



Expression and significance of EMMPRIN, MMP-9 and TIMP-1 in non-small cell lung carcinoma tissues of Guangxi Province, China

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Background: Although radical operation to treat lung cancer is thought to be an important treatment measure, prognosis of advanced lung cancer is still poor. A reasonable treatment procedure for advanced lung cancer is an issue worthy of investigating nowadays. The further research on the role and the co-relationship of EMMPRIN, MMP-9, TIMP-1 will not only help to approach the mechanisms of the non-small cell lung carcinoma (NSCLC) incidence and development in depth, but also offer help for the gene targeted therapy of NSCLC. This study was to examine the expression and significance of tumor metastasis genes *EMMPRIN*, *MMP-9*, *TIMP-1* in Guangxi NSCLC tissues, cancer adjacent tissues and benign lung tissues.

Methods: Reverse transcription polymerase chain reaction (RT-PCR) was used to measure the expression level of EMMPRIN, MMP-9, TIMP-1 in 80 cases of NSCLC, 40 cases of tumor adjacent tissues and 10 cases of benign lesion lung tissues, then we analysed the sequence of PCR products of these gene.

Results: The positive expression rate of EMMPRIN, MMP-9 and TIMP-1 in lung cancer cells was higher than in the cancer adjacent tissues ($P < 0.05$), no expression in benign lung tissues. In NSCLC, the expression of EMMPRIN, MMP-9, TIMP-1 showed an increasing trend with the increase of pathological tumor classification and TNM staging and these gene expression was positively correlated with the lymph node metastasis ($P < 0.05$). EMMPRIN, MMP-9, TIMP-1 expression had no relationship with gender, age, smoking or not, ethnic group, and pathological type ($P > 0.05$). Patients with EMMPRIN or MMP-9 negative expression had a significantly longer OS ($P = 0.016$ and $P = 0.026$) than those with positive expression.

Conclusions: In NSCLC, the detected level of EMMPRIN, MMP-9 and TIMP-1 was higher than in cancer adjacent tissue and benign lung tissue, suggesting that they were involved in NSCLC incidence and development; EMMPRIN, MMP-9, TIMP-1 expression can not only serve as the indicator to determine the pathological classification, metastasis and prognosis of NSCLC, but also offer help for targeted gene therapy of NSCLC.

Keywords: Non-small cell lung cancer (NSCLC); reverse transcription polymerase chain reaction (RT-PCR); EMMPRIN; MMP-9; TIMP-1

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Introduction

The invasion and metastasis are the most important biological characteristics of malignant tumors, and also are the major reasons for patient treatment failure and mortality.

The invasion and metastasis of lung cancer is a multi-stage, multi-step, sequential process involved with multiple factors, including invasion, circulating pervasion, jump clone and angiogenesis (1). The existing research recognized that

such genes as *EMMPRIN*, *MMP-9*, *TIMP-1* etc. are ones of the primary regulation genes functioning in the invasion and metastasis of lung cancer. The tumor cells destruct the basement membrane on its primary site, go through the interstitial, enter and pierce the blood vessels and being located in the secondary site, which all are associated with the hydrolysis and remodeling of extracellular matrix (ECM) (2). *MMP-9* is the main component of ECM degradation, which can degrade almost all ingredients of the ECM, making them play an important role in cell proliferation, tumor invasion and angiogenesis, etc. Its inhibitor (*TIMP-1*) can form 1:1 complexes together with *MMP-9*, inhibiting the *MMP-9* roles in lung cancer invasion and metastasis (3). *EMMPRIN* is an induce factor of *MMP-9*, and it is closely related with the tumor evolution: it can promote tumor growth by increasing the production quantity of the transparent methane, and can degrade the basement membrane and ECM by stimulating stromal cells and tumor cells to produce a variety of matrix metalloproteinases, thereby to promote the tumor invasion and metastasis (4). For understanding the changes that occur in gene mRNA expression during carcinogenesis may result in improvement of the diagnosis, treatment, and prevention of non-small cell lung carcinoma (NSCLC). We examined, by the way of reverse transcription polymerase chain reaction (RT-PCR), 80 cases of NSCLC patient cancer tissues, 40 cases of cancer adjacent tissues and 10 cases of benign lung tissue in Guangxi, China, to explore the role of the *EMMPRIN*, *MMP-9*, *TIMP-1* expression in NSCLC incidence and development, which will offer help for the clinical diagnosis, therapy and to predict overall survival in this cohort of patients.

