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Expression of chromosomal regional maintenance protein-1 may be associated with subcellular survivin expression in human gastric and colorectal carcinoma

MICHIKO SHINTANI[1], AKITO TASHIRO[1], AKIKO SANGAWA[2], NAOKI YAMAO[3] and SHINGO KAMOSHIDA[1]

[1]Laboratory of Pathology, Division of Medical Biophysics, Kobe University Graduate School of Health Sciences, Kobe, Hyogo 654-0142; [2]Department of Diagnostic Pathology, Osaka Red Cross Hospital, Osaka 543-8555; [3]Department of Clinical Laboratory, Kuma Hospital, Kobe, Hyogo 650-0011, Japan

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Abstract. Survivin, a member of the inhibitor of apoptosis protein family, is a potential prognostic marker and molecular target for anticancer therapies. Chromosomal regional maintenance protein-1 (CRM-1) mediates the nuclear export of proteins such as survivin. The aims of the present study were to compare the expression and subcellular localization of CRM-1 in human gastric and colorectal carcinomas and to assess the association between CRM-1 and survivin expression in these tumor types. The nuclear and cytoplasmic CRM-1 expression rates in gastric carcinoma were 61% (42/69) and 29% (20/69), respectively, while the nuclear and cytoplasmic CRM-1 expression rates in colorectal carcinoma were 55% (43/78) and 37% (29/78), respectively. Nuclear and cytoplasmic CRM-1 expression was found to be significantly correlated with nuclear and cytoplasmic survivin expression in colorectal carcinoma, but not gastric carcinoma. These results indicate that CRM-1 expression patterns differ between gastric and colorectal carcinomas and thus, we hypothesize that CRM-1-mediated nuclear export of survivin may be deregulated in gastric carcinoma. Therefore, CRM-1 may exhibit different functions in gastric and colorectal carcinoma.

Introduction

As a member of the inhibitor of apoptosis protein family, survivin resides in both the nucleus and cytoplasm of cells. Survivin controls both cell division (when localized to the nucleus) and cell survival (when localized to the cytoplasm) and thus may exhibit a dual function (1-3). In normal adult tissues survivin is usually undetectable, however it is overexpressed in the majority of human tumors, including lung, colon, breast, stomach and liver cancer (4-9). Due to its involvement in tumorigenesis, survivin has been proposed as a prognostic marker and a molecular target for anticancer therapies (10-13).

Survivin shuttles between the nucleus and cytoplasm, and several studies have demonstrated that chromosomal regional maintenance protein-1 (CRM-1) regulates its subcellular distribution (2,3,12,13). CRM-1, a member of the importin B superfamily, exports both proteins and mRNAs from the nucleus and is the main nuclear exporter in humans. It recognizes a leucine-rich nuclear export signal in proteins and exports proteins, including survivin, via the CRM-1/Ras-related nuclear protein-guanosine-5'-triphosphate axis (14-17). High CRM-1 expression has been identified in several malignancies, including stomach, pancreatic, ovarian, cervical, renal and esophageal cancers. Its presence in tumor cells indicates that it predicts an unfavorable prognosis (18-23).

At present, cancer is the primary cause of mortality in Japan (24). In 2011, a total of 132,000 and 125,000 cases of gastric and colorectal cancer were diagnosed, respectively (25). Furthermore, gastric and colorectal cancer accounted for 47,903 and 48,500 mortalities in 2014, respectively (25). Gastric cancer remains a significant cause of cancer-related mortality (24). Our previous study demonstrated that nuclear and cytoplasmic survivin expression was higher in colorectal carcinomas than gastric carcinomas (26). The levels of nuclear and cytoplasmic survivin are directly correlated in colorectal carcinoma, but not gastric carcinomas. However, the effect of survivin localization remains unclear and thus, further study of survivin/CRM-1 interaction with regard to tumorigenesis is required (27-29).

