Histol Histopathol (2011) 26: 663-671
DOI: 10.14670/HH-26.663

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Overexpression of GRP78 and GRP94 is involved in colorectal carcinogenesis

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Summary. Glucose-related proteins (GRPs) are ubiquitously expressed in the endoplasmic reticulum and assist in protein folding and assembly, consequently considered to be molecular chaperones. GRP78 and GRP94 expression was induced by glucose starvation and up-regulated in samples taken from several different malignant tissues. To clarify the roles of both molecules in tumorigenesis and progression of colorectal carcinomas, immunohistochemistry (IHC) was performed on tissue microarrays containing colorectal carcinomas, adenomas and the non-neoplastic mucosa (NNM) using antibodies against GRP78 and GRP94. Their expression was correlated with the clinicopathological parameters of carcinomas. Both proteins were also studied in colorectal carcinoma cell lines (DLD-1, HCT-15, SW480 and WiDr) by IHC and Western blot. There was a gradually increased GRP78 expression from colorectal NNMs, carcinomas, to low-grade and high-grade adenomas ($P<0.05$), while up-regulated GRP94 expression from NNM, low-grade adenoma, high-grade adenoma, to carcinoma ($P<0.05$). The expression was similar in all the carcinoma cell lines. GRP78 expression was negatively correlated with lymphatic invasion or low GRP94 expression of the carcinomas ($P<0.05$), while there was no correlation of GRP94 expression with other parameters of carcinomas ($P>0.05$). Multivariate analysis showed that venous invasion, lymph node metastasis and UICC staging ($P<0.05$), but not age, sex, tumor size, differentiation, depth of invasion, lymphatic invasion, GRP78 and GRP94 expression ($P>0.05$), were independent prognostic factors for carcinomas. It is suggested that up-regulated expression of GRP78 and GRP94 could possibly be involved in the pathogenesis of colorectal carcinomas.

Key words: Colorectal carcinoma, GRP78, GRP94, Pathogenesis, Progression, Prognosis

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Introduction

Colorectal carcinoma (CRC) ranks as the world's second leading cause of cancer mortality behind lung cancer, despite a sharp worldwide decline in both its incidence and mortality since the second half of the 20th century. Japan has experienced a marked increase in the incidence of colorectal cancer, and has recently been listed in the group of countries with the world's highest incidence rates (Boyle and Leon, 2002; American Cancer Society, 2005; Yoshida et al., 2007). Tumorigenesis and progression of CRCs is a multistage process with the involvement of a multifactorial etiology. Recently, it has been reviewed that the endoplasmic reticulum (ER) plays an important role in regulating the synthesis, folding, and targeting of secretory and membrane proteins. Oxidative stress, glucose deprivation, chemical toxicity, alterations in intracellular Ca^{2+} levels, blockade of glycosylation and hypoxia induce ER stress, in which the expression of glucose-related proteins(GRPs) is activated (Baldwin et al., 1987; Chen et al., 2002; Morishima et al., 2004).

GRPs are ubiquitously expressed in ER and able to assist in protein folding and assembly, consequently considered as molecular chaperones. GRP78 was discovered in the late 1970s together with GRP94, as cellular proteins induced by glucose starvation, and almost shows 50% homology with heat shock protein (HSP) 70. In non-stressed cells, GRP78 binds to ER

transmembrane sensor proteins PERK, IRE1, and ATF-6 to maintain their inactive form. Additionally, it is involved in protein folding in the ER lumen, translocation of newly synthesized secretory precursors across the ER membrane, presentation of aberrant proteins to the proteolytic machinery by the proteosome, and regulation of apoptosis (Baldwin et al., 1987; Schmidt and Perlmutter, 2005). GRP94 is representative of the HSP90 family of stress-induced proteins and plays an important role in stabilizing calcium homeostasis and suppressing cell death. It was found to influence the biosynthesis of such secreted and membrane-bound proteins as immunoglobulins or Toll-like receptors, and also act as a tumor vaccine, delivering the peptides for presentation to T lymphocytes (Argon and Simen, 1999; Chen et al., 2002).

