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Expression of reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) as a prognostic indicator in gastric cancer

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ABSTRACT

In this study the expression levels of reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) in gastric cancer cell lines and tissues have been analysed in order to assess their value as a prognostic indicator. The expressions of RECK, activated matrix metalloproteinase (MMP)-7, and vascular endothelial growth factor (VEGF) in gastric cancer tissues and cell lines were evaluated by Western blot analysis; and MMP-2 and MMP-9 were evaluated by gelatin zymography. RECK expression in the context of gastric cancer was also compared with various clinicopathologic parameters and compared to the expression of activated MMP-7, MMP-2, and MMP-9. Fifty-two percent of the 102 gastric cancer tissues and 81.8% of the 11 gastric cancer cell lines exhibited reduced RECK expression. We also detected a significant inverse correlation between RECK expression and macroscopic tumour growth ($P = 0.018$), lymphatic invasion ($P = 0.018$), lymph node metastasis ($P = 0.000$), stage ($P = 0.000$), and MMP-9 ($P = 0.039$). No correlation between RECK expression and MMP-7 and MMP-2, VEGF were detected. Our data strongly supports the hypothesis that RECK is a suppressor of malignancy, and constitutes a good prognostic indicator in gastric cancer.

1. Introduction

The gene for reversion-inducing-cysteine-rich protein with Kazal motifs (*RECK*) was initially isolated using an expression cloning strategy, which was designed to detect transformation-suppressor genes against activated *ras* oncogenes [1]. Also, the *RECK* gene is expressed widely in a variety of normal human tissues and non-neoplastic cell lines, and its expression is either low or undetectable in oncogene-transformed fibroblasts and many tumour-derived cell lines [2,3]. Previous experimental studies have revealed that *RECK* is able to inhibit tumour angiogenesis, invasion, and metastasis [2–5]. It also inhibits matrix metalloproteinase (MMP)-9, MMP-2, and MT1-MMP (MMP-14) secretion and activity, via an as-yet-unknown mechanism [2,4]. Vascular endothelial growth factor (VEGF) is considered a key mediator of tumour angiogenesis, including neovascularization in human cancer. Several VEGF isoforms are produced from a single gene as a result of alternative splicing [6]. The isoforms differ in their biological properties and in their abilities to bind heparan sulfate proteoglycans [7]. VEGFs are bioactive as freely diffusible proteins in the extracellular space where they become available to

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endothelial cells, and in one report it has been suggested that soluble isoforms VEGF 121 or VEGF 165 have greater angiogenic and tumourigenic properties than the heparin-bound form [8]. In support of this hypothesis, RECK expression in a variety of human tumours, as compared with normal tissues, was correlated with prolonged survival in patients suffering from hepatocellular carcinoma [9], pancreatic cancer [10], breast cancer [11], non-small cell lung cancer [12], and colorectal cancer [13].

Gastric cancer is the second most common cause of cancer death in the world, and is also one of the most common cancers in East Asia and South America, although its incidence in Western Europe and North America is now declining. Most patients who are diagnosed with gastric cancer exhibit advanced disease, and prognoses are extremely poor, with survival rates rarely exceeding 50%. However, patients suffering from cancers which are limited to the mucosae and submucosae, namely early gastric cancer (EGC), have a five-year survival rate of approximately 95%. Therefore, the management of gastric cancer is intimately dependent on tumour stage. In addition, lymph node metastasis is also very important, even in patients with EGC. Five-year survival rates of the node negative and positive EGC patients are significantly different, with a 93–99% survival rate for the node-negative patients, and a 73–90% survival rate for the node-positive patients [14]. It is therefore important to delineate a novel set of parameters which can be used to target lymph node metastasis and staging.

In this study, we have analysed RECK expression levels in gastric cancer cell lines and tissues, in order to assess their status in gastric cancer tissues, and their value as prognostic indicators. We have also analysed MMP-7 activation, and compared the data with those for RECK expression. Our results indicate that the level of RECK expression in gastric cancers correlates inversely with certain metastasis-related clinicopathological and molecular features.