The aims of the present study were to compare the expression levels and intracellular localization of CRM-1 in human gastric and colorectal carcinomas and to investigate the association between CRM-1 and survivin expression. In this study, CRM-1 expression was assessed

Correspondence to: Dr Michiko Shintani, Laboratory of Pathology, Division of Medical Biophysics, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe, Hyogo 654-0142, Japan
E-mail: mtshin@kobe-u.ac.jp

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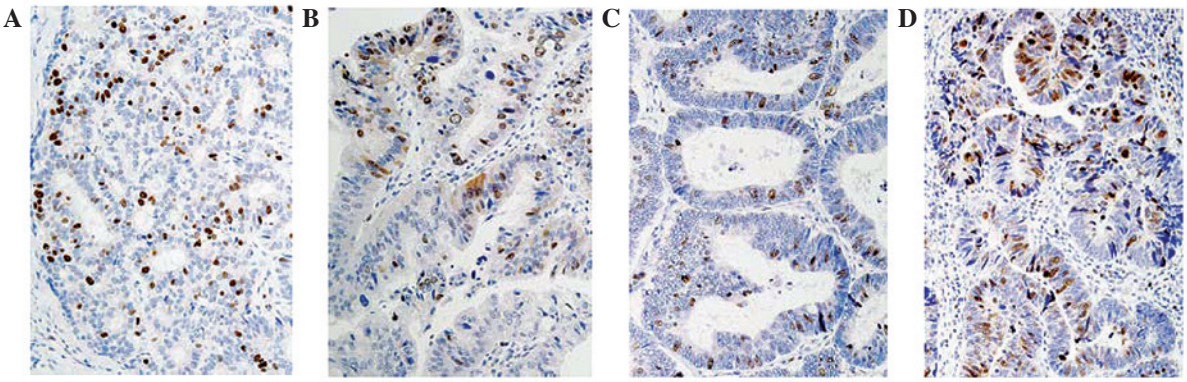

Figure 1. Immunohistochemical staining of CRM-1. Representative images of (A) nuclear CRM-1 and (B) cytoplasmic CRM-1 expression in gastric carcinoma, (C) nuclear CRM-1 expression in colon carcinoma and (D) cytoplasmic CRM-1 expression in rectal carcinoma (magnification, x200). CRM-1, chromosomal regional maintenance protein-1.

using immunohistochemistry and to the best of our knowledge, this method has not been used to detect this protein previously.

Materials and methods

Human tissue samples. A total of 69 advanced gastric adenocarcinoma specimens (33 intestinal type and 36 diffuse type) and 78 colorectal adenocarcinoma specimens (68 well- or moderately-differentiated and 10 poorly-differentiated) collected between April 2004 and October 2009 were obtained from the archives of the Department of Diagnostic Pathology, Osaka Red Cross Hospital (Osaka, Japan) and the Kobe Central Hospital of Social Insurance (Kobe, Japan). The histological typing was classified according to the Japanese Classification of gastric (30) or colorectal carcinoma (31). Histologically, papillary adenocarcinomas and tubular adenocarcinomas (well- or moderately-differentiated) were classified as intestinal type adenocarcinoma, whereas poorly-differentiated adenocarcinomas and signet ring cell carcinomas were classified as diffuse type adenocarcinoma. The median ages of the gastric and colorectal carcinoma patients were 72 (range, 41-94 years) and 67.5 years (range, 27-86 years), respectively. The gastric carcinoma group consisted of 48 male (70%) and 21 female (30%) patients, whereas the colorectal carcinoma group consisted of 44 male (56%) and 34 female (44%) patients. The present study was approved by the Ethics Committee of Kobe University Graduate School of Health Sciences (Kobe, Japan).

Immunohistochemistry. Formalin-fixed, paraffin-embedded surgically-resected tumor tissues were cut into 3-μm sections, and mounted on aminopropyltriethoxysilane-coated glass slides (Matsunami Glass, Osaka, Japan).

Slides were deparaffinized using xylene and dehydrated using a graded ethanol series. For antigen retrieval, the slides were immersed in 10-mM citrate (pH 6.0) and heated to >100˚C for 10 min in a pressure cooker (Groupe SEB, Écully, France). After heating, the sections were cooled at room temperature in soaking solution for 30 min and washed with running tap water followed by 10 mM phosphate-buffered saline (PBS; pH 7.2). After blocking in 0.25% casein in PBS (Dako, Glostrup, Denmark), the sections were incubated overnight in PBS (negative control) or PBS containing a rabbit anti-CRM-1 monoclonal antibody (dilution, 1:1,200; catalog no. ab77977; Abcam, Cambridge, UK) at room temperature. To detect CRM-1, the sections were rinsed with PBS and incubated with Histofine Simple Stain MAX-PO (Nichirei Biosciences Inc., Tokyo, Japan) for 1 h at room temperature. Reaction products were then developed with 3,3'-diaminobenzidine and counterstained with Mayer's hematoxylin (Nacalai Tesque, Kyoto, Japan).

