Histol Histopathol (2011) 26: 1111-1120

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Up-regulated expression of Ezrin and c-Met proteins are related to the metastasis and prognosis of gastric carcinomas

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Summary. Recent publications demonstrated that abnormal expression of Ezrin and c-Met proteins were related to carcinogenesis, metastasis and prognosis of various sorts of tumors. In this study we detected the expressions of Ezrin and c-Met proteins in normal gastric mucosa, chronic atrophic gastritis, intestinal metaplasia, dysplasia and gastric carcinoma and analyzed the correlations with metastasis and prognosis of gastric carcinomas. The results demonstrated that both Ezrin and c-Met overexpression were related to the occurrence and progression of gastric carcinoma. Our findings also demonstrated that combined detection of these two tumor-specific biomarkers in gastric carcinomas can provide additional efficacy in predicting the patients' outcomes.

Key words: Ezrin, c-Met, Gastric Carcinoma, Metastasis, Prognosis

Introduction

Gastric carcinoma is one of the most common malignancies and the leading cause of cancer-related death worldwide (Jemal et al., 2008), particularly in Asia (Coburn et al., 2010). As other malignant tumors, the key reason of patients' death is extensive metastasis in gastric carcinoma. However, the molecular mechanism of tumor metastasis, heretofore, is still indistinct. Ezrin protein, a member of the ezrin-radixin-moesin (ERM) cytoskeleton-associated protein family of species-conserved protein in the band 4.1 superfamily, is a membrane cytoskeleton linker and regulates cytoskeletal-related functions such as cell adhesion, cell survival and cell motility, all of which are important in tumor progression and metastasis (Guo et al., 2008; Rasmussen et al., 2008; Nuesch et al., 2009). C-Met is the receptor of hepatocyte growth factor (HGF), which has mitogenic and morphogenic functions in various types of cells (Guo et al., 2008). Several studies showed that c-Met protein influences tumor invasion and metastasis on binding with HGF, which activates diverse intracellular signaling pathways, including phosphatidylinositol 3-kinase (PI3K)/serine-threonine protein kinase B (Akt), mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 (MAPK/ERK1/2), p38, and signal transducer and activation of transcription 3 (STAT3). Resent publications demonstrated that abnormal expression of Ezrin and c-Met proteins were related to carcinogenesis, metastasis and prognosis of various sorts of tumors. In current study, we investigated both Ezrin and c-Met protein expression in human gastric carcinoma and its precancerous lesions, and analyzed correlations of the both protein expression with gastric mucosa cancerization, lymph node metastasis and prognosis, in order to explore the correlations and significance of the both with gastric cancer progression and prognosis.

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Materials and methods

Clinicopathological data

One hundred and eighty-two patients with primary gastric carcinoma (GC) who underwent curative resection without radiotherapy or chemotherapy at the first Affiliated Hospital of China Medical University and Tumor Hospital of Liaoning Province between December 2003 and April 2007 were studied. The patients were comprised of 121 males and 61 females

with a median age of 58.4 years (range from 30 to 81 years). Specimens involved in this study comprised of 182 cases of primary gastric carcinoma including 2 early (EGC) and 180 advanced carcinoma (AGC), 108 cases of matched normal gastric mucosa (obtained at greater than 5 cm apart from the edge of primary tumor focus), 36 chronic atrophic gastritis (CAG), 62 dysplasia (Dys) and 55 intestinal metaplasia (IM). According to Borrmann's classification, gross types of AGC were classified as follows: 5 cases of Borrmann I, 33 cases of Borrmann II, 130 cases of Borrmann III, and 12 cases of Borrmann IV. In the light of the WHO's histological classification of GC, 182 cases were classified as follows: 4 papillary adenocarcinoma, 16 well and 57 moderately and 78 poorly differentiated adenocarcinoma, 3 undifferentiated carcinoma, 16 mucinous adenocarcinoma and 8 signet ring cell carcinoma (SRC). There were 77 cases of intestinal, 89 diffuse and 16 mixed type tumors according to Lauren's classification. There were 45 cases without and 137 cases with lymph node metastasis.

Tissue microarray construction and immunohistochemical staining

Samples were fixed in 10% formalin, embedded in paraffin and cut into 4 μm thick sections. All the samples were evaluated by two experienced pathologists to comfirm diagnoses, and marked various target lesions. Six blocks of tissue microarray containing gastric cancers and their precancerous lesions, lymph node metastasis were constructed using Microarrayer (USA), 4 μm consecutive sections were cut, one was preformed conventional H&E staining, the others were reserved at room temperature for further immunohistochemistry. Expression of Ezrin and c-Met proteins were detected using PV-9000 two-step immunohistochemical method. Mouse monoclonal antibody against human Ezrin was purchased from Neomarkers Company (clone number 3C12, working dilution 1:200). Rabbit polyclonal antibody against human c-Met (ready to use) and PV-9000 kit were from Beijing Zhongshan Goldenbridge Biotechnology Company (China). DAB kit was from Fuzhou Maixin Company (China). Tissue microarray slides were deparaffinized in xylene and hydrated with alcohol before being placed in 3% H_2O_2 methanol blocking solution, which was followed by heat-induced antigen retrieval. The slides were incubation with primary antibodies overnight at 4°C, then stained using the PV-9000 detection system and counterstained with hematoxylin. All procedures were implemented according to the manufacturer's instructions. For negative controls, sections were treated with 0.01 mol/L phosphate-buffered saline instead of primary antibodies.

Immunohistochemistry assessment

Both the intensity and the extent of staining were assessed. The positive cells of Ezrin and c-Met proteins were defined as that there was clearly brown granules located in cytoplasm or cytoplasmic membrane. Staining intensity initially was recorded on a four-point scale: 0, no staining; 1, light brown; 2, brown; 3, dark brown. The extent of staining also was initially assessed on a four-point scale: 0, no positive cell; 1, 1-10% positive cells; 2, 11%–50% positive cells; 3, 51%-75% positive cells and 4, >75% positive cells. According to above assessing criterion, the immunostaining results were classified into: 0-2, negative (-); 3-4, weakly positive (+); 6-8, moderately positive (++) and 9-12, strongly positive (+++). In present study, it was defined as specific positive case that the product of staining intensity and the percentage of positive cells was ≥3.

