Targ Oncol (2013) 8:231–235
DOI 10.1007/s11523-012-0239-4

ORIGINAL RESEARCH

High expression of epithelial cellular adhesion molecule in peritoneal metastasis of gastric cancer

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Received: 16 May 2012 / Accepted: 5 November 2012 / Published online: 17 November 2012

Abstract Intraperitoneally administrated epithelial cellular adhesion molecule (EpCAM) monoclonal antibody is a therapeutic agent in patients with malignant effusion in several types of carcinoma. However, the role of EpCAM in peritoneal metastasis (PM) lesions and primary lesions of gastric cancer (GC) is still unclear. Therefore, in this study, we investigated EpCAM expression in GC patients with PM. We investigated the expression of EpCAM in 35PM lesions and 104 biopsy samples as primary lesions. Immunohistochemical staining was performed using the Ventana Benchmark XT (Roche Diagnostics) system. EpCAM expression was evaluated by calculating the total immunostaining score, which is the product of the proportion score and the intensity score. Overexpression was defined as a total score greater than 4. All PM specimens showed overexpression of EpCAM, and GC cells in both the surface layer and the deep layer of the PM showed a high expression of EpCAM. Meanwhile, in the biopsy sample, the expression of EpCAM ranged from none to strong. The EpCAM score results for PM specimens and biopsy samples were 11.0±2.0 and 6.9±3.9, respectively. The difference between the scores was statistically significant ($P<0.05$). The intraperitoneally administrated EpCAM antibody might have a anti-cancer effect in PM lesions of GC. Additionally, it can be assumed that only GC cells which express a high level of EpCAM might metastasize to the peritoneum.

Keywords Gastric cancer · Peritoneal metastasis · Epithelial cellular adhesion molecule (EpCAM) · Target therapy

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Introduction

Gastric cancer (GC) is the second most common cause of cancer-related death worldwide [1]. Although surgery is the only curative procedure for localized advanced GC, for metastatic or recurrent GC patients, chemotherapy is the only therapeutic approach.

Recently, a number of new drugs to treat GC have become available. Unfortunately, these agents are not particularly effective, resulting in a high recurrence rate, a low survival rate, and a poor prognosis for metastatic or recurrent GC patients [2]. Additionally, GC patients with peritoneal metastasis (PM) have lower survival rates than other GC patients. In a multicenter prospective study, the median survival time was only 3.1 months for GC patients with PM [3]. Thus, another type of treatment for GC patients, particularly those with PM, is required. For example, target therapies that are associated with the expression of a particular gene may open up a new avenue for cancer treatments.

Table 1 Clinicopathological features of patients

Clinicopathological factors	No. of cases
Gender	
Males	25
Females	10
Average age (range), years	58.6 (22–75)
Borrmann type	
I	0
II	1
III	14
IV	20
Laurens system	
Intestinal type	8
Diffuse type	27
Number of biopsy samples	104

For histopathology typing, gastric cancers were classified as being intestinal or diffuse on the basis of the Laurens system

The epithelial cellular adhesion molecule (EpCAM) is a 39–42-kDa, 314-amino-acid type I transmembrane glycoprotein [4]. EpCAM is detected in the basolateral membrane of the majority of epithelial tissues, and overexpression of EpCAM has been demonstrated in a variety of epithelial cancers [5, 6].

EpCAM has been reported to have effects on cell adhesion, signaling, migration, proliferation, and differentiation, each of which are properties related to metastasis of several types of cancer [7]. In addition, an EpCAM monoclonal antibody, catumaxomab, has been licensed for clinical use in the European Union since 2009 for the intraperitoneal treatment of malignant effusion in patients with EpCAM-positive cells where standard therapy is not available or no longer feasible. Heiss et al. have reported that catumaxomab conferred a puncture-free survival in a prospective randomized phase II/III trial [8]. Furthermore, a subsequent analysis of the report by Heiss et al. revealed that catumaxomab had a significant overall survival benefit to GC patients [9]. However, the expression of EpCAM on the primary lesions and PM lesions of GC is still unclear. Therefore, in this study, we investigated EpCAM expression in GC patients with PM.

Materials and methods

Surgical specimens

Biopsy samples and specimens of PM were obtained from 35 GC patients during upper gastrointestinal endoscopy and staging laparoscopy conducted in our department between 2008 and 2011. All GC patients lacked non-curative factors, such as distant metastasis to liver, lung, or lymph nodes except for PM. In accordance with the Department of Surgery Kinki University Faculty of Medicine policy, written informed consent was obtained from the patients at the time of initial treatment.

Initial treatment

The initial treatment of these patients consisted of single intraperitoneal administration of paclitaxel followed by sequential systemic chemotherapy with S-1 plus paclitaxel. The details of the treatment regimen were described previously [10].