Although GRP94 and GRP78 are localized constitutively within the ER and perform normal physiological functions under moderate levels of basal expression, they might be induced under pathological conditions, such as tumor growth or toxic damage. Tumor cells are confronted with oxygen deprivation and nutrient stress often, even with extensive angiogenesis, which causes the activation of GRPs gene expression (Lee, 2001; Fu and Lee, 2006). The induction of GRP94 or GRP78 may be a defence mechanism for the survival of cancer cells exposed to these stress conditions. Recent studies have indicated a potential role for altered GRP78 expression and function in tumor development and progression (Lee, 2001, 2007; Fu and Lee, 2006). Moreover, investigators have revealed that GRP78 expression is significantly high in a variety of malignancies, such as breast and hepatocellular carcinoma, lung and prostate cancer, and more recently gastric cancer (Shuda et al., 2003; Uramoto et al., 2005; Zhang et al., 2006; Daneshmand et al., 2007; Zheng et al., 2008). GRP94 overexpression was recorded in oral carcinoma, lung cancer, esophageal, gastric and colonic carcinomas (Wang et al., 2005a-c; Nomura et al., 2007; Zheng et al., 2008).

Our previous work has indicated that up-regulated expression of GPR78 and GRP94 might be involved in pathogenesis, growth, invasion and metastasis of gastric carcinomas (Zheng et al., 2008). Here, expression of GRP78 and GRP94 was examined in colorectal carcinoma, adenoma and non-neoplastic mucosa, and compared with the clinicopathological parameters of carcinomas, as well as prognosis to explore the clinicopathological significance and molecular roles of both GRPs in stepwise development of colorectal carcinomas.

Materials and methods

Cell lines and culture

Colorectal carcinoma cell lines were kindly presented by Prof. Sugiyama, Department of Gastroenterology, Graduate School of Medical and Pharmaceutical Sciences, University of Toyama. They were maintained in RPMI 1640 (HCT-15, DLD-1 and SW480) and DMEM (WiDr) medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2 at 37°C. All cells were harvested by centrifugation, rinsed with PBS, and subjected to total protein extraction by sonication in RIPA lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 5 mM dithiothreitol, 10 mM NaF, protease inhibitor cocktail [Sigma]). All cells were collected by centrifugation, rinsed with phosphate-buffered saline (PBS), fixed by 10% formalin and then embedded in paraffin as routinely processed.

Western Blot

The denatured protein was separated on an SDS-polyacrylamide gel (10% acrylamide) and transferred to Hybond membrane (Amersham, Germany), which was then blocked overnight in 5% skim milk in TBST (10mM Tris-HCl, 150mM NaCl, 0.1% Tween 20). For immunobloting, the membrane was incubated for 1h with the goat antibody against GRP78 (sc-1050, Santa Cruz, USA; 1:1000) and GRP94 (sc-1794, Santa Cruz, USA; 1:1000). Then, it was rinsed by TBST and incubated with anti-goat IgG conjugated to horseradish peroxidase (DAKO, USA, 1:1000) for 1h. Bands were visualized with LAS4000 (Fujifilm, Japan) by ECL-Plus detection reagents (Amersham, Germany). After that, membrane was washed with WB Stripping Solution (pH2-3, Nacalai, Tokyo, Japan) for 20min and treated as described above, except mouse anti-ß-actin antibody (Santa Cruz, USA, 1:1000) and anti-mouse IgG conjugated to horseradish peroxidase (DAKO, USA, 1:1000) as an internal control.

Subjects

Colorectal carcinomas (n=312) and adjacent non-neoplastic mucosa (NNM, n=301) were collected from surgical resection, adenoma (n=88) from endoscopic biopsy or polypectomy from endoscopic biopsy in the affiliated hospital, University of Toyama and Kouseiren Takaoka Hospital between 1993 and 2002. All carcinomas were adenocarcinomas and the adenoma group was free from non-neoplastic polyp types, leiomyomas and benign GIST's. The patients with colorectal carcinoma were 172 men and 140 women (18~90years, mean=68.7 years). Among them, 122 cases have carcinomas accompanied with lymph node metastasis. None of the patients underwent chemotherapy, radiotherapy or adjuvant treatment before surgery. They all provided consent for use of tumor tissue for clinical research and our Ethical Committee approved the research protocol. We followed up all patients by consulting their case documents or by

telephone.

Pathology

All tissues were fixed in 10% neutral formalin, embedded in paraffin and cut into 4 μm sections. These sections were stained by haematoxylin-and-eosin (HE) to confirm their histological diagnosis and other microscopic characteristics. The staging for each colorectal carcinoma was evaluated according to the Union Internationale Contre le Cancer (UICC) system for the extent of tumor spread (Sobin and Wittekind, 2002). Histological architecture of colorectal carcinoma was expressed in terms of WHO classification (Hamilton and Aaltonen, 2000). Furthermore, tumor size, depth of invasion, lymphatic and venous invasion were determined.