2. Materials and methods

2.1. Cell lines and tissue samples

The following human gastric caner cell lines were studied: SNU1, SNU5, SNU16, SNU620, SNU601, SNU638, SNU668, M1, M28, M45, and M74. All cancer cell lines were cultured in RPMI 1640, supplemented with 10% FBS, 1 mM $NaCO_3$, 2 mM L-glutamine, penicillin–streptomycin, in a 5% CO_2 atmosphere, at 37 °C. 102 patients with gastric adenocarcinoma, who had undergone curative surgery (total or subtotal gastrectomy with tumour-free resection margins, with regional lymphadenectomy), were included in this study. Patients suffering from other gastric malignancies, including malignant lymphoma and stromal tumour, were excluded from the study. The resected specimens were measured routinely, examined grossly, and dissected from the representative tumour and non-tumour areas for western blot analysis. The remaining samples were then fixed in 10% neutrally buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin for pathologic diagnosis. The gross gastric cancer types were determined by Borrmann's classifications. For this study, we adhered to the above classifications, with minor modifications: infiltrative growth, Borrmann types III and IV; and non-infiltrative growth, Borrmann types I and II. For the microscopic examinations, however, we adhered to Lauren's classifications. Gastric cancers were staged according to the recent TNM staging system approved by the UICC [15] and the following clinicopathological features were recorded: tumour size, histologic tumour grade, and depth of tumour invasion.

2.2. Western blot analysis

Tissues or cells were frozen in liquid nitrogen, extracted with lysis buffer (0.1 M Tris–HCl/0.4% Triton X-100), and then centrifuged at 12000 rpm. Samples containing 15 μg of protein was separated on 10% SDS-polyacrylamide gel, and transferred to nitrocellulose membranes. These membranes were blocked in Tris-buffered saline containing 5% skim milk and 0.05% Tween-20 (TBST) overnight at 4 °C, before incubation with primary antibody. Western blotting was conducted using a 1:500 dilution of a monoclonal antibody against human RECK (PharMingen, San Diego, CA), a 1:3000 dilution of antibody against MMP-7 (Chemicon, Temecula, CA) and a 1:1000 dilution of antibody against human VEGF antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Peroxidase-conjugated goat antimouse IgG diluted 1:5000 was used as a secondary antibody. Peroxidase was visualized via an enhanced chemiluminescence assay (Amersham, Piscataway, NJ). Images on the gels were scanned using a Gel Documentation System (Gel Doc 1000, Bio-Rad, Hercules, CA) and the relative densities were analysed using a Multi-analyst fingerprinting program (version 1.1). The RECK protein expression level was calculated as the intensity ratio of the band from the tumour area per band from the non-tumour area from the same specimen.

2.3. Gelatin zymography

The tissue sample lysates were diluted with non-reducing sample buffer (0.5 M Tris/HCl, pH 6.8, SDS, glycerol and bromophenol blue). Gelatin zymography was performed to determine the presence of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) in the tissue samples. Each sample (20 μl) was run in parallel with a molecular weight marker and MMP-2 and MMP-9 protein standards on a SDS polyacrylamide gel (10%) containing 0.1% gelatin as the substrate, at 100 V for 3 h. After electrophoresis, the gel was washed in 2% Triton X-100 for one hour at room temperature on an orbital shaker. The substrate gel was then incubated overnight with MMP incubation buffer (0.05 M Trizma base, 0.2 M NaCl, 5 mM $CaCl_2$, pH 7.4). After incubation, gels were stained with a 0.2% solution of Coomassie blue for 30 min and then destained (10% acetic acid and 30% methanol) for 30 min. Proteolytic activity was represented by clear lysis bands of degraded protein on a uniformly blue background.