Methods

Materials

We collected, from First Affiliated Hospital of Guangxi Medical University since May, 2007 to October, 2009, 80 resected fresh specimens of NSCLC patients, among these, 51 males and 29 females; age between 40–76 (58.0±6.7) years; 55 cases of ethnic Han, 25 cases of Zhuang; all without any preoperative radiotherapy and chemotherapy. According to 2004 WHO Classifications of Lung Tumor, these specimens were re-classified into 45 cases of squamous carcinoma and 35 cases of adenocarcinoma; and by the differentiation degree, divided into 49 cases of well and medium differentiation, 31 cases of poorly differentiated. According to 1997 Revisions in the International System for Staging Lung Cancer (I–IV), the specimens were divided into 48 cases of early (I–II period),

and 32 cases of advanced (III–IV period); 33 cases with lymph node metastasis pathologically confirmed, and 47 cases without lymph node metastasis; 47 cases of long-term smoking (daily smoking >20 cigarettes, over 20 years of continuous smoking, smoking index >400), 33 cases of non-smokers; 40 cases of cancer adjacent tissues (more than 5 cm tumor, the lung tissues without cancer invasion pathologically confirmed) and 10 cases of benign lung tissues were as the control group.

Total RNA extraction and reverse transcription

According to Trizol reagent instructions, total RNA was extracted from the experiment group and the control group. After the reverse transcription reaction referring to M-MLV reverse transcriptase kit instructions, the synthesized cDNA was reserved at –20 °C storage.

PCR reaction

The cDNA as a template, we proceed the amplification according to the appropriate primers and reaction conditions. The upstream primer of *EMMPRIN* was 5'-GAGAGCAGGTTCTTCGTGAGTTC-3', the downstream primer was 5'-GCCTTTGTTCATTCTGGTGCTG-3', and the PCR product was 318 bp. With 3 min at reaction conditions 95 °C, 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C, 5 min at 72 °C, we proceed 40 cycles. The upstream primer of *MMP-9* was 5'-TTGACAGCGACAAGAAGTGG-3', the downstream primer was 5'-GCCATTCACGTCGTCCTTAT-3', and the PCR product was 179 bp. With 3 min at reaction conditions 94 °C, 45 s at 94 °C, 50 s at 54.5 °C, 60 s at 72 °C, 5 min at 72 °C, we proceed 32 cycles. The upstream primer of *TIMP-1* was 5'-GGCATCCTGTTGTTGCTGTGG-3', the downstream primer was 5'-GACGGGACTGGAAGCCCTTTT-3', PCR product was 529 bp. With 3 min at reaction conditions 94 °C, 45 s at 94 °C, 30 s at 54 °C, 60 s at 72 °C, 8 min at 72 °C, we proceed 35 cycles. Reaction system was 25 µL, the reaction products were put in 2% agarose gel for electrophoresis, and observing the target band in gel imaging system. Beijing Nuosai Genome Research Center Co., Ltd. sequenced the reaction products, and the target products were confirmed after comparing the detected sequence in PUB MED.

