the efficacy of adjuvant GS or G alone in patients with PDAC has not been reported.

Accordingly, the aim of this study was to investigate whether SPARC expression could predict survival in patients with PDAC treated with adjuvant GS or G alone.

Materials and methods

Study design

This was a retrospective single institutional study based on a prospectively maintained institutional database of all PDAC patients. Of consecutive patients with PDAC who underwent surgical resection with curative intent (R0 or R1 resection) at the Department of Surgery, Hiroshima University Hospital, Hiroshima, Japan between January 2000 and December 2014, patients treated with adjuvant GS or G alone after resection were enrolled in this study. Patients with distant metastasis were excluded from this analysis, however, those with para-aortic lymph node metastasis, which was diagnosed by postoperative histologic examination and not by preoperative imaging examinations, were included. All patients had a confirmed pathological diagnosis of PDAC. Dissection of regional lymph nodes and para-aortic lymph nodes was routinely performed for all patients. Each tumor was classified as well-differentiated, moderately differentiated, or poorly differentiated adenocarcinoma according to the predominant histology. Tumor stage, lymph node metastasis, and final stage were assessed according to the seventh edition of the International Union Against Cancer (UICC) tumor-node-metastasis classification [19]. Resectability status of each tumors ware assessed based on the resectability status of the National Comprehensive Cancer Network (NCCN) 2016, version 2 [20]. Notably, borderline resectable tumors were classified into two groups. Tumors that had tumor contact with the portal vein or super mesenteric vein of $>180^{\circ}$ or thrombosis of the vein but with suitable vessel proximal and distal to the site of involvement, allowing for safe and complete resection and vein reconstruction were defined as borderline resectable with portal vein or superior mesenteric vein contact (BR-PV) group. Also, tumors that had tumor contact with the superior mesenteric artery of $\leq 180^{\circ}$ or tumor contact with the common hepatic artery without extension to the celiac axis or hepatic artery bifurcation, allowing for safe and complete resection and reconstruction were defined as borderline resectable with arterial contact (BR-A) group. Pancreatic body tumors with tumor contact with the celiac axis of $\leq 180^{\circ}$ or tumor contact with the celiac axis of $>180^{\circ}$ without involvement of the aorta and with intact and uninvolved gastroduodenal artery were also defined as BR-A group. The study protocol was consistent with the recommendations of the Declaration of Helsinki and was approved by the Institutional Review Board of Hiroshima University. All patients provided informed consent for participation in the study.

Immunohistochemical analysis of SPARC expression

Sections of the formalin-fixed paraffin-embedded tissue including the biggest volume of cancer cell the pathologist diagnosed were selected for use in a tissue microarray. To evaluate SPARC expression, we used an affinity-purified monoclonal mouse antibody (clone ON1-1) against SPARC (Takara Bio Inc., Otsu, Japan). Tissue sections cut as 4-μm-thick from formalin-fixed, paraffin-embedded tissue blocks were deparaffinized and rehydrated. Following antigen retrieval by autoclaving (121 °C for 10 min in 0.01 M citrate buffer), sections were immersed in methanol containing 3% hydrogen peroxide for 15 min and incubated in protein blocking solution (Dako, Carpinteria, CA, USA) for 10 min. Sections were incubated with appropriate dilutions of the anti-SPARC antibody (final concentration, 4 μg/mL) for 60 min at room temperature. Then, samples were incubated in labeled streptavidin-biotin polymer (Envision Plus; Dako) at room temperature for 60 min as a secondary antibody. The samples were immersed for 10 min in 0.01% 3,3-diaminobenzidine solution in 50 mM Tris-HCl buffer with 10 mM hydrogen peroxide as a substrate. Sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. Negative control samples consisted of sections incubated without the primary antibodies.

Immunohistochemical evaluation of cytoplasmic SPARC in PDAC cells and peripheral stromal SPARC expression was carried out by 2 observers (R.S. and N.K.) in a blinded manner. According to previous reports [16], the staining intensity was scored as follows; grade 0 (no staining), grade 1 (weak staining, light yellow), grade 2 (moderate staining, yellow brown), grade 3 (strong staining, brown). The samples were considered as having high stromal or cytoplasmic expression if grade 2 or 3 staining was observed over 10%. Samples that did not fulfill these criteria were considered to have low stromal or cytoplasmic expression (Fig. 1).

Adjuvant gemcitabine-based chemotherapy

During the study period, adjuvant GS or G alone, including gemcitabine alone and gemcitabine in combination with S-1, was administered to patients who met the following institutional criteria: older than 20 years, an Eastern Cooperative Oncology Group performance status of 0–1, adequate bone marrow reserve (white blood cell count $>3000/mm^3$, platelet count $>100{,}000/mm^3$, hemoglobin level >8 g/dL), and adequate renal (serum creatinine concentration <1.5 mg/dL) and liver function (total serum bilirubin concentration <3 mg/dL). Patients who received postoperative adjuvant chemotherapy had two options after resection: intravenous chemotherapy alone or intravenous and oral chemotherapy. The selection of gemcitabine alone or gemcitabine in combination with S-1 was determined based on the physical condition of each patient. The regimens of these chemotherapies were reported previously [4]. Postoperative adjuvant chemotherapy was started between 2 and 6 weeks after surgery. Intravenous chemotherapy consisted of gemcitabine 700 mg/m2 administered biweekly for 30 min by intravenous drip infusion. Patients who received intravenous and oral chemotherapy were given intravenous gemcitabine 700 mg/m2 on day 1 and oral S-1 50 mg/m2 for 7 consecutive days, followed by a 1-week pause in chemotherapy. Patients received 10 cycles of adjuvant chemotherapy every 2 weeks. Toxicity was assessed according to the common toxicity criteria adverse event (CTCAE) version 4.0. An additional course was delayed if toxicity of grade 3 or 4 was observed or if the patient's condition did not improve sufficiently to fit eligibility criteria. Neither external-beam radiation nor intra-operative irradiation was administered to any of the patients. Patients who had to switch to other chemotherapies due to recurrent disease before the 10 cycles were included in the adjuvant GS or G alone group. Patients who received gemcitabine-based chemotherapy because of recurrent disease after completion of adjuvant GS or G alone were also included in this study.

Postoperative follow-up

All patients were followed up regularly by blood testing and

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