Tissue Microarray(TMA)

Representative areas of solid tumors were identified in HE stained sections of the selected tumor cases and a four mm-in-diameter tissue core per donor block was punched out and transferred to a recipient block with a maximum of 24 cores using a Tissue Microarrayer (AZUMAYA KIN-1, Japan). Four-μm-thick sections were consecutively incised from the recipient block and transferred to poly-lysine-coated glass slides. HE staining was performed on TMA for confirmation of tumor tissue.

Immunohistochemistry

Consecutive sections were deparaffinised with xylene, dehydrated with alcohol, and subjected to antigen retrieval by irradiating in target retrieval solution (TRS, DAKO, Carpinteria CA93013, USA) for 15 minutes with microwave oven (Oriental rotor Lmt. Co., Tokyo, Japan). Five percent bovine serum albumin was then applied for 1min to prevent non-specific binding. The sections were incubated with primary antibodies for 15minutes, then treated with the anti-goat conjugated to horseradish peroxidase (DAKO, USA; 1:100) antibodies for 15minutes. All the incubations were performed in a microwave oven to allow intermittent irradiation as described previously (Kumada et al., 2004). After each treatment, the slides were washed with TBST three times for 1 minute Goat anti-GRP78 (sc-1050, 1:50) and goat anti-GRP94 (sc-1794, 1:50) antibodies were employed to detect the individual proteins. Binding sites were visualized with 3,3'-diaminobenzidine (DAB). After counterstaining with Mayer's haematoxylin, the sections were dehydrated, cleared and mounted. Omission of the primary antibody was used as a negative control.

One hundred cells were randomly selected and counted from 5 representative fields of each section blindly by two independent observers (Takahashi and Zheng). The inconsistent data were confirmed by both persons until final a final agreement was reached. The positive percentage of counted cells was graded semi-quantitatively according to a four-tier scoring system: negative (-), 0~5%; weakly positive (+), 6~25%; moderately positive (++), 26~50%; and strongly positive (+++), 51~100%.

Statistical analysis

Statistical evaluation was performed using Spearman correlation test to analyze the rank data. *Kaplan-Meier* survival plots were generated and comparisons between survival curves were made with the log-rank statistic. The Cox's proportional hazards model was employed for multivariate analysis. $P<0.05$ was considered as statistically significant. SPSS 10.0 software was employed to analyze all data.

Results

Western blot showed that there were unique strong bands of GRP78 and GRP94 proteins in DLD-1, HCT-15, SW480 and WiDr cells (Fig. 1A), which ensure the immustaining reliability of both proteins. Immunohistochemically, their expression was positive in the cytoplasm of these cancer cells (Fig. 1B). Both proteins were homogeneously distributed in the cytoplasm of colorectal epithelial cells, adenomas and

Table 1. GRP78 expression in NNMs, adenomas and CRCs.

Groups	n	GRP78 expression				
		-	+	++	+++	PR(%)
NNMs	301	59	221	20	1	80.4
Low-grade adenomas	72	2	22	27	21	97.2*,†
High-grade adenomas	16	0	3	5	8	100.0*,†
CRCs	312	7	160	111	34	97.8*

GRP: glucose-regulated protein; PR: positive rate; NNM: non-neoplastic mucosa; CRC: colorectal carcinoma; *: Compared with NNM, P<0.001; †: Compared with carcinoma or NNM; P<0.001

Table 2. GRP94 expression in NNMs, adenomas and CRCs.

Groups	n	GRP94 expression				
		-	+	++	+++	PR(%)
NNMs	289	39	234	16	0	86.5
Low-grade adenomas	72	0	30	37	5	100.0*
High-grade adenomas	16	0	4	7	5	100.0*,†
CRCs	305	0	160	111	34	100.0*,†

GRP: glucose-regulated protein; PR: positive rate; NNM: non-neoplastic mucosa; CRC: colorectal carcinoma; *: Compared with NNM, P<0.001; †: Compared with low-grade adenoma, P<0.05

carcinomas, and were frequently observed in the infiltrating inflammatory cells (Fig. 2). Overall, GRP78 expression was detected in 242 of 301 NNM samples (80.4%), 70 of 72 low-grade adenomas (97.2%), all high-grade adenoma(16 cases) and 305 of 312 colorectal carcinoma patients (97.8%), respectively. However, GRP78 was highly expressed in colorectal adenomas and carcinomas, compared to the NNM ($P<0.05$). Positive labeling of GRP78 was higher in adenoma than in carcinoma samples regardless of dysplastic grade of adenoma ($P<0.05$, Table 1). GRP94 expression was positive in all 305 colorectal carcinomas (100.0%) and low-grade (100.0%, 72 cases) and high-grade adenomas (100.0%, 16 cases), and at a higher level than in the NNMs (86.5%, 250/289; $P<0.05$). High-grade adenomas and carcinoma showed greater GRP94 expression than low-grade carcinomas ($P<0.05$, Table 2)