Statistical analysis

Categoric data are described using frequencies and percentages. Continuous data are described using means and standard deviations for normally distributed data. Statistical analysis was performed using SPSS 13.0 Package and χ^2 test or Fisher's exact test was used to differentiate the rates of different groups. Time-to-event data was estimated by the Kaplan-Meier method and analyzed with the log-rank test. The cumulative overall survival rates were calculated using life table techniques, illustrated by Kaplan-Meier plots. Multivariable analysis model was fit using a cox proportional hazards regression model using SPSS13.0. All statistical analysis were two sided, and significance was assigned at $P<0.05$.

Results

Ezrin protein expression in normal gastric mucosa, CAG, IM, Dys and GCs and the correlation with clinicopathological parameters

The positive rates of Ezrin protein expression in intestinal metaplasia (94.55%, 52/55), dysplasia (87.10%, 54/62) and gastric carcinoma (83.52%, 152/182) were significantly higher than that in normal gastric mucosa (59.25%, 64/108), respectively ($P<0.05$). In Lauren's types of gastric cancer, the positive rate of Ezrin protein in gastric cancer of intestinal type (93.51%, 72/77) was significantly higher than that in diffuse type (79.78%, 71/89), $P<0.05$. The positive expression of Ezrin protein in well-differentiated tubular adenocarcinoma (100.00%, 16/16) was significantly higher than that in moderately-differentiated tubular adenocarcinoma (91.23%, 52/57) and poorly-differentiated adenocarcinoma (78.21%, 61/78), $P<0.05$. In gastric cancer with lymph node metastasis, the positive rate of Ezrin protein (88.32%, 123/137) was significantly higher than that in the group without lymph node metastasis (68.89%, 31/45), $P<0.05$. There was no significant difference between the Ezrin expression in primary gastric cancer (71.43%, 15/21) and relevant lymph node metastatic tumor (76.19%, 16/21), and neither between Ezrin protein expression and patients' gender, age and Borrmann's type (Tables 1, 2, 5. Fig. 1).

C-Met protein expression in normal gastric mucosa, AGC, IM, Dys and GCs and the correlation with clinicopathological parameters

The positive rate of c-Met protein in chronic atrophic gastritis (55.56%, 20/36), intestinal metaplasia (63.64%, 35/55), dysplasia (61.29%, 38/62) and gastric cancer (65.93%, 120/182) were significantly higher than that in normal gastric mucosa (49.07%, 53/108), respectively (P<0.05) in Lauren's types of gastric cancer, the positive rate of c-Met protein in gastric cancer of intestinal type (80.52%, 62/77) was significantly higher than that in diffuse type (59.55%, 53/89), P<0.05. The positive expressive rate of c-Met protein in well-differentiated tubular adenocarcinoma (87.50%, 14/16) was significantly higher than that in moderately-differentiated tubular adenocarcinoma (78.95%, 45/57) and poorly-differentiated adenocarcinoma (60.26%, 47/78), P<0.05. In the group of gastric cancer with lymph node metastasis, the positive rate of c-Met protein (71.53%, 98/137) was significantly higher than that in the group without lymph node metastasis (48.89%, 22/45), P<0.05. There was no significant difference between the primary gastric cancer (66.67%, 14/21) and relevant lymph node metastases (74.13%, 15/21), and neither between c-Met protein expression and patients' gender, age and Borrmann's type, P>0.05 (Tables 3, 4, 5. Fig. 2).

Correlation of Ezrin peotein with c-Met protein expression in gastric carcinoma

The expression of Ezrin protein was statistically correlated with the expression of c-Met protein in 182 cases of gastric cancer, r_k=0.602, P<0.05 (Table 5, Figs. 1D, 2D).

Correlation between expressions of Ezrin and c-Met proteins in primary gastric carcinoma and relevant lymph node metastasis