Statistical

All experimental data applied SPSS 13.0 statistical analysis software for statistical analysis, the test methods used χ^2 test, the

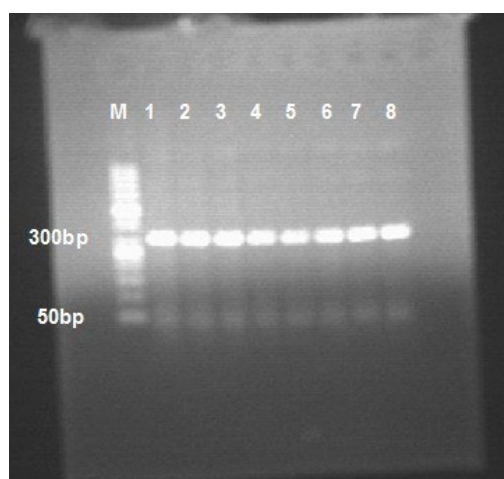


Figure 1 The expression of EMMPRIN in NSCLC tissues. Through examining *EMMPRIN* gene in NSCLC tissues and cancer adjacent lung tissues by RT-PCR, 318bp fragment was obtained as a result. M, marker; 1,2 NSCLC tissues, 1,2 positive expression. NSCLC, non-small cell lung carcinoma.

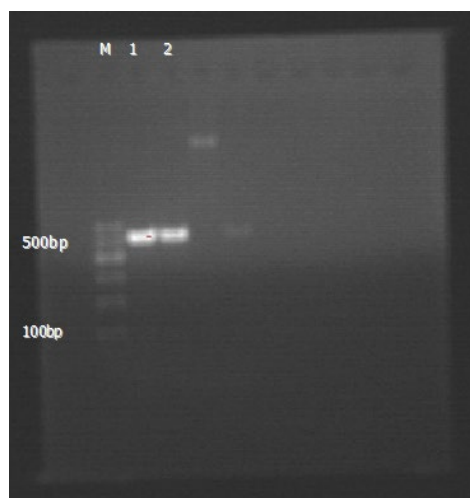


Figure 2 The expression of TIMP-1 in NSCLC tissues. Through examining *TIMP-1* gene in NSCLC tissues and cancer adjacent lung tissues by RT-PCR, 518bp fragment was obtained as a result. M, marker; 1,2 NSCLC tissues, 1,2 positive expression. NSCLC, non-small cell lung carcinoma.

relationship of EMMPRIN, MMP-9, TIMP-1 expressions in NSCLC tissues was analyzed through the correlation matrix, the Kaplan-Meier method and Log-rank test were used to analyze the correlation of patient survival with gene expression, $P < 0.05$ showed the statistically significant difference.

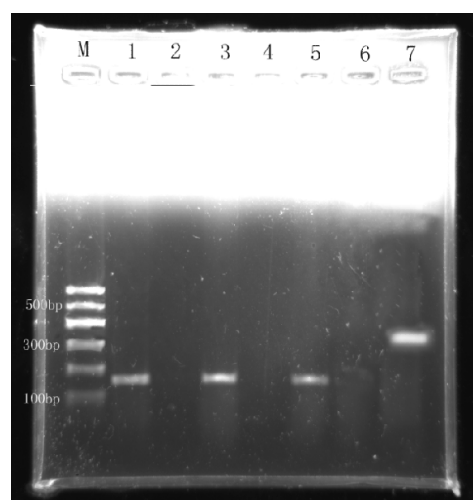


Figure 3 The expression of MMP-9 in NSCLC tissues. Through examining *MMP-9* gene in NSCLC tissues and cancer adjacent lung tissues by RT-PCR, 179bp fragment was obtained as a result, with an internal control fragment 334bp. M, marker; 7, β -actin; 1–6 NSCLC tissues, 1,3,5 positive expression; 2,4,6 negative expression. NSCLC, non-small cell lung carcinoma.

Results

The expression of *EMMPRIN*, *MMP-9* and *TIMP-1* gene: RT-PCR results (Figures 1-3).

Sequence comparison of PCR results and sequence figures (Figures 4-6).

The *EMMPRIN* gene expression levels in lung cancer and cancer adjacent lung tissues were 68.7% (53/80), 42.5% (17/40) respectively, the *MMP-9* gene expression levels in lung cancer and cancer adjacent lung tissues were 71.25% (57/80), 45.0% (18/40) respectively. The *TIMP-1* gene expression levels in lung cancer and cancer adjacent lung tissues were 50.0% (60/80), 25.0% (10/40) respectively. All three genes had no expression in benign lung tissues. The expression levels of three genes in the lung cancer tissues were higher than in the cancer adjacent lung tissues, and the difference was statistically significant.