2.4. Determination of lymphatic tumour invasion

Blood vessels were visualized via immunohistochemical staining with anti-CD34 antibody (DakoCytomation, Carpinteria, CA), to verify endothelial cells, and to exclude pseudo-invasion due to artifacts of the tumour-tissue separation technique. For the immunohistochemical analysis, 5 μm thick

sections were cut from formalin-fixed, paraffin-embedded blocks, and affixed to slides. After deparaffinization, the sections were incubated in 3% H_2O_2 for 20 min, in order to inactivate the endogenous peroxidase. Deparaffinized and rehydrated specimens were heated in 10 mM citrate buffer (pH 6.0) for 20 min at 95 °C. After being cooled to room temperature for 30 min, the specimens were incubated with normal horse serum for 20 min at room temperature, followed by incubation with primary antibody diluted in 1:100 PBS. In order to preclude the possibility of background staining by secondary antibodies, adjacent sections from the same tumours were similarly treated with non-specific mouse IgG. The sections were washed three times for 5 min in PBS and incubated for 1 h, after which the avidin–biotin-complex method (LSAB kit, DakoCytomation) was utilized for detection. The immune complexes were then visualized with 0.03% diaminobenzidine used as a chromogen, and the sections were counterstained with haematoxylin and mounted. Identification of lymphatic tumour emboli was performed independently by an experienced pathologist, who was blinded with regard to the clinical outcomes and clinicopathological features of the studied cases.

2.5. *Statistical analysis*

Clinicopathological parameters were compared with RECK expression levels, using either χ^2 tests or Fisher's exact tests (two-sided). The comparative evaluations between RECK expression and parameters were also conducted with the Fisher's exact test (two-sided). The results were considered statistically significant at P values of less than 0.05. All statistical analyses were performed using the SPSS statistical software package (SPSS, Chicago, IL).

3. Results

3.1. *RECK expression in gastric cancer cell lines and tissues*

Of the 11 gastric cancer cell lines examined, nine (81.8%) did not show RECK protein expression by western blot analysis (Fig. 1A). Only two cell lines, S16 and M74, exhibited RECK protein expression. Of the 102 gastric cancer tissues, 53 (52.0%) displayed a reduced expression of RECK protein (Fig. 1B).

3.2. *General clinicopathologic characteristics of studied cases*

The 102 patients studied included 61 males (59.8%) and 41 females (40%), and ranged in age from 30 to 78 years, with a mean age of 57.7 years. Tumour size ranged from 3.0 to 17.0 cm, with a mean of 8.1 cm. Of the macroscopic types of tumours (Fig. 2), 30 proved to be expansile (29.4%) and 72 were infiltrative (70.6%). With regard to histological differentiation,

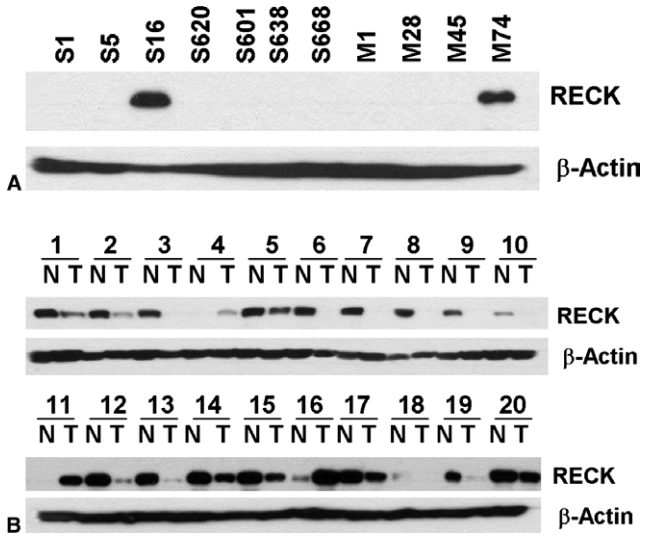

Fig. 1 – Expression levels of reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) according to Western blot analysis. (A) Among 11 different gastric cancer cell lines (SNU1, SNU5, SNU16, SNU620, SNU601, SNU638, SNU668, M1, M28, M45 and M74), two cell lines, S16 (lane 3) and M74 (lane 11), expressed RECK protein. (B) Representative gastric tumour specimens (T) evidence decreased RECK expression, as compared with corresponding normal tissues (N). The size of the RECK protein is around 110 kDa. β-Actin is shown as a loading control.