In 21 cases of gastric carcinoma with lymph node

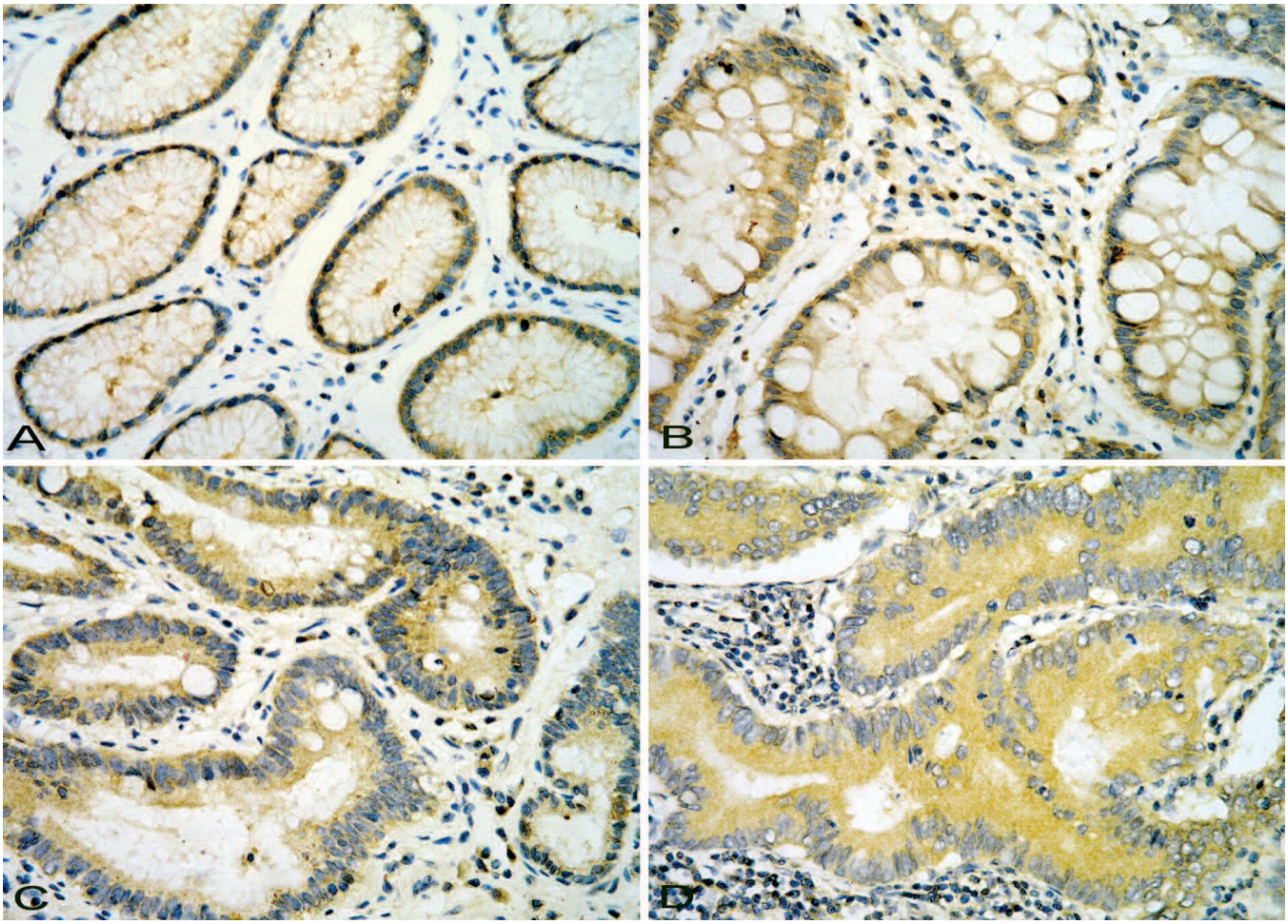

Fig. 1. The weakly positive expression of Ezrin protein in normal mucosa **(A),** strongly positive expression in intestinal metaplasia **(B)**, dysplasia **(C)** and gastric carcinoma (**D**). PV9000. A, x 200; B-D, x 400

metastasis, from which both primary and relevent lymph node metastatic tumors were collected for comparative study, the expression of Ezrin protein was closely correlated with c-Met expression in primary tumors (coefficient of correlation was 0.869), and so did in the relevant lymph node metastatic tumors (coefficient of correlation was 0.735) (P<0.05. Figs. 3, 4, Table 5).

Survival curves and Multivariate cox proportional hazards regression of patients with gastric carcinoma of different Ezrin and c-Met expressive patterns

Kaplan-Meier analysis of overall survival rates for 136 cases of gastric cancer patients (46 out of the 182 patients were lost from follow-up) categorized with positive and negative expression for Ezrin and c-Met expression. With a total follow-up of 65 months, 57 of the 136 assessable patients were still alive and 79 patients died. The overall survival (OS) for all patients was 41.91%. The OS for patients with negative and positive Ezrin expression was 50.00% and 41.12%, respectively. Patients with Ezrin positive tumors tended to have poorer prognosis than those with negative tumors (P=0.038). The OS of patients with negative and positive c-Met tumor was 44.44% and 41.00%, respectively. The patients with c-Met positive tumor

Table 1. Correlation between Ezrin expression and clinicopathological features of gastric carcinoma.

Groups	n	Ezrin expression				+~+++ (%)	χ^2	*P*
		-	+	++	+++			
Gender							3.117	0.374
Male	121	24	35	31	31	80.17		
Female	61	6	20	16	19	90.16		
Age (years old)							8.866	0.450
≤44	22	2	5	8	7	90.91		
45~59	60	9	15	21	15	85.00		
60~74	74	15	26	13	20	79.73		
≥75	26	4	9	5	8	84.62		
Gross types								
ECG:IIc	2	0	1	0	1	100.00		
AGC:Borrmann's type							6.976	0.069*
Bor.I+Bor.II	38	12	9	9	8	68.42		
Bor.III+Bor.IV	142	18	45	38	41	87.32		
WHO's histological types							12.741	0.005
Papillary. ade.	4	0	0	2	2	100.00		
Well-diff. ade.	16	0	3	7	6	100.00		
Moderately-diff.ade.	57	5	18	15	19	91.23		
Poorly-diff. ade.	78	17	21	20	20	78.21		
Undiff. ade.	3	0	3	0	0	100.00		
SRC	8	1	3	2	2	87.50		
Mucinous ade.	16	7	7	1	1	56.25		
Lauren types								0.002*
Intestinal type	77	5	21	24	27	93.51	7.869	0.049
Diffuse type	89	18	27	22	22	79.78		
Mixed type	16	7	7	1	1	56.25		
Lymph node metastasis							15.775	0.001
No	45	14	5	11	15	68.89		
Yes	137	16	50	36	35	88.32		

*: Fisher's exact test; ade.: Adenocarcinomas; diff.: Differentiated.

Table 2. Expression of Ezrin protein in normal gastric mucosa, CAG, IM, Dys and GC.

Groups	n	Ezrin protein expression				+~+++ (%)	χ^2	*P*
		-	+	++	+++			
							48.250[w]	0.0001[w]
Normal	108	44	31	18	15	59.25	5.648[a]/30.171[b]	0.130[a]/0.0001[b]
CAG	36	7	14	7	8	80.56	7.808[c]/1.249[d]	0.005[c]/0.741[d]
IM	55	3	15	13	24	94.55	6.727[e]/7.428[f]	0.081[e]/0.059[f]
Dys	62	8	23	17	14	87.10	14.809[g]/1.532[h]	0.002[g]/ 0.675[h]
GC	182	30	55	47	50	83.52	23.798[i]/1.674[j]	0.0001[i]/0.643[j]

[w]: overall compared; [a]: Normal mucosa vs CAG; [b]: Normal mucosa vs IM; [c]: CAG vs IM; [d]: CAG vs Dys; [e]: IM vs Dys; [f]: IM vs GC; [g]: Normal mucosa vs Dys; [h]: Dys vs GC; [i]: Normal mucosa vs GC; [j]: CAG vs GC.