The relationship between EMMPRIN, MMP-9, TIMP-1 expressions and clinical pathologic parameters

The expression of *MMP-9* and *TIMP-1* genes in NSCLC tissues had no relation with patient age, gender, smoking or not, ethnic group, pathological types ($P > 0.05$), and had relationship with tumor differentiation degree, lymph node metastasis and the clinical staging ($P < 0.05$) (Table 1).

```
>[ref|NM_004994.2|] UEGM Homo sapiens matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase,
92kDa type IV collagenase) (MMP9), mRNA
Length=2387

GENE ID: 4318 MMP9 | matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase,
92kDa type IV collagenase) [Homo sapiens] (Over 100 PubMed links)

Sort alignments for this subject sequence by:
E value Score Percent identity
Query start position Subject start position

Score = 254 bits (137), Expect = 3e-65
Identities = 140/141 (99%), Gaps = 1/141 (0%)
Strand=Plus/Plus

Query 9 GATA-AGTTTGTCTCCTCGTGGCGGCGCATGAGTTCGGCCACGCGCTGGGCTTAGATCATT 67
      |||
Sbjct 1194 GATACAGTTTGTCTCCTCGTGGCGGCGCATGAGTTCGGCCACGCGCTGGGCTTAGATCATT 1253

Query 68 CCTCAGTGCCGGAGGCGCTCATGTACCCATATGTACCGCTTCACTAGGGGGCCCCCTTGC 127
      |||
Sbjct 1254 CCTCAGTGCCGGAGGCGCTCATGTACCCATATGTACCGCTTCACTAGGGGGCCCCCTTGC 1313

Query 128 ATAAGGACGACGTGAATGGCA 148
      |||
Sbjct 1314 ATAAGGACGACGTGAATGGCA 1334
```

Figure 4 The homologous comparison result of PCR products of *MMP-9* gene mRNA in NSCLC tissues. PCR, polymerase chain reaction; NSCLC, non-small cell lung carcinoma.

```
>[ref|NM_198589.1|] UEGM Homo sapiens basigin (Ok blood group) (BSG), transcript variant
2, mRNA
Length=1676

GENE ID: 682 BSG | basigin (Ok blood group) [Homo sapiens]
(Over 100 PubMed links)

Score = 505 bits (273), Expect = 2e-140
Identities = 285/290 (99%), Gaps = 4/290 (1%)
Strand=Plus/Plus

Query 8 CGGTC-GAGCTACACATTGAG-ACCTGAACATGGAGGCGGACCCCGGCCATACCGGTGC 65
      |||
Sbjct 594 CGGTCAGAGCTACACATTGAGAACCTGAACATGGAGGCGGACCCCGGCCATACCGGTGC 653

Query 66 AACGGCACCAGCTCCAAGGGCTCCGACGAGGCCATCATCAGCTCCGCGTGCGCAGCCAC 125
      |||
Sbjct 654 AACGGCACCAGCTCCAAGGGCTCCGACGAGGCCATCATCAGCTCCGCGTGCGCAGCCAC 713

Query 126 CTGGCCGCCCTCTGGCCCTTCTGGGCATCGTGGCTGAGGTGCTGGTGTGGTCAACCATC 185
      |||
Sbjct 714 CTGGCCGCCCTCTGGCCCTTCTGGGCATCGTGGCTGAGGTGCTGGTGTGGTCAACCATC 773

Query 186 ATCTTCATCTACGAGAAGCGCGGAGCCCGAGGACGCTCCTGGATGATGACGACGCCGGC 245
      |||
Sbjct 774 ATCTTCATCTACGAGAAGCGCGGAGCCCGAGGACGCTCCTGGATGATGACGACGCCGGC 833

Query 246 TCTGCACCCCTGAAGAGCAGCGGGCAGCACCAGAA-GTACAAAGGCCAGA 294
      |||
Sbjct 834 TCTGCACCCCTGAAGAGCAGCGGGCAGCACCAGAAATG-ACAAAGGCCAGA 882
```