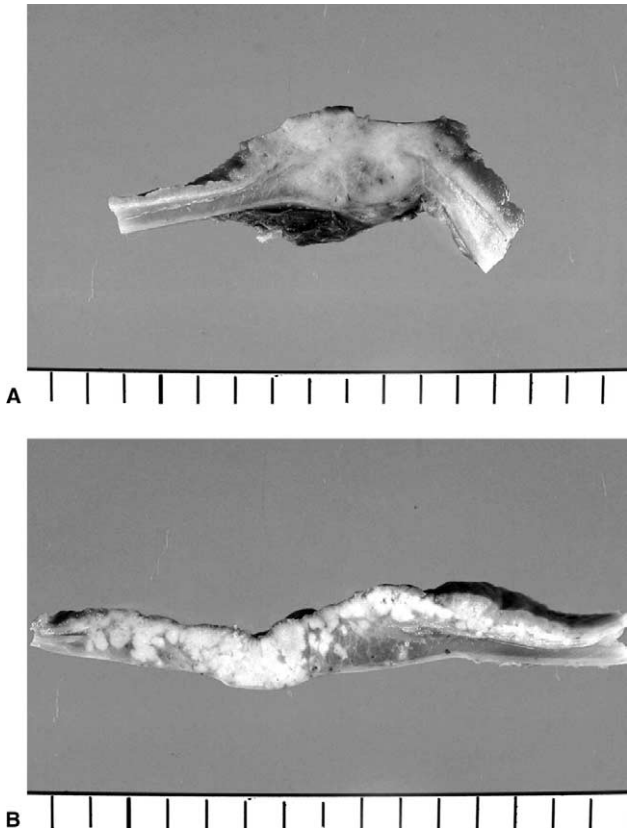

Fig. 2 – Macroscopic growth pattern of gastric cancers. (A) Cut section of expansile tumour exhibited typical pushing type tumour growth, with no satellite nodules or infiltrations. (B) Cut section of infiltrative tumour exhibited many satellite nodules and infiltrations at the periphery of the main tumour.

according to Lauren's tumour classifications, 33 were intestinal-type (32.4%) and 69 were diffuse-type (67.6%). The depth of tumour invasion in 60 samples was T2 (58.8%) and in 42 was either T3 or T4 (41.2%). With regard to lymph node metastasis, 13 were absent (12.7%) and 89 were present (87.3%); and tumour staging showed 29 were stage 1 or 2 (28.4%) and 73 were stage 3 or 4 (71.6%).

3.3. *RECK expression in gastric cancer tissues and its correlation with clinicopathological parameters*

In our comparison between the patients with RECK-preserved tumours and patients with RECK-reduced tumours, we determined there to be a significant inverse correlation between RECK expression and macroscopic tumour growth ($P = 0.018$), lymph node metastasis ($P = 0.000$), and tumour stage ($P = 0.000$), according to the results of Fisher's exact test (Table 1). Reduced RECK expression was more commonly found in tumours with macroscopically infiltrative growth (59.7%), positive lymph node metastasis (59.6%) and higher tumour staging (63.0%) than in tumours with macroscopically expansile growth (33.3%), negative lymph node metastasis (0%), and lower tumour staging (24.1%). No other factors, including age, sex, tumour size, Lauren classification, or depth of tumour invasion, were significantly different between the two groups. However, the diffuse-type tumours (58.0%) exhibited a marked tendency toward reduced RECK expression, as compared to the intestinal tumours (39.4%) with borderline significance ($P = 0.0973$).

3.4. *RECK expression in gastric cancer tissues and its correlation with lymphatic tumour invasion, as detected by CD34 immunohistochemistry*

As RECK expression was determined to be inversely correlated with tumour metastasis in gastric cancer, we further assessed its relationship with lymphatic invasion using CD 34 immunohistochemistry. Of the 102 gastric cancer tissues, 77 (75.5%) exhibited lymphatic tumour invasion (Fig. 3). Fisher's exact test indicated a significant inverse correlation in evidence between RECK expression and lymphatic tumour invasion ($P = 0.018$) (Table 2). Reduced RECK expression was more commonly found in tumours exhibiting lymphatic tumour invasion (59.0%) than in tumours exhibiting no lymphatic tumour invasion (29.2%).