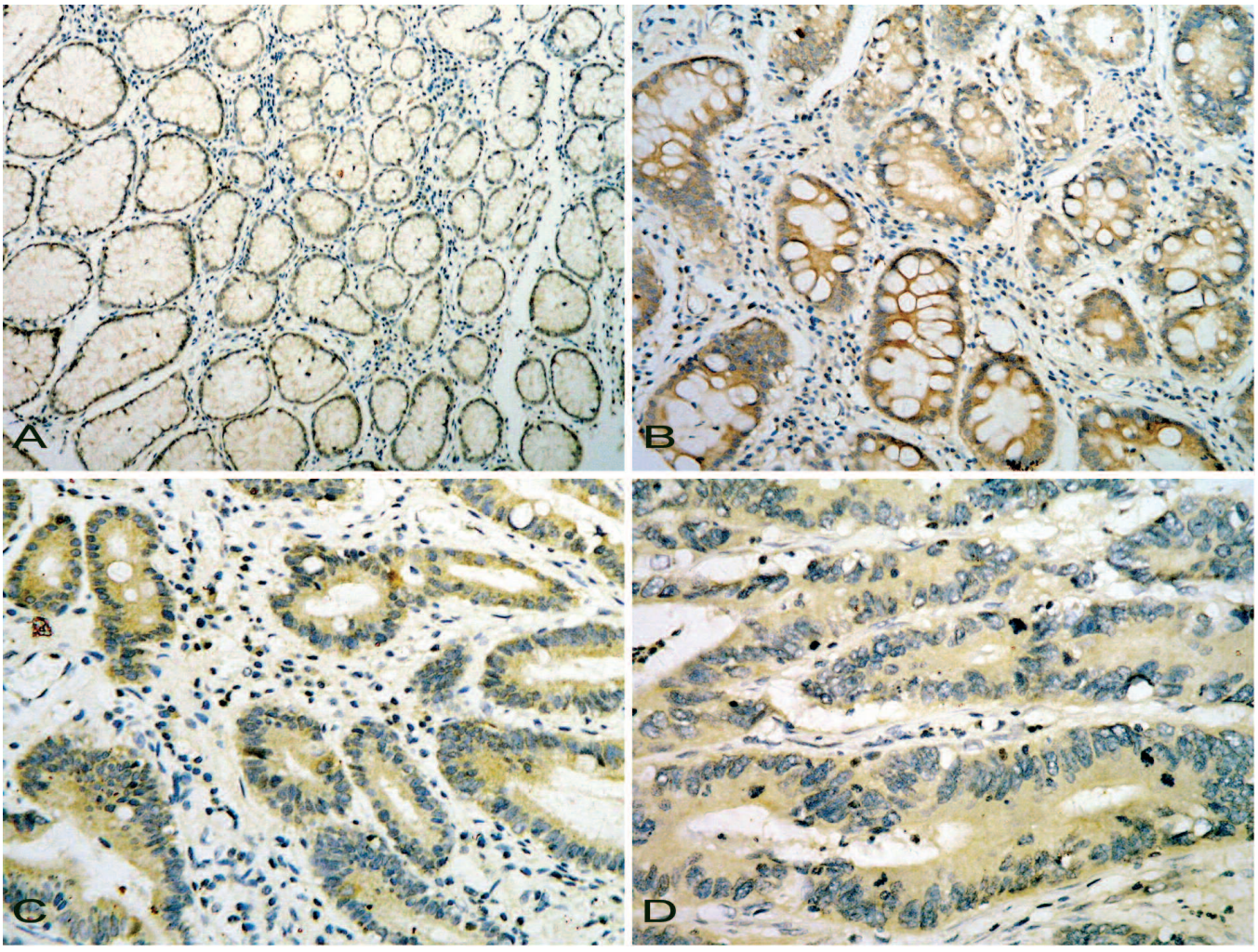

Fig. 2. The weakly expression of c-Met protein in normal mucosa **(A)**, strongly positive expression in intestinal metaplasia **(B)**, dysplasia **(C)** and gastric carcinoma **(D)**. PV9000. A, x 200; B-D, x 400