Immunostaining evaluation. At least five randomly selected fields were examined (magnification, x400) to determine the mean percentage of tumor cells expressing nuclear and/or cytoplasmic CRM-1. Samples in which ≥30% of the tumor cells exhibited nuclear staining or in which ≥15% of the tumor cells exhibited cytoplasmic staining were considered positive for CRM-1 protein expression. These cutoff values were based on the median observed values. All slides were evaluated by two independent investigators blinded to the experimental design.

Statistical analysis. χ^2 test and Fisher's exact test were used to assess the CRM-1 expression levels of two groups. Spearman's rank correlation was used to assess correlations between two variables. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed with Statcel 3 (OMS, Tokyo, Japan).

Results

Expression of CRM-1 in gastric carcinoma. CRM-1 was predominantly localized to the nucleus in gastric carcinoma tissue samples (Fig. 1). Nuclear expression of CRM-1 was observed in 61% (42/69) of samples and cytoplasmic CRM-1 expression was observed in 29% (20/69) of samples (Table I). Nuclear CRM-1 was identified in 73% (24/33) of intestinal type tumors and 50% (18/36) of diffuse type tumors. Cytoplasmic CRM-1 was detected in 36% (12/33) of intestinal type tumors and 22% (8/36) of diffuse type tumors. No significant differences were identified between CRM-1 expression in intestinal type and diffuse type tumors.

Table I. Nuclear and cytoplasmic expression of CRM-1 in gastrointestinal and colorectal adenocarcinomas.

	CRM-1 expression	
Cancer type	Nucleus, n (%)	Cytoplasm, n (%)
Gastric adenocarcinoma (n=69)	42 (61)	20 (29)
Intestinal type (n=33)	24 (73)	12 (36)
Diffuse type (n=36)	18 (50)	8 (22)
Colorectal adenocarcinoma (n=78)	43 (55)	29 (37)
Well- to moderately-differentiated (n=68)	40 (59)	29 (43)
Poorly-differentiated (n=10)	3 (30)	0 (0)

Integers indicate the number of CRM-1-positive samples. CRM-1, chromosomal regional maintenance protein-1.

Table II. Correlation between CRM-1 and survivin expression in the nucleus and cytoplasm in gastric and colorectal carcinoma.

	Nuclear survivin		Cytoplasmic survivin	
Cancer type	r	P-value	r	P-value
Gastric carcinoma, total				
N-CRM-1	0.484	<0.001[a]	0.322	0.008[a]
C-CRM-1	0.192	0.113	0.127	0.296
Gastric carcinoma, intestinal type				
N-CRM-1	0.204	0.249	0.292	0.098
C-CRM-1	0.096	0.588	0.267	0.131
Gastric carcinoma, diffuse type				
N-CRM-1	0.649	0.001[a]	0.371	0.028[a]
C-CRM-1	0.245	0.147	0.078	0.645
Colorectal carcinoma, total				
N-CRM-1	0.338	0.003[a]	0.299	0.009[a]
C-CRM-1	0.254	0.026[a]	0.268	0.019[a]

[a]P<0.05. CRM-1, chromosomal regional maintenance protein-1; N-CRM-1, nuclear CRM-1; C-CRM-1, cytoplasmic CRM-1.

Expression of CRM-1 in colorectal carcinoma. Nuclear CRM-1 expression was observed in 55% (43/78) and cytoplasmic CRM-1 expression in 37% (29/78) of colorectal carcinoma samples (Table I). Nuclear CRM-1 was identified in 59% (40/68) of well to moderately-differentiated tumors and 30% (3/10) of poorly-differentiated tumors. Cytoplasmic CRM-1 was observed in 43% (29/68) of well to moderately-differentiated tumors and none of the poorly-differentiated tumors.

Association between CRM-1 and survivin expression in gastric and colorectal carcinomas. The correlation between nuclear or cytoplasmic CRM-1 expression and nuclear or cytoplasmic survivin expression in gastric carcinoma (intestinal type and diffuse type) and colorectal carcinoma was evaluated (Table II). In intestinal type gastric carcinoma, no correlations were identified between any parameters. In diffuse type gastric carcinoma, nuclear CRM-1 expression was significantly correlated with nuclear (r=0.649; P<0.001) and cytoplasmic (r=0.371; P=0.028) expression of survivin. In colorectal carcinoma, nuclear and cytoplasmic CRM-1 significantly correlated with nuclear and cytoplasmic survivin (nuclear CRM-1 and nuclear survivin, r=0.338; P=0.003; nuclear CRM-1 and cytoplasmic survivin, r=0.299; P=0.009; cytoplasmic CRM-1 and nuclear survivin, r=0.254; P=0.026; cytoplasmic CRM-1 and cytoplasmic survivin, r=0.268; P=0.019).