3.5. *RECK expression in gastric cancer tissues and its correlation with MMPs and VEGF*

As RECK expression was determined to be inversely correlated with tumour invasion into lymphatic spaces, we further analysed MMP-7 activation. Of the 74 gastric cancer tissues, 17 (23.0%) evidenced expression of activated MMP-7 (Fig. 4). No correlation was detected between RECK expression and MMP-7 activation (Table 2). After gelatin zymography, a maximum of six lysis bands was observed, with molecular masses of 205 kDa, 116 kDa, 92 kDa (latent MMP-9), 84 kDa (active MMP-9), 72 kDa (latent MMP-2), and 68 kDa (active MMP-2). The 72 kDa band corresponding to latent MMP-2 was present in gastric tumour and normal samples. The 68 kDa band corresponding to active MMP-2 was found in 46 of 80 tumour samples but not in normal tissue samples. Similarly, the lysis band migrating at 92 kDa, corresponding to latent MMP-9, was present in all tumour and normal tissue samples analysed. However, the 84 kDa band corresponding to active MMP-9 was absent in normal samples (Fig. 5). As determined by densitometry, MMP-9 expression was significantly greater in 37 of 80 tumours compared to normal tissue. Our results showed that RECK and active MMP-9 were negatively associated ($P = 0.039$), whereas RECK and MMP-2 are not (Table 2). Western blot analysis shows that VEGF 165 had been detected in both normal and tumour samples, whereas VEGF 121

Table 1 – Comparison of clinicopathological parameters between gastric cancers with or without expression of reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) protein

Parameters	Category	RECK expression		P
		Preserved	Reduced	
		$n = 49$	$n = 53$	
Sex	Male	32	29	0.316[a]
	Female	17	24	
Age (year)	Mean ± SD	57.4 ± 12.7	58.0 ± 11.0	0.783[b]
Macroscopic growth	Expansile	20	10	**0.018**[a]
	Infiltrative	29	43	
Tumour size (cm)	Mean ± SD	8.4 ± 3.7	7.8 ± 3.2	0.358[b]
Lauren classification	Intestinal	20	13	0.093[a]
	Diffuse	29	40	
Depth of invasion	Within serosa	31	29	0.425[a]
	Serosa or beyond serosa	18	24	
Lymph node metastasis	Absent	13	0	**0.000**[a]
	Present	36	53	
Stage	1–2	22	7	**0.000**[a]
	3–4	27	46	

SD, standard deviation.
a *P* was obtained by Fisher's exact test.
b *P* was obtained by one-way ANOVA test.