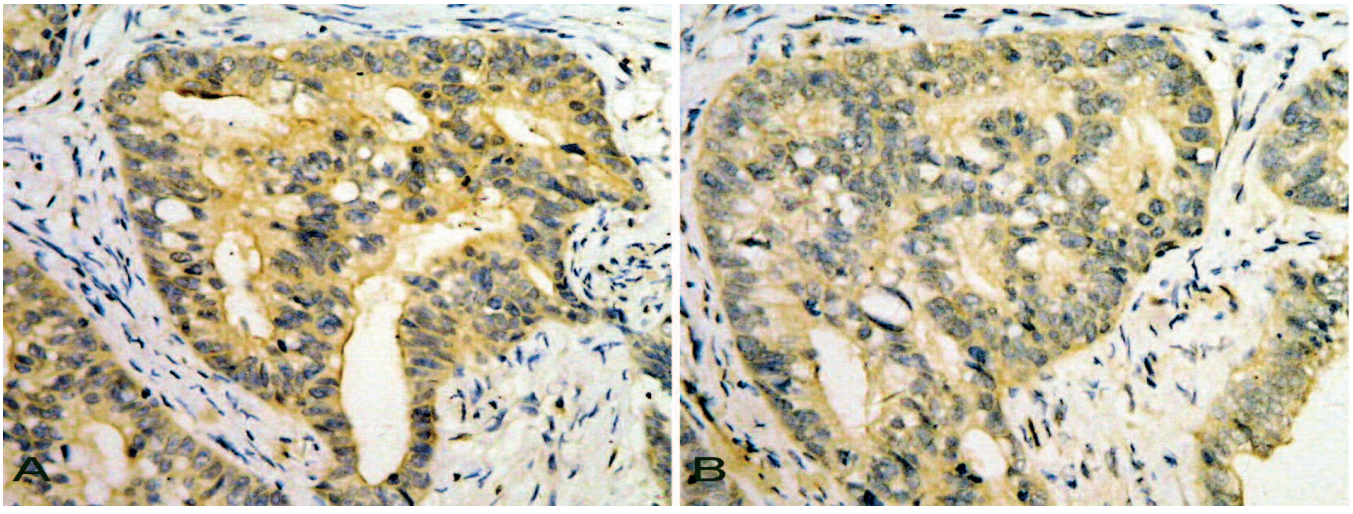

Fig. 3. The strongly positive expression of Ezrin **(A)** and c-Met **(B)** protein in primary gastric carcinoma. PV9000. x 400

showed significantly poorer survival than those with c-Met negative tumor (P=0.031). Of three expressive patterns of Ezrin and c-Met proteins, the patients with $Ezrin^+/c\text{-}Met^+$ expressive pattern always showed the worst outcome, and those with $Ezrin^-/c\text{-}Met^-$ expressive pattern always showed the best outcome, whereas the patients with either $Ezrin^+/c\text{-}Met^-$ or $c\text{-}Met^+/Ezrin^-$ tumors showed different outcomes between $Ezrin^+/c\text{-}Met^+$ and $Ezrin^-/c\text{-}Met^-$ groups, P=0.035 (Fig. 5).

Multivariate cox proportional hazards regression of

Table 3. Correlation between c-Met expression and clinicopathological features of gastric carcinoma.

Groups	n	c-Met expression				+~+++ (%)	χ^2	*P*
		-	+	++	+++			
Gender							0.677	0.879
Male	121	43	50	16	12	64.46		
Female	61	19	25	9	8	68.85		
Age(years old)							7.373	0.288
≤44	22	5	9	2	6	77.27		
45~59	60	18	30	8	4	70.00		
60~74	74	26	29	11	8	64.86		
≥75	26	13	7	4	2	50.00		
Gross types								
ECG:IIc	2	1	1	0	0	50.00		
AGC:Borrmann's type							5.391	0.145
Bor.I+Bor.II	38	18	14	2	4	52.63		
Bor.III+Bor.IV	142	43	60	23	16	69.72		
WHO's histological types							19.101	0.0001
Papillary. ade.	4	1	2	1	0	75.00		
Well-diff. ade.	16	2	7	4	3	87.50		
Moderately-diff.ade.	57	12	24	9	12	78.95		
Poorly-diff. ade.	78	31	33	10	4	60.26		
Undiff. ade.	3	1	2	0	0	66.67		
SRC	8	4	3	1	0	50.00		
Mucinous ade.	16	11	4	0	1	31.25		
Lauren types								0.001*
Intestinal type	77	15	33	14	15	80.52	14.938	0.002
Diffuse type	89	36	38	11	4	59.55		
Mixed type	16	11	4	0	1	31.25		
Lymph node metastasis							8.467	0.037
No	45	23	12	5	5	48.89		
Yes	137	39	63	20	15	71.53		

*: Fisher's exact test; ade.:adenocarcinoma; diff.: Differentiated.

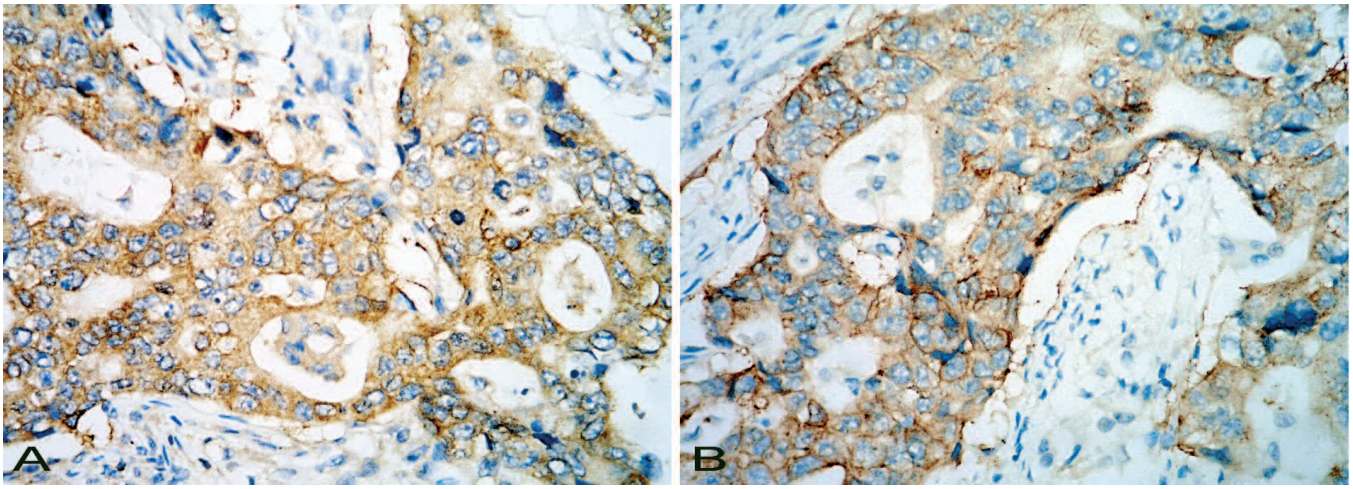

Fig. 4. The strongly positive expression of Ezrin **(A)** and c-Met **(B)** protein in lymph node metastatic tumor from relevant primary gastric carcinoma. PV9000. x 400

136 patients with gastric carcinoma was used to adjust the survival for the effect of independent predictors of prognosis. These included the presence of Ezrin positive expression (P=0.029, hazard ratio [HR] 2.594), c-Met positive expression (P=0.023 HR 1.879), and lymph node metastasis of gastric cancer (P=0.021; HR 2.029

Table 4. Expression of c-Met protein in normal gastric mucosa, CAG, IM, Dys and GC.