Figure 5 The homologous comparison result of PCR products of *EMMPRIN* gene mRNA in NSCLC tissues. PCR, polymerase chain reaction; NSCLC, non-small cell lung carcinoma.

```
>[ref|NM_003254.2|] UEGM Homo sapiens TIMP metalloproteinase inhibitor 1 (TIMP1), mRNA
Length=931

GENE ID: 7076 TIMP1 | TIMP metalloproteinase inhibitor 1 [Homo sapiens]
(Over 100 PubMed links)

Score = 870 bits (471), Expect = 0.0
Identities = 476/478 (99%), Gaps = 1/478 (0%)
Strand=Plus/Plus

Query 13 CTGCACCTGTGT-CCACCCACCCACAGACGGCCTTCTGCAATTCGACCTCGTCATCAG 71
      |||
Sbjct 261 CTGCACCTGTGTCCACCCACCCACAGACGGCCTTCTGCAATTCGACCTCGTCATCAG 320

Query 72 GGCCAAGTTCGTGGGGACACCAGAAGTCAACCAGACCACTTATACCAGCGTTATGAGAT 131
      |||
Sbjct 321 GGCCAAGTTCGTGGGGACACCAGAAGTCAACCAGACCACTTATACCAGCGTTATGAGAT 380

Query 132 CAAGATGACCAAGATGTATAAAGGGTTCCAAGCCTTAGGGGATGCCGCTGACATCCGGTT 191
      |||
Sbjct 381 CAAGATGACCAAGATGTATAAAGGGTTCCAAGCCTTAGGGGATGCCGCTGACATCCGGTT 440

Query 192 CGTCTACACCCCGCCATGGAGAGTGTCTGCGGATACTTCCACAGTCCCACAAACCGCAG 251
      |||
Sbjct 441 CGTCTACACCCCGCCATGGAGAGTGTCTGCGGATACTTCCACAGTCCCACAAACCGCAG 500

Query 252 CGAGGAGTTTCTCATTGCTGGAAAAGTGCAGGATGGACTCTTGACATCACTACCTGCAG 311
      |||
Sbjct 501 CGAGGAGTTTCTCATTGCTGGAAAAGTGCAGGATGGACTCTTGACATCACTACCTGCAG 560
```

Figure 6 The homologous comparison result of PCR products of *TIMP-1* gene mRNA in NSCLC tissues. PCR, polymerase chain reaction; NSCLC, non-small cell lung carcinoma.

Association between gene expression and survival after surgical resection

The Kaplan-Meier survival curves in patients positive and negative for EMMPRIN, MMP-9 and TIMP-1 expression. Patients negative for EMMPRIN expression had a significantly longer median overall (more than 33.60 *vs.* 24.31 months; $P=0.016$) survival, compared with those positive for EMMPRIN expression. Patients negative for MMP-9 expression had a significantly longer median overall survival (more than 33.94 *vs.* 24.47 months; $P=0.026$), than those positive for MMP-9 expression. Patients negative for TIMP-1 expression had a longer median overall survival (more than 29.46 *vs.* 25.23 months; $P=0.172$), than those positive for TIMP-1 expression, but the difference was not statistically significant. In this study, TNM stage ($P=0.008$) and metastasis of lymph node ($P=0.007$) were also prognostic (Table 2; Figures 7-9).

The relationship among EMMPRIN, MMP-9 and TIMP-1 expressions

Through the correlation matrix, the result was: through the correlation analysis between EMMPRIN and MMP-9, the contingency coefficient was 0.475 ($P<0.05$). Through the correlation analysis between EMMPRIN and TIMP-1, the contingency coefficient was 0.473 ($P<0.05$). And the correlation analysis between MMP-9 and TIMP-1 got the contingency coefficient of 0.454 ($P<0.05$). It showed that the expressions of EMMPRIN, MMP-9, TIMP-1 in non-small cell lung cancer were positively correlated, and in low positive correlation.