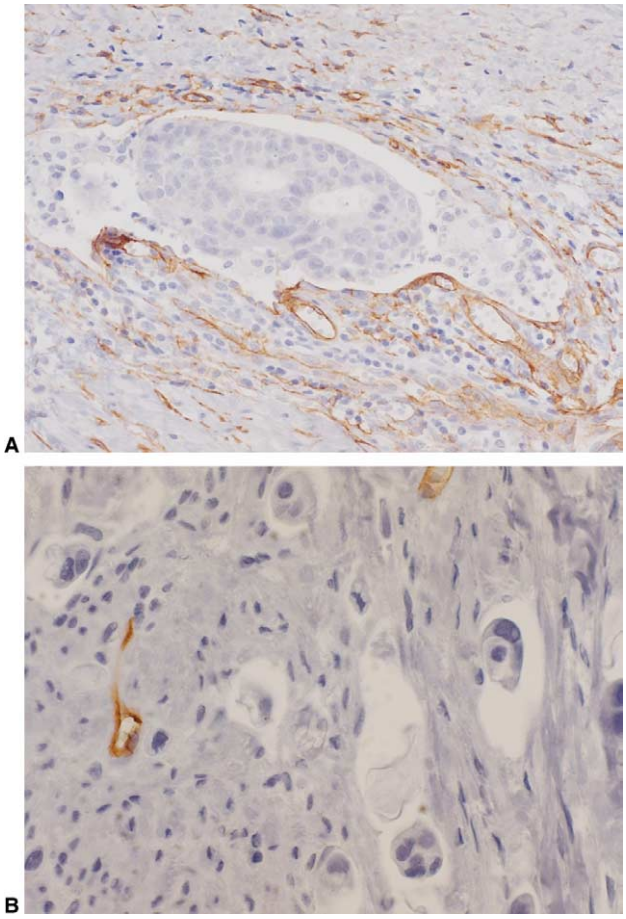

Fig. 3 – Immunohistochemical detection of lymphatic tumour invasion using anti-CD34 antibody. (A) True tumour embolus in the CD34-positive vascular space. (Original magnification ×200). (B) Pseudoemboli of tumour cells, a result of a tumour-stroma contraction artifact. These lesions were occasionally reminiscent of true tumour emboli. Note the internal positive control of the CD34-positive vascular space. Immune complexes were visualized via the avidin–biotin-complex method, and were counterstained with haematoxylin (Original magnification ×400).

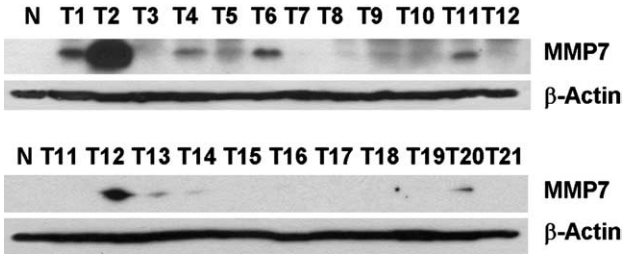

Fig. 4 – Expression of activated MMP-7 by Western blot analysis in gastric tissues. Representative specimens of a gastric tumour (T) exhibit activated MMP-7 expression, as compared with corresponding normal tissues (N). The size of the activated MMP-7 is approximately 18 kDa. It is different from the non-activated, 28 kDa-sized pro MMP-7. β-Actin is shown as a loading control.

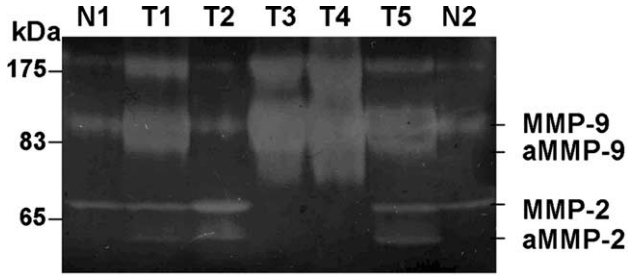

Fig. 5 – Gelatin zymogram illustrating the gelatinolytic activity of gastric tumour (T) and normal (N) tissues samples. The first lane contains the molecular weight markers (MW). Latent and active forms of matrix metalloproteinase 2 (MMP-2) and MMP-9 are detected in tumour samples; however, only the latent forms of these enzymes are detected in the normal samples.

expression was restricted to tumour samples (Fig. 6). However, our study did not uncover any significant correlations between RECK and VEGF 121 or VEGF 165 (Table 2).

Table 2 – Correlation between Reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) expression and other parameters

	Category	RECK expression		P
		Preserved	Reduced	
Lymphatic tumour invasion	Absent	34.7% (17/49)	13.2% (7/53)	**0.018**[a]
	Present	65.3% (32/49)	86.8% (46/53)	
MMP-7	Not activated	76.3% (29/38)	77.8% (28/36)	1.000[a]
	Activated	23.7% (9/38)	22.2% (8/36)	
MMP-2	Not activated	40.5% (17/42)	42.1% (16/38)	0.531
	Activated	59.5% (25/42)	57.9% (22/38)	
MMP-9	Not activated	64.3% (27/42)	42.1% (16/38)	**0.039**[a]
	Activated	35.7% (15/42)	57.9% (22/38)	
VEGF 121	Absent	64.3% (27/42)	51.3% (20/39)	0.169
	Present	35.7% (15/42)	48.7% (19/39)	
VEGF 165	Preserved	42.9% (18/42)	51.3% (20/39)	0.296
	Increased	57.1% (24/42)	48.7% (19/39)	

a P was obtained by Fisher's exact test.