Groups	n	c-Met expression				+~+++ (%)	χ^2	*P*
		-	+	++	+++			
								0.001[w*]
Normal	108	55	40	13	0	49.07		0.003[a*]/0.006[b*]
CAG	36	16	9	7	4	55.56		0.36[c*]/0.732[d*]
IM	55	20	17	16	2	63.64	0.083[e]/9.435[f]	0.959[e]/0.024[f]
Dys	62	24	19	15	4	61.29	5.675[h]	0.005[g*]/0.129[h]
GC	182	62	75	25	20	65.93	17.091[i]	0.001[i] /0.321[j*]

*: Fisher's exact test; [w]: overall compared; [a]: Normal mucosa vs CAG; [b]: Normal vs IM; [c]: CAG vs IM; [d]: CAG vs Dys; [e]: IM vs Dys; [f]: IM vs GC; [g]: Normal mucosa vs Dys; [h]: Dys vs GC; [i]: Normal mucosa vs GC; [j]: CAG vs GC.

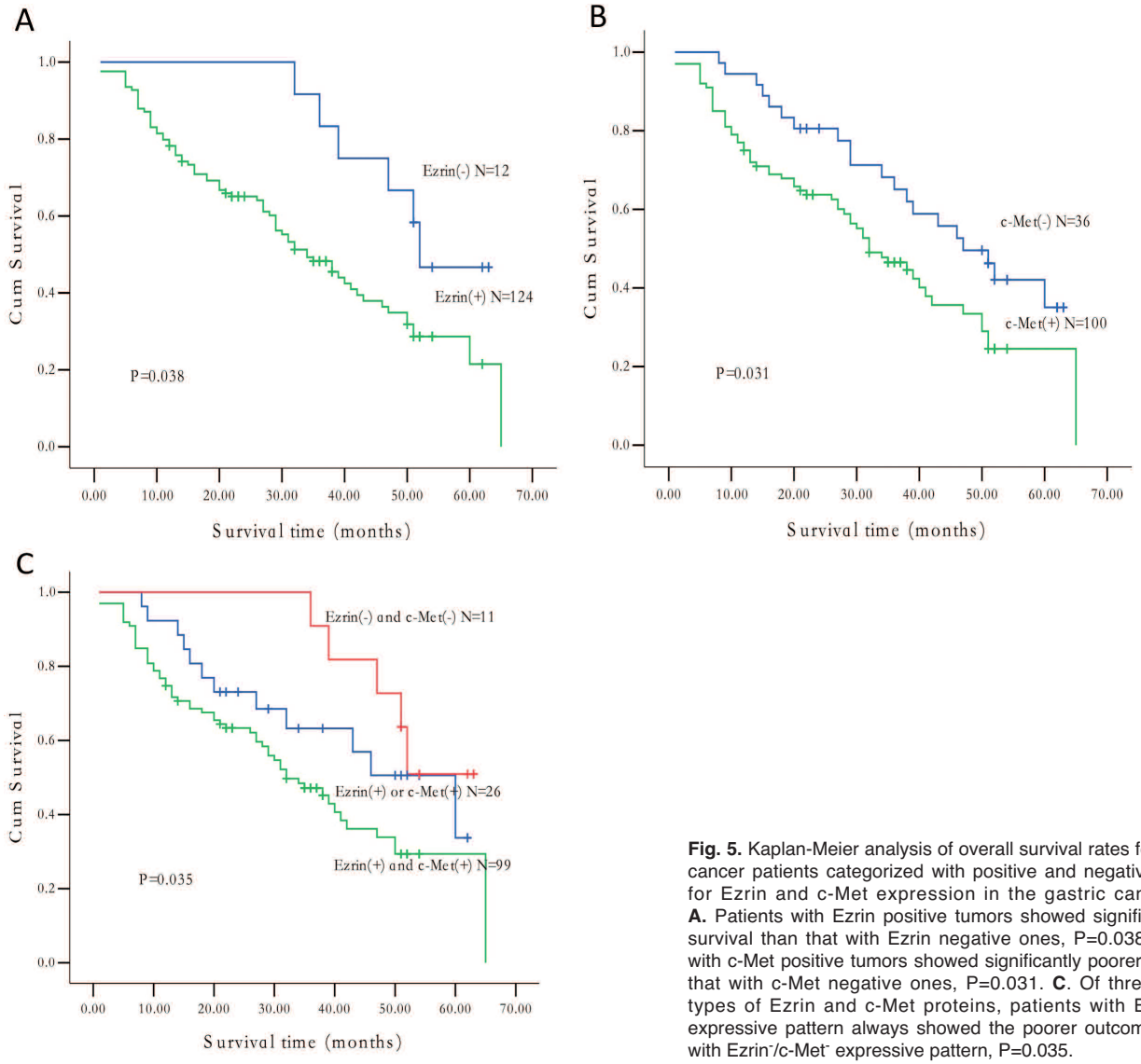

Fig. 5. Kaplan-Meier analysis of overall survival rates for 136 gastric cancer patients categorized with positive and negative expression for Ezrin and c-Met expression in the gastric cancer tissues. **A.** Patients with Ezrin positive tumors showed significantly poorer survival than that with Ezrin negative ones, P=0.038. **B.** Patients with c-Met positive tumors showed significantly poorer survival than that with c-Met negative ones, P=0.031. **C**. Of three expressive types of Ezrin and c-Met proteins, patients with $Ezrin^+/c\text{-}Met^+$ expressive pattern always showed the poorer outcome than those with $Ezrin^-/c\text{-}Met^-$ expressive pattern, P=0.035.