Discussion

The invasion and the metastasis are the most important biological characteristics of malignant tumors, but also the major reasons for patient treatment failure and mortality. Therefore, screening specific tumor-marker is necessary and valuable for early diagnosis and special treatment of tumors. The existing study recognized that, during the lung cancer invasion and metastasis, such genes as *EMMPRIN*, *MMP-9*, and *TIMP-1* etc. were currently acknowledged as ones of several largest regulatory genes functioning in the invasion and metastasis of lung cancer.

MMP is a group of zinc, calcium-dependent endopeptidases with very similar structure and function, which can almost degrade all protein compositions in ECM. Some studies

showed that the degradation of ECM by MMP-9 played a key role in tumor invasion and metastasis, including NSCLC (5). In lung cancer tissues at different staging and classifications, the positive MMP-9 expression was different, the higher staging and the poorer differentiation, the stronger expression (6). Our findings indicate that MMP-9 expression might correlate with OS ($P<0.05$). The above results suggested that MMP-9 could be used as a marker of the metastatic potential of lung cancer, and the malignancy degree and the metastatic ability of lung cancer could be determined by detecting MMP-9. Foreign literature confirmed this view (7,8). So MMP-9 could be used as the indicator to determine the prognosis of lung cancer patients, and the high MMP-9 expression will indicate a poor prognosis (9,10).

TIMP-1 is the inhibitor of MMP-9, TIMP family is an encoding protein of multi-gene families, which is the specific inhibitor of MMP. TIMP-1, by inhibiting MMP-9, plays the role on anti-tumor growth and preventing the tumor invasion and metastasis (11). In our study, the result showed that the expression rate of TIMP-1 in NSCLC tissues was higher than in cancer adjacent tissues ($P<0.05$), which kept consistent with Jumper *et al.* (12) research findings. Even though the negative regulatory role of TIMP-1 on tumor growth was recognized, but TIMP-1 was also highly expressed with increased tumor staging (13-15). The above results suggested that the positive TIMP-1 expression was related with the prognosis of lung cancer patients, patients whose tumors had TIMP-1 expression would have significantly poorer survival (16,17). But our study shows that TIMP-1 expression not correlate to prognosis in NSCLC. It might be caused by the limitation of number of samples (18).

EMMPRIN is an immunoglobulin super family (IgSF) member, which is a transmembrane glycoprotein of about 58 kD molecular weight, as a gene with multiple functions, in a variety of malignant tumors, it not only stimulates the tumor-associated stromal fibroblasts and the endothelial cell to produce MMPs, but also has a significant effect on tumor invasion and metastasis (19-21). In NSCLC tissues, the high EMMPRIN expression induced the production of MMPs, which led to the degradation of ECM, thereby promoting the invasion and metastasis of cancer cell (22). This study found that the EMMPRIN expression was related with tumor differentiation degree, whether there was lymph node metastasis, TNM staging and other pathological factors, the difference was statistically significant ($P<0.05$). This study results kept consistent with others research results (23,24).

Table 1 The relationship of EMMPRIN, MMP-9, TIMP-1 expressions with NSCLC clinical pathologic parameters