Immunohistochemical study

All sections were placed on the Ventana Benchmark XT (Roche Diagnostics) for detection of the EpCAM oncoprotein. The sections were dewaxed and then subjected to pretreatment with cell conditioning 1 solution (Roche Diagnostics) for 30 min. Sections were then washed with reaction buffer followed by incubation with the mouse monoclonal primary antibody EpCAM (0.1 μg/mL, Vu1D9, Cell Signaling Technology, USA) for 32 min. On-board detection using ultraView Universal DAB kit (Roche Diagnostics), used in accordance with the manufacturer's instructions, was used to detect the location of the primary antibody EpCAM.

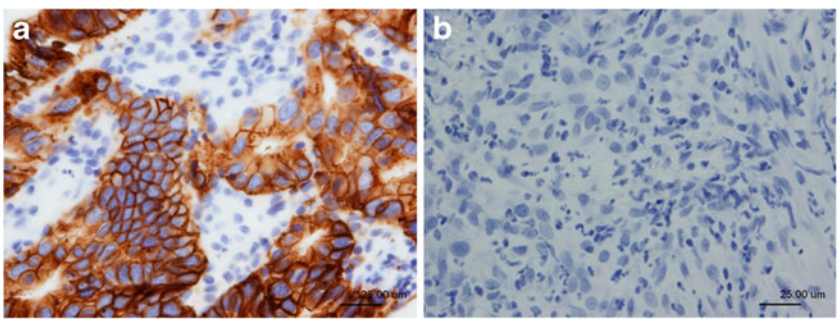

Fig. 1 EpCAM expression in a biopsy sample of gastric cancer. **a** Strong reactivity of EpCAM was visible in most gastric cancer cell membranes in biopsy samples. A representative samples with a score of 12 is shown. **b** Representative sample of gastric cancer cells in a biopsy sample with no reactivity of EpCAM (scored as 0). *EpCAM* epithelial cellular adhesion molecule

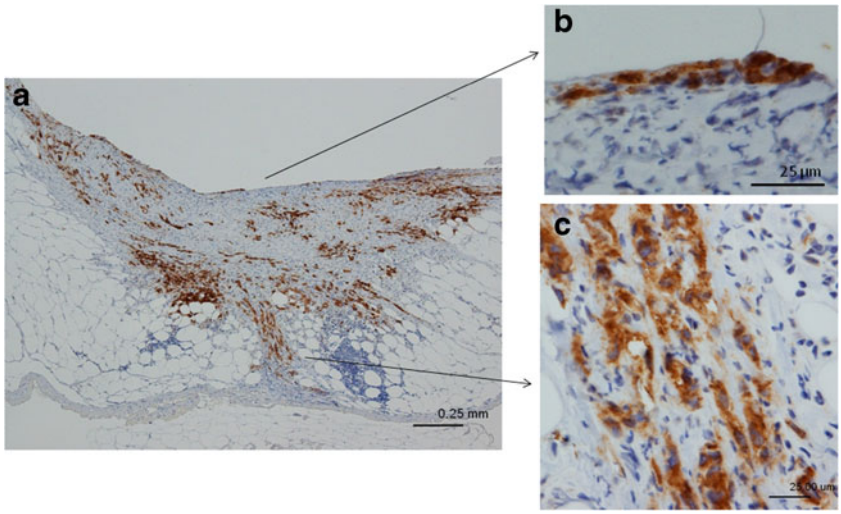

Fig. 2 EpCAM expression of gastric cancer cells in a peritoneal metastasis lesion. **a** High expression of EpCAM is observed in most gastric cancer cells in the peritoneum, scored as 12. **b** Gastric cancer cells show a high expression of EpCAM in the surface layer of the peritoneum. **c** Gastric cancer cells also show a high EpCAM expression in the deep layer of the peritoneum. EpCAM reactivity shows the membrane and cytoplasm of tumor cells. *EpCAM* epithelial cellular adhesion molecule

Immunohistochemical analysis

EpCAM expression was evaluated by calculating the total immunostaining score, which was defined as the product of the proportion score and the intensity score. EpCAM expression was evaluated by the following formula [11]: the proportion score described the estimated fraction of positively stained tumor cells (0, none; 1, <10 %; 2, 10–50 %; 3, 50–80 %; 4, >80 %). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). The total score ranged from 0 to 12. EpCAM overexpression was defined as a total score greater than 4 [12].

Statistical analyses

The statistical software GraphPad Prism 5 (GraphPad Software Inc, USA) was used to analyze data by Fisher's exact test. A difference of $P<0.05$ was considered as significant.

Results

Patient characteristics

The patients had a median age of 58.6 years (range 22–75 years). There were ten female and 25 male patients. Borrmann III and IV types accounted for the majority. The details of the main clinicopathological features of patients are presented in Table 1. The median survival time of the 35 patients was 23.4 months.