Table 5. Correlation between Ezrin and c-Met expression in primary gastric carcinoma and lymph node metastasis.

C-Met expression	Ezrin expression				Total	r_k	*P*
	-	+	++	+++			
In 182 primary GCs						0.602	<0.01
-	29	20	6	7	62		
+	1	32	24	18	75		
++	0	2	14	9	25		
+++	0	1	3	16	20		
Total	30	55	47	50	182		
In 21 primary GCs with Ln Mets						0.869	<0.01
-	6	0	1	0	7		
+	0	4	1	0	5		
++	0	0	6	0	6		
+++	0	0	1	2	3		
Total	6	4	9	2	21		
In 21 relevent Ln Mets						0.735	<0.01
-	4	1	0	1	6		
+	1	3	1	0	5		
++	0	0	6	0	6		
+++	0	0	1	3	4		
Total	5	4	8	4	21		

*Ln Mets: lymph node metastasis.

Table 6. Multivariate cox regression analysis of survival of 136 GC patients with Ezrin expression.

Variable	Regression Coefficient (± SE)	*P*	Hazard Ratio (95.0% CI)
Ezrin expression	0.953±0.438	0.029	2.594 (1.100-6.115)
Gender	0.087±0.248	0.726	1.091 (0.671-1.774)
Age	-0.220±0.235	0.349	0.803 (0.507-1.272)
Borrmann's type	-0.106±0.198	0.590	0.899 (0.610-1.324)
Histological types	0.024±0.192	0.901	1.024 (0.704-1.491)
Lauren's types	0.196±0.408	0.631	1.217 (0.547-2.709)
Lymph node metastasis	0.708±0.307	0.021	2.029 (1.111-3.705)

Table 7. Multivariate cox regression analysis of survival of 136 GC patients with c-Met expression.

Variable	Regression Coefficient (± SE)	*P*	Hazard Ratio (95.0% CI)
c-Met expression	0.631±0.278	0.023	1.879 (1.089-3.241)
Gender	0.190±0.248	0.445	1.209 (0.743-1.960)
Age	-0.289±0.240	0.228	0.749 (0.468-1.199)
Borrmann's types	-0.057±0.196	0.772	0.945 (0.643-1.388)
Histological types	0.074±0.196	0.708	1.076 (0.732-1.582)
Lauren's types	0.197±0.408	0.630	1.217 (0.547-2.710)
Lymph node metastasis	0.612±0.304	0.044	1.843 (1.016-3.344)

and P=0.044, HR 1.843, respectively) (Tables 6, 7).

Discussion

Human Ezrin gene maps to chromosome 6q25.2-q26 and the total length of mRNA is 3166 bp, encoding 585 amine acids. Ezrin protein, a cytoplasmic peripheral membrane protein serves as an intermediate between the plasma membrane and the actin cytoskeleton. It promotes cell motility by linking the actin cytoskeleton to the plasma membrane through the membrane-spanning ECM receptor CD44 and plays a key role in cell surface structure, adhesion, migration, and organization (Crepaldi et al., 1997; Ma et al., 2003). The protein is also a downstream effector of the Rho kinase signaling pathway (Orian et al., 2002; Suzuki et al., 2003) and frequently overexpressed in metastatic tumor cells. Overexpression of Ezrin protein and the loss of RhoGDP-dissocialion inhibitor (Rho-GDI) might alter the balance of the intracellular signaling cascade and result in amplification of metastasis-associated signaling from the plasma membrane through the Rho-associated signal transduction pathways, finally, leading to tumor metastasis (Hunter, 2004). Ezrin has been shown to bind directly to PI3K and influence a number of signaling pathways that affect cellular functions related to tumorigenesis and metastasis, including the MAPK-ERK1/2, PI3K-Akt and Rho pathways. Ezrin-mediated effects on Akt and ERK 1/2 activity have been linked to the ability of Ezrin to interact with the Akt-mediated cellular apoptotic mechanism (Sizemore et al., 2007). Recently, some researches also showed that Ezrin protein play an important role in tumor progression and development of metastasis (Zhai et al., 2010; Zhou et al., 2010).

In this study, we demonstrated that the positive rates of Ezrin protein in intestinal metaplasia and intestinal type gastric cancer were significantly higher than those in normal gastric mucosa, whereas there was not significant difference between the expression of Ezrin protein in intestinal metaplasia and intestinal type gastric cancer, suggesting that Ezrin protein was associated with histiocytic intestinal phenotype and may be involved in the occurrence of intestinal metaplasia and intestinal type gastric cancer. Bal detected Ezrin expression by immunohistochemistry and found that the expression of Ezrin protein in the intestinal type adenocarcinoma was higher than that in the diffuse type, which was consistent with the current research (Bal et al., 2007). Shi found strong Ezrin immunreactivity in gastric cancer tissues and the positive rate of Ezrin protein had positive correlation with lymph node metastasis of gastric cancer (Shi et al., 2006). We found a similar expression pattern of Ezrin using immunohistochemistry: among 182 cases of gastric cancer, 88.32% of cases with lymph node metastasis showed positive expression of Ezrin protein and 68.89% in the group without lymph node metastasis. Recently, many researches showed that Ezrin protein might be associated with the occurrence, metastasis and prognosis of several kinds of tumors, such as hepatocellular carcinoma (Yeh et al., 2009), lung cancer (Deng et al., 2007), osteosarcoma (Kim, et al., 2009), etc. The results presented in the current study suggested

that Ezrin protein may be involved with the occurrence of gastric carcinoma and have a promotive effect on the process of lymph node metastasis. The potential for Ezrin to coordinate and amplify metastasis-associated cell-surface signals, and to alter the balance of the intracellular signaling cascade, suggests it mediates many of the changes that are required for a tumor cell to successfully form a secondary lesion. Ezrin protein may be served as an adjuvant factor for predicting lymph node metastasis of gastric cancer.