As summarized in Table 3, GRP78 expression was negatively correlated with lymphatic invasion and low GRP94 expression ($P<0.05$), but not with age, sex, tumor size, differentiation, depth of invasion, venous invasion, lymph node metastasis or UICC staging ($P>0.05$). Additionally, GRP94 expression was not associated with the aggressive parameters mentioned above ($P>0.05$). Follow-up information was available on 262 colorectal carcinoma patients for periods ranging from 0.9 months to 12.1 years (median = 66.4 months) after surgery. Survival curves stratified according to GRP78 (A) and GRP94 (B) status for colorectal carcinomas are shown in Figure 3. Univariate analysis, using the *Kaplan-Meier* method, indicated that the cumulative survival rate of patients with moderate to strong GRP78 or GRP94 expression was a little higher than in patients with little or weak expression of both proteins, despite there being no statistical difference ($P>0.05$). Multivariate analysis showed that venous invasion, lymph node metastasis and UICC staging ($P<0.05$), but not age, sex, tumor size, differentiation, depth of invasion, lymphatic invasion, GRP78 and GRP94 expression ($P>0.05$) were independent

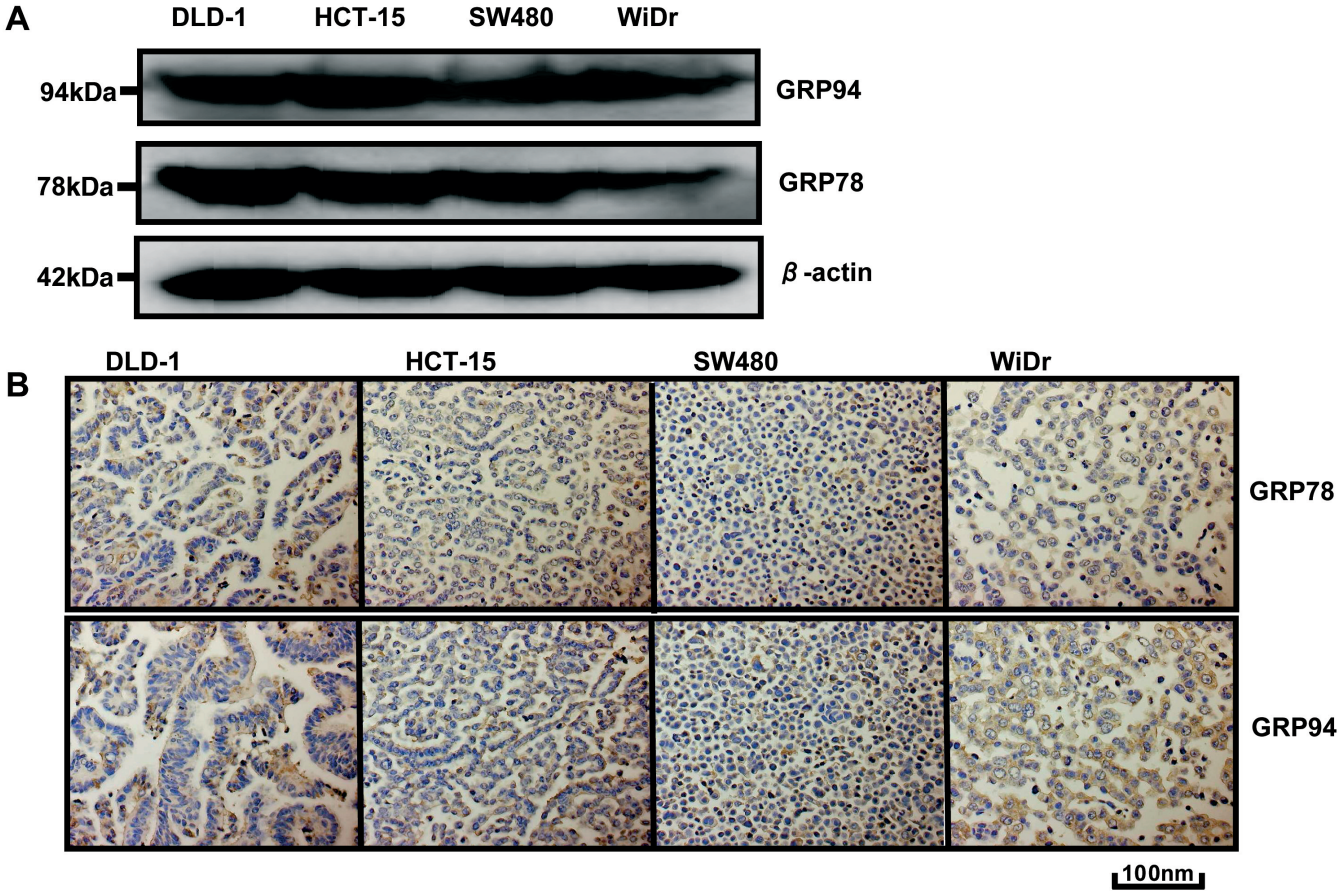

Fig. 1. GRP78 and GPR94 expression in colorectal carcinoma cell lines. **A.** Western blot analysis of GRP78 and GRP94 proteins in colorectal carcinoma cell lines. Cell lysate was loaded and probed with anti-GRP78 (Panel 1, 78kDa) and GRP94 (Panel 2, 94kDa) antibody with ß-actin (Panel 3, 42kDa) as an internal control. **B.** GRP78 and GRP94 proteins were positively immunostained in the cytoplasm of the four colorectal carcinoma cell lines (x 100) as mentioned in Fig. 1A.

prognostic factors for the carcinomas (Table 5).

Discussion

Both GRP78 and GRP94 play essential roles in the ER linked to nuclear signaling, protein folding, sorting and secretion, protection against Ca^{2+} depletion stress, and antigen presentation (Baldwin et al., 1987; Chen et al., 2002; Morishima et al., 2004; Schmidt et al., 2005). In this study, both molecules were observed in the cytoplasm of colorectal adenomas and carcinomas, and in several carcinoma cell lines, which is consistent with their specific distribution in the ER. The carcinomas displayed stronger expression of GRP78 and GRP94 than the NNM, indicating that their up-regulated expression is involved in colorectal carcinogenesis, in agreement with previous findings (Shuda et al., 2003; Uramoto et al., 2005; Wang