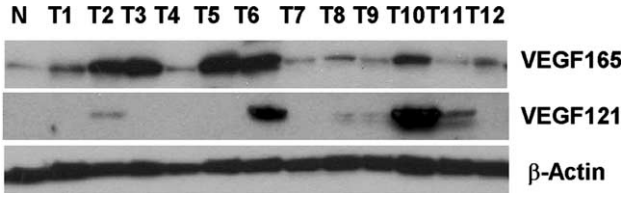

Fig. 6 – Western blot analysis of VEGF in gastric tissues. Specimens of a gastric tumour (T) exhibit VEGF expression, as compared with representative normal tissues (N). Representative gastric tumour specimens (T) evidence significant amount of VEGF expression, as compared with corresponding normal tissues (N). The size of the VEGF165 and 121 is approximately 42 and 28 kDa, respectively. β-Actin is shown as a loading control.

4. Discussion

The inverse relationship between RECK expression in various human tumours and their prognoses has been amply demonstrated in patients suffering from hepatocellular carcinoma [9], pancreatic cancer [10], breast cancer [11], non-small cell lung cancer [12], and colorectal cancer [13]. However, the significance of RECK expression, not only in gastric cancer but also in non-cancerous gastric tissue, has yet to be elucidated and warrants pilot studies in other types of human tumours [12]. In this study, we determined that the RECK protein was not expressed in the majority of the gastric cancer cell lines, and also that the patients with gastric cancer tissues with reduced RECK expression, as compared with the surrounding non-tumour tissues, exhibited more infiltrative macroscopic tumour growth, more metastasis into regional lymph nodes, and more advanced staging overall. Reduced RECK expression was also related to frequent lymphatic tumour cell invasion, which may constitute an early parameter of metastasis. The presence of lymphatic tumour emboli was significantly associated by multivariate analysis with increased lymph node metastasis in EGC [16]. Our present data strongly supports a previous report, where nude mice inoculated with HT1080 fibrosarcoma cells transfected with a RECK expression vector, exhibited lower lung and lymph node metastasis [2].

Metastasis formation is a complex multi-step process, which includes the invasion of tumour cells into the stroma, migration through the stroma, probably coupled with protease activity, association with angiogenesis, penetration of the circulation, and settling in the 'new world', or the metastatic site. RECK is a promising molecule with regard to the prediction of tumour invasion and metastasis via the dual mechanisms of angiogenesis [2,4,12,13] and the MMP pathways [4,5,11–13,17–19]. These mechanisms may, therefore, be a common process which explains the role of RECK in the multitude of human tumours with which it is associated. Takenaka and colleagues have suggested that RECK-expression might be inversely correlated with microvessel density, thereby carrying great import in active angiogenesis [12]. Interestingly, the effects of RECK were determined to be most robust in the tumours which expressed VEGF at higher levels, raising the possibility that RECK may suppress the tumour angiogenesis induced by VEGF. VEGF is a potent angiogenic factor, and bioactive as freely diffusible proteins in the extracellular space, where it acts on endothelial cells by stimulating cell proliferation, migration, and tubular organization and increase vascular permeability [20]. The shorter isoforms of VEGF 121 and 165 are soluble secreted proteins, although a portion of the 165 form remains bound to the cell surface, whereas the larger isoforms bind tightly to heparin and are sequestered in the extracellular matrix [20,21]. VEGF 121, considered to be the most potent stimulator of angiogenesis in vivo and the predominate isoform in primary human cancer, diffuses into the extracellular space from the cells producing it and cannot be detected by immunostaining of tumour sections [8,22]. VEGF measured in blood has been considered as an alternative to these methods, but the interpretation of such studies has been complicated by the fact that most serum VEGF is derived from platelets, which are activated on coagulation [23]. Western blot analysis shows that VEGF 165 has been detected in both normal and tumour samples, whereas VEGF 121 expression was found only in tumour samples. However, our study did not uncover any significant correlations between RECK and VEGF 121 or VEGF 165, although they were increased in tumour tissues compared with normal tissues.