C-Met gene, a proto-oncogene, maps to human chromosome 7q21-31 and is about 120kb long. C-Met encodes a transmembrane tyrosine kinase receptor for hepatocyte growth factor (HGF), which has mitogenic and morphogenic functions in various types of cells (Guo et al., 2008). C-Met plays an important role in tumorigenesis. The pleiotropic cellular effects of HGF are transduced through activation of its transmembrane receptor tyrosine kinase c-Met. On HGF binding, c-Met undergoes dimerization and autophosphorylation on tyrosine residues, generating multidocking sites, which activate diverse intracellular signaling pathways. ERK1/2 and PI3K/Akt signaling pathways are two important kinase cascades that mediate HGF-induced invasion and metastasis (Ye et al., 2008). Several studies have found that c-Met protein influences tumor invasion and metastasis by the following mechanisms: 1) By inducing tumor proliferation and angiogenesis of stroma cells, c-Met protein promotes the progression of tumor directly or indirectly (Ide et al., 2006). 2) By inducing the production of matrix catabolic enzymes, impairing the basal membrane and upregulating proteolytic activity, c-Met protein promotes tumor invasion and metastasis (Sawada et al., 2007). 3) By up-regulating the synthesis of cyclooxygenase-2 (COX-2) and prostaglandin (PGE). PGE enhances activation of c-Met, possibly via activation of prostanoid receptors, in turn cross-linking growth factor receptors and subsequent downstream signal transduction, thus influencing the growth and migration of tumor cells (Tuynman et al., 2008). The results presented in the current study suggested that the positive rates of c-Met protein in intestinal metaplasia, dysplasia and gastric cancer were significantly higher than that in normal gastric mucosa. This finding was consistent with previous report of Chen (Chen et al., 2007). Uen detected the levels of c-Met mRNA expression by RT-PCR approach and indicated that the levels of c-Met was significantly correlated with lymph node metastasis, agreeing with the present results, suggesting that the possibility of tumor metastasis is greater when c-Met protein is expressed at a higher level (Uen et al., 2006).

Our results showed that a significant association between Ezrin protein and c-Met protein expressions in 182 cases of gastric carcinoma (r_k=0.602, P<0.01), so we presumed that overexpressed Ezrin and c-Met proteins together participated in occurrence and progression of gastric cancer through activating diverse intracellular signaling transduction pathways, including Rho, PI3K/Akt, Ras/Erk, etc. Ezrin has been shown to associate with the product of the Met gene (Crepaldi et al., 1997), the hepatocyte growth-factor receptor, which has previously been implicated in the progression of several human cancers (Ma et al., 2003). Hunter (2004) has reviewed that Ezrin functions as a downstream target for cell-surface receptors, including the c-Met receptor and CD44. Both Ezrin and c-Met protein have been implicated in cell motility, suggesting that Ezrin is involved in mediating cellular invasion. In the meantime, stimulation of the CD44 receptor also results in both the upregulation and activation of c-Met protein (Suzuki et al., 2003). Recent evidence has also demonstrated that these proteins cooperate in signaling to the MEK/ERK pathway through the ERM-binding domain of CD44 (Orian et al., 2002). The over-expression of Ezrin protein would disrupt the normal balance of the cellular signaling network by sequestering negative regulators of the signal transduction pathway and amplify the pro-metastasis signals originating from cell-surface molecules, such as CD44 or the c-Met (Steeg, 2003).

In this study, Kaplan-Meier analysis demonstrated that the overall survival rate of 136 patients with Ezrin or c-Met positive gastric cancers were significantly lower than those in patients with Ezrin or c-Met negative tumors (P<0.05). The results showed that Ezrin and c-Met were prognostic factors in gastric cancer and patients with high Ezrin or c-Met expression tumors had a significantly poorer prognosis than those with Ezrin or c-Met negative ones. Of three expressive patterns of Ezrin and c-Met expression, the patients with $Ezrin^+/c\text{-}Met^+$ expressive pattern always showed the worst outcome, and those with $Ezrin^-/c\text{-}Met^-$ expressive pattern always showed the best outcome, whereas the patients with either $Ezrin^+/c\text{-}Met^-$ or $c\text{-}Met^+/Ezrin^-$ tumors showed different outcomes between $Ezrin^+/c\text{-}Met^+$ and $Ezrin^-/c\text{-}Met^-$ groups, P=0.035. The median overall survival in patients with $Ezrin^+/c\text{-}Met^+$ tumor was 33 months (1~65 months), while 49.5 months (36~63 months) in patients with $Ezrin^-/c\text{-}Met^-$ tumor. Multivariate cox proportional hazards regression of 136 patients with gastric carcinoma indicated that the presence of Ezrin positive expression, c-Met positive expression and lymph node metastasis of gastric cancer were high hazard factors for gastric carcinoma. Our findings demonstrate that combined detection of these two tumor-specific biomarkers in gastric carcinomas can provide additional efficacy in predicting patients' outcomes. So far, there has been no similar description compared with our study, although several previous papers described the correlation between Ezrin and c-Met protein in gastirc carcinomas.

In conclusion, the results of our study demonstrated that both Ezrin and c-Met overexpression were related to the occurrence and progression of gastric carcinoma. The up-regulated expression of Ezrin and c-Met, as prognostic markers, could be used in predicting the outcome in patients with gastric carcinoma. However, it

is unlikely that all of the functions and mechanisms of Ezrin and c-Met have been investigated. For example, Ezrin is known to be involved in Fas-mediated apoptosis in lymphocytes (Lozupone et al., 2004), but whether it have a role in metastasis-associated apoptosis resistance have not been clear. A large amount of work is still required to explore the functions and mechanisms of Ezrin and c-Met. The regulation of interaction between Ezrin and c-Met proteins is unidentified and requires further investigation.

Acknowledgements. This research was supported by National Natural Science Foundation of China (No.30973503; No.81071650) and Special foundation for Climbing Scholars of Universities in Liaoning Province (2009-2011).

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Accepted December 12, 2010