et al., 2005a-c; Zhang et al., 2006; Daneshmand et al., 2006; Xing et al., 2006; Nomura et al., 2007). Glucose deprivation, or hypoxia, can also induce the expression of both proteins as a protective response mechanism to allow the cells to adjust to potentially lethal stress in the tumor microenvironment, because neovascularization does not satisfy the nutrient demand during growth of the carcinoma cells (Fu et al., 2006; Lee et al., 2007). Zheng et al. (2006) found that gastrointestinal adenomas and adenocarcinomas also displayed higher proliferation levels which correlated with elevated expression of GRP78 and GRP94 in colorectal adenomas and carcinomas because of their anti-apoptotic function (Biroccoi et al., 2001). Reportedly, GRP94 overexpression could cause the activation of NF-kappaB, which alone resulted in expression of anti-apoptotic proteins and blocked apoptosis induced by tumor-necrosis-related apoptosis- inducing ligand (Lee et al., 2008). Additionally, forced expression of GRP78 stimulated cell proliferation and prevented apoptosis, including that induced by endoplasmic reticulum stress and chemotherapy in cancer cells (Yeung et al., 2008). It

Table 3. Relationship between GRP78 expression and clinicopathological features in CRCs.

Clinicopathological features	n	GRP78 expression					
		-	+	++	+++	PR(%)	P value
Age(years)							0.828
<65	117	0	65	39	13	100.0	
≥65	195	7	95	72	21	95.9	
Sex							0.313
male	172	4	94	54	20	97.7	
Female	140	3	66	57	14	97.8	
Tumor size (cm)							0.641
≤5	208	3	111	72	22	98.6	
>5	103	4	48	39	12	96.1	
Depth of invasion							0.479
T_{is}-T_1	22	0	11	7	4	100.0	
T_2-T_4	286	7	147	102	30	97.6	
Differentiation							0.490
Well-differentiated	153	4	73	59	17	97.4	
Moderately-differentiated	146	3	80	50	13	97.9	
Poorly-differentiated	13	0	7	2	4	100.0	
Lymphatic invasion							0.025
-	213	6	100	82	25	97.2	
+	90	1	58	24	7	98.9	
Venous invasion							0.072
-	256	7	127	91	31	97.3	
+	47	0	31	15	1	100.0	
Lymph node metastasis							0.431
-	183	5	90	66	22	97.3	
+	122	2	66	43	11	98.4	
UICC staging							0.660
I-II	65	1	38	21	5	98.5	
II-IV	190	4	98	65	23	97.9	
GRP94 expression							<0.001
-	0	0	0	0	0		
+	65	6	40	16	3	98.3	
++	160	1	91	54	14	99.4	
+++	87	0	29	41	17	100.0	

GRP: glucose-regulated protein; PR: positive rate; T_{is}: carcinoma in situ; T_1: lamina propria and submucosa; T_2: muscularis propria; T_3: subserosa and exposure to serosa ; T_4: invade other organs or perforate visceral peritoneum; UICC: Union Internationale Contre le Cancer.