Expression of EpCAM in biopsy samples of gastric cancer

EpCAM expression in 104 biopsy samples from 35 GC patients was determined with immunohistochemical staining. On average, we investigated 2.97 biopsy samples per patient. EpCAM was located on the membrane of GC cells. We observed a diverse range of EpCAM expression intensities. The staining scores of EpCAM ranged from 0 to 12, with an average score of 6.9±3.9. Eighty samples showed overexpression of EpCAM. Figure 1a, b shows representative cases.

Expression of EpCAM in PM of gastric cancer

EpCAM expression in 35PM specimens from 35 GC patients was determined with immunohistochemical staining. EpCAM was located not only on the membrane; diffuse staining was also found in the cytoplasm. Strongly positive-staining tumor cells were found in both the surface layer and the deep layer of the peritoneum. The resulting staining scores of EpCAM ranged from 8 to 12, with an average score of 11.0±2.0. All PM specimens were classified as having EpCAM-overexpressing tumors. Figure 2 shows a representative case.

A significant difference in immunoreactive intensity and average staining score of EpCAM was found between the PM specimens and the biopsy samples ($P<0.05$; Table 2).

Discussion

Between 70 and 100 % of tumor cells in malignant effusions from gastric, ovarian, breast, and colorectal cancer have

Table 2 Overexpression of EpCAM in PM lesions and biopsy samples

	EpCAM overexpression		*P*
	Positive	Negative	
PM lesions	35	0	0.004
Biopsy samples	80	24	

EpCAM epithelial cellular adhesion molecule, *PM* peritoneal metastasis

been found to express EpCAM [13–15]. However, the expression of EpCAM in PM lesions has not been defined. In our study, all specimens of PM with GC showed EpCAM overexpression. This is the first report to reveal these results.

In our study, the expression of EpCAM was stronger in the PM lesions than in the primary lesions. The expression of EpCAM in primary lesions was investigated in biopsy samples. The biopsy samples showed a wide range of EpCAM expression. Conversely, in the PM lesions, almost all GC cells showed a strong EpCAM expression. Furthermore, in vitro studies of EpCAM showed enhanced cell proliferation independent of c-myc and cyclin D_1/E [16, 17].

Additionally, it was reported that EpCAM negatively modulated cadherin-mediated cell adhesion by disruption of the link between α-catenin and F-actin [18]. Furthermore, EpCAM loosened the tight junctions between cells and modulated proliferation, differentiation, and tissue maintenance [19]. Similar phenomena have already been confirmed in breast and renal cancer [19]. In gastric cancer, overexpression of EpCAM might also disrupt cell–cell contact, enabling the cellular migration that is required for metastasis [19]. Thus, only GC cells whose proliferation was enhanced by EpCAM might metastasize to the peritoneum, as this is one of the most frequent metastatic sites of GC.

GC patients with PM have poorer survival outcomes than other GC patients [3]. To improve the survival rate of GC patients with PM, multidisciplinary methods, including intraperitoneal chemotherapy, hyperthermia, and aggressive surgery, have been used to treat PM [20] [21]. However, these trials did not result in a satisfactory clinical outcome. One of the reasons that PM resists multidisciplinary therapy is due to the stem cell characteristics of the cancer cells. Cancer stem cells are responsible for cancer relapse as they are resistant to conventional cancer therapy, such as chemotherapy and radiation [22, 23]. In our results, all PM specimens showed EpCAM overexpression. EpCAM expression is a biologically and clinically relevant characteristic of cancer stem cells from primary GC tissue [24].Therefore, GC cells in PM lesions may have stem cell-like characteristics. The very poor clinical outcomes in GC patients with PM are consistent with these findings.

To improve treatment outcomes of GC with PM, antibody-based cancer therapies are required. Catumaxomab, which is specific for the EpCAM target antigen, is used to treat cancer patients with malignant ascites in the European Union. The clinical benefit of catumaxomab administered by the intraperitoneal route was demonstrated by prospective randomized phase II/ III trials [8]. The antibody can deliver a deadly signal to the cancer cell only by binding to the surface target. However, it seems that the unsatisfactory antitumor effect of catumaxomab on disseminated lesions in the peritoneum is due to the limited penetration of intraperitoneal catumaxomab into the peritoneal surfaces. Additionally, in our study, GC cells in PMs that expressed EpCAM were present not only in the surface layer but also in the deep layer of the peritoneum. Therefore, intraperitoneally administered catumaxomab may only be effective to treat cancer cells in malignant ascites and in the surface layer of the peritoneum.

To further improve treatment outcomes, the investigation of combination therapies comprising systemic chemotherapy plus intraperitoneal catumaxomab and/or intravenously administered catumaxomab may be necessary. Further investigations are required in the future.

Acknowledgments The authors express their appreciation to Dr. Harumasa Ohyanagi, vice board director of the University of KinDAI Himeji for his expert comments on the manuscript. We also wish to thank Mr. Tadao Uesugi and Miss Fusako Kamada for technical assistance.

Conflict of interest The authors have no conflicts of interest to declare.

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