During pathologic vasculogenesis, RECK was found to inhibit proper blood vessel formation, probably via the suppression of angiogenic sprouting. Previous studies have reported that RECK inhibits the secretion and activity of MMP-2, MMP-9, and MMP-14 [2,4]. The tumour-suppressive role of RECK, as it is an MMP inhibitor, is less counterintuitive than that of the TIMPs, and has been proposed and characterized in many of those earlier studies. However, we failed to detect MMP-2 and MMP-9 in gastric cancer tissues by Western blot analysis. Instead, MMP-7 was detected, but our study did not uncover any remarkable correlations. However, gelatin zymography on biopsies from gastric carcinomas found a greater expression of active forms of MMP-2 and MMP-9 in tumour tissues compared with normal tissues. Thus, some kind of activating event seems to occur for MMP-2 and MMP-9 in gastric tumour tissues. We could show that decrease of RECK is correlated with activation of MMP-9, but not MMP-2, in tumour tissue in vivo. Thus, loss of RECK might be to some extent responsible for the particular up-regulation of MMP-9 activity in gastric tumour compared to normal tissues. As such, any prognostic contribution of RECK and MMP-9 will not be independent, whereas RECK and MMP-2 could be.

One of the more interesting findings in our results was the correlation between RECK expression and macroscopic infiltrative growth. It is difficult to demonstrate a relationship between macroscopic growth and molecular events, as macroscopic tumour growth is such a complex phenomenon, and also because gastric cancer appears to be a heterogeneous phenomenon [24]. There have, however, been few valid reports which have correlated gross morphology of gastric cancer with molecular alterations. Ohene-Abuakwa and colleagues reported that the expression of the E-cadherin/catenin complex was correlated with the macroscopic appearance of EGCs [25]. We also reported that the abnormal expression of E-cadherin, α- and β-catenins was significantly associated with depressed tumour growth, as well as diffuse histological type [24]. If we consider macroscopic infiltrative growth to be a

complex phenomenon, the meaning of its correlation with RECK expression must then be emphasized, as it would then overcome any other variables related to macroscopic growth.

We demonstrated RECK expression in two gastric cancer cell lines, using Western blot analysis. The expression of the RECK protein in cancer tissues is inconsistent with the fact that *RECK* gene expression is suppressed in several tumour-derived cell lines and *ras*-transformed fibroblasts [26]. Pancreatic cancer cell lines did not exhibit RECK expression, according to the results of Western blot analysis [10]. However, we did find that the RECK protein was expressed in 52% of gastric cancer tissues, at the same level as in the surrounding non-tumour tissues, again by Western blot analysis. Also, strong or high level RECK protein expression was demonstrated in 52% of pancreatic cancer tissues [10] and 56.6% of colorectal cancers [13], in immunohistochemical studies. Taking into account these results, about half of the tested advanced cancers evidenced RECK protein expression. Tumours which retained RECK expression were less prone to recur [9], probably due to reduced angiogenesis and inhibited ECM remodeling. These results support the view that *RECK* is an important tumour suppressor gene [11]. Therefore, a pharmaceutical mimetic, or drugs which activate endogenous RECK expression, should be assessed for their efficacy as possible therapeutic or preventive agents for various advanced human cancers. Liu and colleagues suggested that the induction of RECK expression may be one of the mechanisms by which non-steroidal anti-inflammatory drugs suppress MMP activity to block angiogenesis and metastasis, and that this may provide a new strategy for the treatment of cancer [27].

In conclusion, we determined there to be a significant correlation between RECK expression in human gastric cancer tissues, and the prognostic parameters of the patients. These data are consistent with the notion that RECK plays an active role in the suppression of malignant phenotypes. Our data, together with that obtained in previous reports, strongly supports the hypothesis that RECK is a suppressor of malignancy, and constitutes a good prognostic indicator in gastric cancers. Our results also suggest that certain therapeutic strategies based on RECK or its mechanism of action may be of value in the treatment of this disease.

Conflict of interest statement

None declared.

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