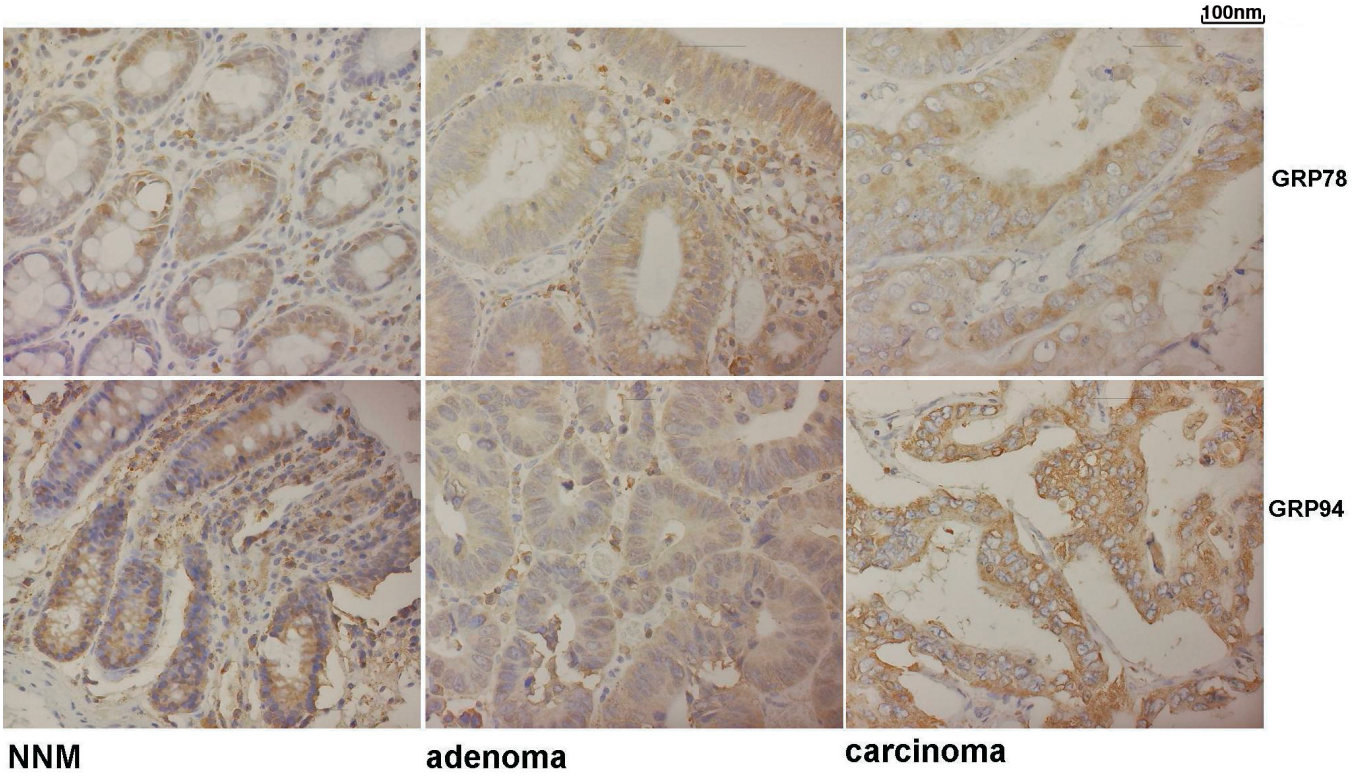

Fig. 2. Immunohistochemical staining of GRP78 and GRP94 proteins in colorectal tissue samples. Note: The expression of GRP78 and GRP94 was positively observed in the cytoplasm of colorectal epithelial cell, adenoma and carcinoma, and infiltrating inflammatory cells. x100

Table 4. Relationship between GRP94 expression and clinicopathological features in CRCs.