Clinical pathology	N	N1	%	EMMPRIN (+)		N2	%	MMP-9 (+)		N3	%	TIMP-1 (+)	
				χ^2	P			χ^2	P			χ^2	P
Age (years)				0.07	0.934			0.01	0.970			0.802	0.370
<60	38	25	65.8			27	71.0			17	44.7		
>60	42	28	66.7			30	71.4			23	54.7		
Gender				0.011	0.917			0.472	0.492			0.487	0.485
Male	51	34	66.7			35	68.6			24	47.0		
Female	29	19	65.5			22	65.5			16	55.1		
Smoking				0.498	0.481			3.063	0.080			0.205	0.651
Smoker	46	29	63.0			27	58.9			22	47.8		
Non-smoker	34	24	70.5			30	88.2			18	52.9		
Ethnic group				3.075	0.080			0.401	0.527			2.851	0.091
Zhuang	25	20	80.0			19	76.0			16	64.0		
Han	55	33	60.0			38	69.0			24	43.6		
Lymph node metastasis				14.261	0.000			7.652	0.006			8.717	0.003
Negative	33	14	42.0			18	54.5			10	30.0		
Positive	47	39	82.9			39	82.9			30	63.8		
Pathological type				0.356	0.551			1.116	0.734			0.487	0.485
Squamous carcinoma	29	18	62.0			20	68.9			13	44.8		
Adenocarcinoma	51	35	68.6			37	72.5			27	53.9		
Clinical staging				5.367	0.021			4.561	0.0337			13.333	0.000
Phase a/II	48	27	56.2			29	60.4			16	33.3		
Phase a/IV	32	26	81.2			28	87.5			24	5.0		
Differentiation degree				7.028	0.008			7.532	0.006			8.901	0.003
Well and medium	49	27	55.1			29	59.1			18	36.7		
Poorly	31	26	83.8			28	90.3			22	70.9		

N1 for cases of EMMPRIN positive expression; N2 for MMP-9 positive expression; N3 for TIMP-1 positive expression. NSCLC, non-small cell lung carcinoma.

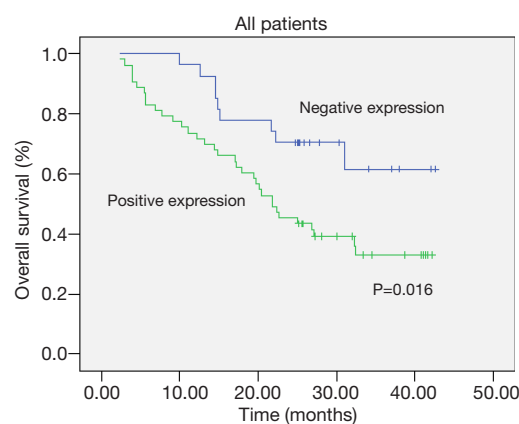
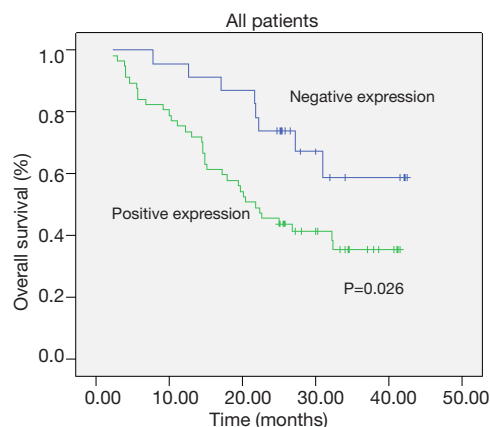
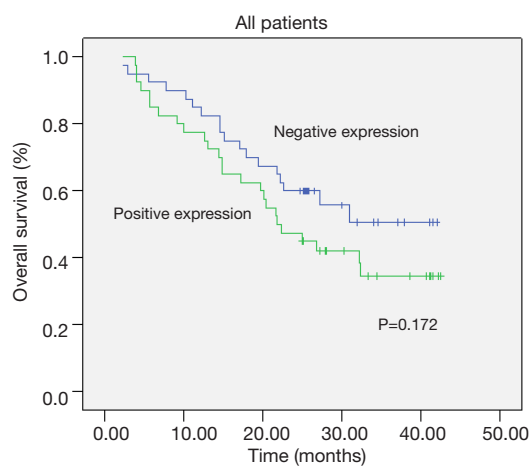
The above results suggested that EMMPRIN might be closely associated with lung cancer invasion and metastasis, it may serve as the important indicator for staging, malignancy degree and prognostic of lung cancer.