Discussion

Previous studies have implicated CRM-1 in carcinogenesis. CRM-1 expression has been found to correlate with a more aggressive tumor status and it has been suggested that CRM-1 may have prognostic value in gliomas, osteosarcomas, ovarian cancers and pancreatic cancers (18,20,32,33). Previous studies have also demonstrated an association between CRM-1 expression and resistance to chemotherapy and thus, potent inhibitors of CRM-1 are currently being developed for use in cancer therapy. At present, members of the CRM-1 inhibitor

family are novel therapeutic targets in renal cell carcinoma, non-small cell lung carcinoma and lymphoma (19,34-36).

The present study compared CRM-1 expression in gastric and colorectal carcinomas. To the best of our knowledge, this is the first study to report a correlation between CRM-1 and survivin expression using immunohistochemistry.

CRM-1 is predominantly found in the nucleus. In the present study, no significant differences in the nuclear or cytoplasmic expression rate of CRM-1 were identified between gastric and colorectal carcinomas. Furthermore, no significant differences in CRM-1 expression rate were identified between gastric carcinomas classified according to tumor type (differentiation status), although expression rates in both the nucleus and cytoplasm were higher in intestinal type gastric carcinomas than diffuse type gastric carcinomas. Zhou *et al* (23) reported that CRM-1 expression did not correlate with tumor differentiation status (poor vs. well to moderate). The authors also reported a higher CRM-1 expression rate in well to moderately-differentiated gastric adenocarcinomas compared with poorly-differentiated gastric adenocarcinomas (23). These results are consistent with those of the present study and suggest that no association exists between CRM-1 expression and tumor differentiation status in gastric carcinoma. A previous immunohistochemical analysis revealed that high CRM-1 expression was correlated with histological differentiation in esophageal squamous cell carcinoma but not pancreatic adenocarcinoma (18,22). The association between CRM-1 expression level and histological tumor differentiation remains unclear. In the present study, the CRM-1 expression rates in well or moderately versus poorly-differentiated colorectal carcinomas could not be investigated accurately due to the large difference in sample sizes in each group.

In the present study, the association between CRM-1 and survivin expression in both the nucleus and cytoplasm was also investigated. In colorectal carcinoma, these parameters correlated in all expression patterns (nuclear and nuclear, cytoplasmic and cytoplasmic, and nuclear and cytoplasmic). In gastric carcinoma, no association between cytoplasmic CRM-1 expression and nuclear or cytoplasmic survivin expression was identified, whereas nuclear CRM-1 expression was found to correlate with nuclear or cytoplasmic survivin expression in diffuse but not intestinal type gastric carcinoma. These results indicate that CRM-1 expression patterns differ in gastric and colorectal carcinomas and suggest that CRM-1-mediated nuclear export of survivin may be deregulated in gastric carcinoma.

Recent studies have demonstrated that the survivin/CRM-1 axis exhibits an important function in regulating cell division and cell survival (1-3). Survivin shuttles proteins between the nucleus and cytoplasm (37,38). When highly expressed in the cytoplasm, survivin may protect cancer cells by inhibiting apoptosis. In the nucleus, survivin predominantly associates with borealin, aurora B kinase and inner centrosome protein to form the chromosome passenger complex, which controls multiple processes during mitosis and is essential for genomic stability. Several studies have revealed that survivin is upregulated in the cytoplasm of cancer cells, where it prevents apoptosis and promotes tumor progression (3-9,13). Elucidation of the mechanism by which CRM-1 modulates survivin expression in several tumor types would be of great importance, and clarification of CRM-1 expression patterns may aid in the selection of effective anticancer agents.

In conclusion, the present study revealed that CRM-1 is involved in survivin expression in gastric and colorectal carcinomas. We hypothesize that CRM-1 exhibits different functions in gastric and colorectal carcinoma. Further studies are required to determine whether CRM-1 expression is a prognostic predictor in these tumor types.

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