Clinicopathological features	n	GRP94 expression					
		-	+	++	+++	PR(%)	P value
Age(years)							0.143
<65	117	0	65	39	13	100.0	
≥65	188	0	95	72	21	100.0	
Sex							0.135
male	168	0	94	54	20	100.0	
Female	137	0	66	57	14	100.0	
Tumor size (cm)							0.780
≤5	206	0	112	72	22	100.0	
>5	99	0	48	39	12	100.0	
Depth of invasion							0.454
T_{is}-T_1	22	0	11	7	4	100.0	
T_2-T_4	279	0	147	102	30	100.0	
Differentiation							0.984
Well-differentiated	149	0	73	59	17	100.0	
Moderately-differentiated	143	0	80	50	13	100.0	
Poorly-differentiated	13	0	7	2	4	100.0	
Lymphatic invasion							0.509
-	207	0	100	82	25	100.0	
+	89	0	58	24	7	100.0	
Venous invasion							0.089
-	249	0	127	91	31	100.0	
+	47	0	31	15	1	100.0	
Lymph node metastasis							0.500
-	178	0	90	66	22	100.0	
+	122	0	68	43	11	100.0	
UICC staging							0.660
I-II	64	0	38	21	5	100.0	
II-IV	186	0	98	65	23	100.0	

GRP: glucose-regulated protein; PR: positive rate; T_{is}: carcinoma in situ; T_1: lamina propria and submucosa; T_2: muscularis propria; T_3: subserosa and exposure to serosa ; T_4: invade other organs or perforate visceral peritoneum; UICC: Union Internationale Contre le Cancer.

was reported that c-Myb is a highly conserved transcription factor required for normal colon development, and c-Myb overexpression is a consistent feature of colon cancer (Mucenski et al., 1991). GRP78 is a c-Myb target gene and c-Myb overexpression may be in part responsible for the high level of expression of GRP78 (Ramsay et al., 2005).

Aberrant GRP expression and activation may play an important role in cancer development and progression (Shuda et al., 2003; Uramoto et al., 2005; Wang et al., 2005a,b,c; Fu et al., 2006; Zhang et al., 2006; Daneshmand et al., 2006; Nomura et al., 2007). In the present study, a negative correlation was observed between GRP78 overexpression and lymphatic invasion, contrary to other reports (Shuda et al. 2003; Uramoto et al., 2005; Wang et al., 2005a-c; Zhang et al., 2006; Daneshmand et al., 2006; Zheng et al., 2008). Knockdown of GRP78 expression inhibited gastric cell invasion in vitro, along with growth and metastasis (Zhang et al., 2006), and up-regulation of GRP78 expression has been implicated in the ability of carcinoma cells to counterattack T-lymphocyte-mediated cytotoxicity or adverse physiological conditions (Song et al., 2001). Some researchers report that elevated GRP expression is related to development of resistance to chemotherapy and photodynamic therapy (Gomer et al., 1991; Fisher et al., 1993; Sugawara et al., 1993; Yun et al., 1995; Tomida et al., 1996 Chatterjee et al., 1994; Kubota et al., 2005; Ryoo et al., 2006). Therefore, we hypothesized that the colorectal well-differentiated and adenoma-derived adenocarcinoma with higher proliferative ability and lower angiogenesis, along with greater GRP78 expression, would show greater resistance to chemotherapy. The fact that no relationship between GRP78 expression and depth of invasion was observed in our study might be attributed to the small number of early carcinoma cases involved. The negative association between GRP78 expression and lymphatic invasion might be due to its inhibitory effect on lymphangiogenesis. Glucose starvation and severe hypoxia characterized poor vascularization, which induced ER stress and expression of GRPs (Lee et al., 1987). Recombinant GRP-78 conferred antiangiogenic resistance of bortezomib to endothelial cells Knockdown of GRP78 gene expression in tumor cells and immunodepletion of GRP-78 protein restored bortezomib sensitivity (Kern et al., 2009).

In this investigation, we analyzed the relationship between GRP78 or GRP94 expression with post-surgical

Table 5. Multivariate analysis of clinicopathological variables for the survival of the patients with CRCs.

Clinicopathological parameters	Relative risk (95%CI)	P value
Age(≥65years)	1.476(0.919-2.370)	0.107
Sex	1.050(0.668-1.652)	0.833
Tumor size(≤5)	0.865(0.540-1.385)	0.546
Depth of invasion (into muscularis propria)	1.650(0.217-12.565)	0.629
Differentiation (well/moderate/poor)	0.896(0.596-1.346)	0.597
Lymphatic invasion(+)	1.562(0.968-2.520)	0.068
Venous invasion(+)	2.146(1.226-3.758)	0.008
Lymph node metastasis(+)	2.675(1.649-4.339)	0.000
UICC staging(I-II)	2.485(1.271-4.857)	0.008
GRP78 expression (+-+++)	0.733(0.466-1.154)	0.180
GRP94 expression (+-+++)	0.832(0.496-1.395)	0.485