The incidence and the development of lung cancer differ from regional distribution and ethnic group, we found that, in the study for EMMPRIN, MMP-9, TIMP-1 in NSCLC tissues of Guangxi Zhuang ethnic, the MMP-9 and TIMP-1 expression rates in Zhuang people were 76% (19/25) and 64% (16/25) respectively, higher than the expression rate

of 69% (33/55) and 43% (24/55) in Han people, but the difference was not statistically significant ($P>0.05$). Chinese Hongyan Wang *et al.* found that, in their research on the MMP-9, TIMP-1 expression in nasopharyngeal carcinoma tissues in Xi'an and Shenzhen regions, the MMP-9, TIMP-1 expression in nasopharyngeal carcinoma tissues of these two regions had no statistically significant difference ($P>0.05$), our study results were similar with Hongyan Wang (25) research results. In this study, the EMMPRIN expression rate in Guangxi Zhuang people was 80% (20/25), higher

Table 2 Survival analysis

Variable	Medians for survival time (months)	χ^2	P
Age (years)		0.455	0.500
<60	31.05		
>60	22.34		
Gender		0.373	0.541
Male	32.26		
Female	22.34		
Smoking		0.00	0.996
Smoker	25.00		
Non-smoker	22.60		
Ethnic group		0.002	0.968
Zhuang	27.17		
Han	22.60		
Lymph node metastasis		5.625	0.018
Negative	32.26		
Positive	15.18		
Pathological type		1.810	0.18
Squamous carcinoma	32.26		
Adenocarcinoma	21.78		
Clinical staging		5.710	0.017
Phase a/II	32.23		
Phase a/IV	20.14		
Differentiation degree		1.271	0.260
Well and medium	25.00		
Poorly	22.43		
EMMPRIN		5.769	0.016
Positive	33.60		
Negative	24.31		
MMP-9		4.973	0.026
Positive	33.94		
Negative	24.47		
TIMP-1		1.868	0.172
Positive	29.46		

**Figure 7** EMMPRIN survival functions. Overall survival according to EMMPRIN expression.**Figure 8** MMP-9 survival functions. Overall survival according to MMP-9 expression.**Figure 9** TIMP-1 survival functions. Overall survival according to TIMP-1 expression.

than in Han people, but the difference was not statistically significant ($P>0.05$). It suggested that the expression of EMMPRIN, MMP-9, TIMP-1 in NSCLC had no regional and ethnic differences.

The EMMPRIN expression and the MMP-9 expression were consistent, MMP-9 also showed high expression when EMMPRIN was highly expressed, conversely both showed lower expression, which indicated that EMMPRIN was the upstream factor of MMP-9, being able to stimulate the MMP-9 secretion (26). With further studies in depth, Tang *et al.* (27) found that the increase in MMP-9 can also promote the soluble EMMPRIN/CD147 expression in tumor matrix. In this study, the expressions of EMMPRIN, MMP-9 and TIMP-1 in NSCLC were positively correlated, and in low positive correlation. It indicated that these three may have mutual induction, synergistic or inhibitory effect in the occurrence and developing process of lung cancer, suggesting that there may be other factors working together, leading to the high expression of these three in lung cancer tissues, but the details need the further study to confirm.

Considering that MMP-9 was the important target for tumor metastasis, vascularization and growth, a number of synthetic MMP inhibitors (MMPIs) were born in succession, such as BB-94, Marimastat and Prinomastat etc. But some research results showed that these MMPIs were not conducive to the improvement of survival rate, and some were filed to terminate experiments because they shortened survival (28,29). Therefore, researching TIMPS reagents to inhibit tumor invasion and metastasis has become a current hot spot.

In summary, to better to improve the clinical outcome of advanced NSCLC patients, relationship between therapy and prognosis of patients must be investigated. Our findings indicate EMMPRIN and MMP-9 are prognostic factors for overall survival, and are predictive biomarkers in NSCLC patients. Accompanied by enlargement of sample size, TIMP-1 might also be an indicator the above-mentioned. The further research on the role and the co-relationship of EMMPRIN, MMP-9, TIMP-1 will not only help to approach the mechanisms of the NSCLC incidence and development in depth, but also offer help for the gene targeted therapy of NSCLC.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2017.12.18>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Institutional ethical approval and informed consent were waived.

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