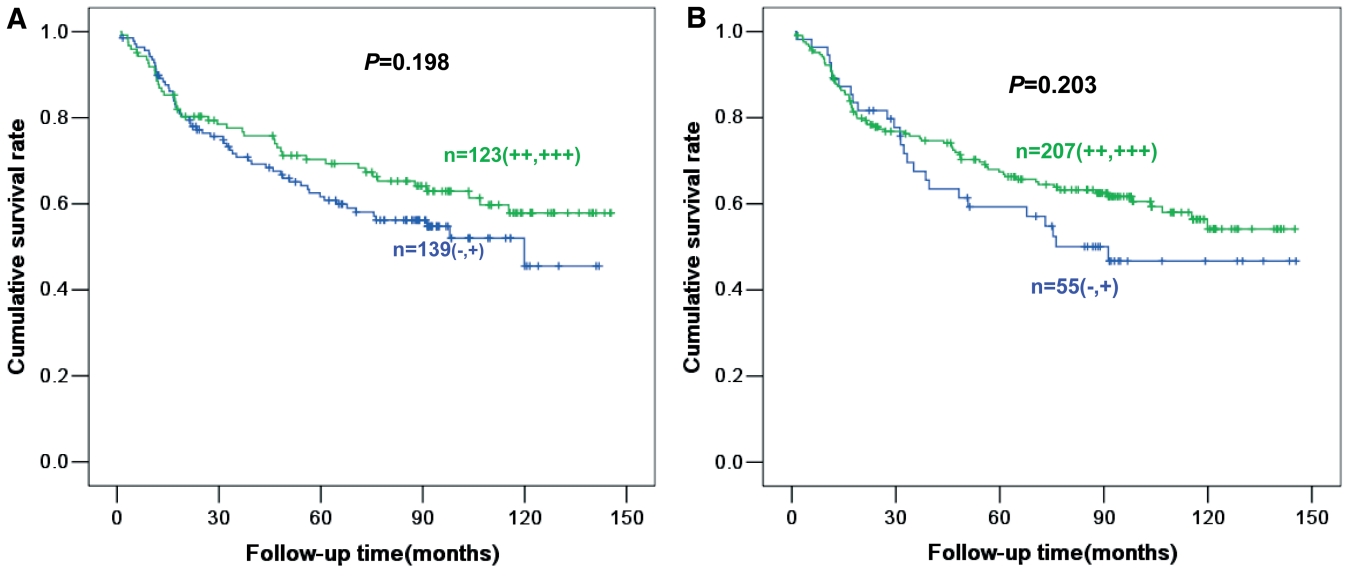

Fig. 3. Correlation between the status of GRP78 or GRP94 and prognosis of the colorectal carcinoma patients. Kaplan-Meier curves for cumulative survival rate of patients with colorectal carcinomas according to the expression of GRP78 **(A)** or GRP94 **(B)**.

survival of more than 262 patients with colorectal carcinoma. The results revealed that the cumulative survival rate of patients with moderate or strong GRP78 or GRP94 expression was slightly higher than those with little or weak expression of both proteins, despite a lack of statistical difference. In contrast, it was previously documented that GRP78 could be employed as a good marker for poor prognosis of gastric and hepatocelluar carcinomas, and prostate cancer (Lim et al, 2005, 2006; Zhang et al,, 2006; Daneshmand et al., 2007; Zheng et al, 2008). Alternatively, GRP78 expression was positively associated with increased survival of neuroblastoma patients as an independent prognostic factor (Hsu et al., 2005). In our study, the multivariate analysis demonstrated that only venous invasion, lymph node metastasis and UICC staging were independent prognostic factors for carcinomas. These findings suggest that GRP78 or GRP94 expression is not an indicator for the prognosis of colorectal carcinoma patients.

In conclusion, up-regulation of GRP78 and GRP94 expression is involved in colorectal pathogenesis and should not be considered as markers to indicate aggressive behavior or prognosis of colorectal carcinomas.

Acknowledgements. We thank Tokimasa Kumada and Hideki Hatta for their technical help, and Toshiro Sugiyama for his gift of colorectal carcinoma cell lines. This study was supported by Shenyang Outstanding Talent Foundation of China, Liaoning BaiQianWan Talents Program, Scientific and Technological Projects for Oversea Returned persons, Ministry of Personnel; Shenyang Science and Technology Grand(1091175-1-00); Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry; Grant-in aid for Scientific Research from the Ministry of Education, Culture, Sports and Technology of Japan (20659109; 21790624), Japanese Smoking Research Foundation and Japanese Uehara Memorial Foundation.

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Accepted December 3, 2010