Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

Correlation analysis of mesenchymal–epithelial transition factor protein and human epidermal growth receptor 2 protein expression in 1479 cases of lung adenocarcinoma in China

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Keywords

Correlation analysis; human epidermal growth receptor 2 protein; immunohistochemistry; lung adenocarcinoma; mesenchymal–epithelial transition factor protein.

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Received: 24 November 2017;
Accepted: 18 December 2017.

doi: 10.1111/1759-7714.12595

Thoracic Cancer **9** (2018) 439–444

Abstract

Background: To investigate the correlation between mesenchymal–epithelial transition factor (C-Met) and human epidermal growth receptor 2 (HER2) protein expression in primary lung adenocarcinoma tissues.

Method: A total of 1479 resected primary lung adenocarcinoma patients were enrolled in the present study for detecting of C-Met and HER2 protein by immunohistochemistry, and correlation analysis was made between the above two biomarkers and related clinicopathological features.

Result: Both C-Met and HER2 proteins were found to stain highly positive in lung adenocarcinomas, and a positive correlation was found between them ($\chi^2 = 118.5$, $P = 2.707 \times 10^{-21}$). In addition, HER2 protein expression was correlated with sex, pathological stage, lymph node metastasis, and major subtypes; and C-Met was correlated with sex ($P < 0.05$).

Conclusion: The expression of C-Met and HER2 protein in lung adenocarcinoma is highly correlated, and whether it is synergistic in the targeted therapy of lung adenocarcinoma deserves further study.

Introduction

In recent years, the incidence of lung cancer has risen in China and worldwide.[1] Lung adenocarcinoma accounts for more than half of non-small cell lung cancer.[2] The evolution of detecting molecular abnormalities, such as epidermal growth factor receptor (EGFR) mutation, ALK fusion and the development of corresponding targeted drugs, has led lung adenocarcinoma to an era of individualized precise treatment.[3,4] However, some cases have been found to be resistant to EGFR inhibitors (EGFR-TKI), so it is necessary to study the mechanism of drug resistance and to explore new therapeutic targets.[5] Human epidermal growth receptor 2 (HER2) and mesenchymal–epithelial transition factor (C-Met) have been found to be mutated and amplified in lung adenocarcinoma, which are associated with EGFR-TKI resistance.[5] There are many studies on HER2 gene mutation and C-Met gene amplification or exon 14 mutation in the literature, but the abnormal expression of protein and the relationship between the two genes are only reported in small samples, and the conclusions are not consistent with clinical pathological features.[6–8]

The present study intended to detect C-Met and HER2 protein expression in a large sample of lung adenocarcinoma cases from the National Cancer Center in China, and to explore the correlation of the two biomarkers with clinicopathological factors.

Methods

Case selection and histological analysis

This study was a retrospective study, which had been approved by the hospital ethics committee to exempt patients' informed consent. All pathologically diagnosed pulmonary adenocarcinoma surgical resection specimens were consecutively collected from the Department of Pathology of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences from July 2013 to September 2014. Clinicopathological data were extracted from medical archives, including the patient's age, sex, AJCC 7th pathological stage, and so on. Histological subtypes were reviewed and distinguished by experienced pathologists according to the 2015 World Health Organization classification of lung tumors (Kappa = 0.72).[9]

A total of 1479 cases of lung adenocarcinoma were retrospectively classified according to predominant components, which included invasive lepidic adenocarcinoma (n = 157; 10.6%), papillary adenocarcinoma (n = 228; 15.4%), acinar adenocarcinoma (n = 843; 57.0%), solid adenocarcinoma with mucin produced (n = 177; 12.0%), mucinous adenocarcinoma (n = 37; 2.5%), micropapillary adenocarcinoma (n = 13; 0.9%), adenocarcinoma *in situ* (n = 8; 0.5%), minimally invasive adenocarcinoma (n = 9; 0.6%), and enteric adenocarcinoma (n = 7; 0.5%). For statistical convenience, we combined those items <1% into a special subgroup including a total of 37 cases, which were 13 cases of micropapillary adenocarcinomas, eight cases of adenocarcinoma *in situ*, nine cases of minimally invasive adenocarcinoma, and seven cases of enteric adenocarcinomas.

Methods

Preprocessing procedures of specimens were as follows: surgically resected tissues were fixed in 10% neutral formalin for 6–48 hours at 10-fold the volume of the tissue liquid, and then embedded in paraffin. Four consecutive 4-μm thick sections were used for HER2 and C-Met immunohistochemical staining, and the others for routine negative control staining for a matched rabbit monoclonal negative immunoglobulin antibody. All sections were heated to 62°C, and then subjected to the fully automated immunohistochemical assay on the Benchmark XT stainer (Roche Company, Basel, Switzerland). According to the manufacturer's scoring algorithm, a four-level scoring system was used for the evaluation of staining results. HER2 protein was deemed positive with the presence of strong granular cytoplasmic and/or membrane staining. C-Met protein staining was deemed positive in cytoplasm (Fig 1a–c). In addition to that, the staining intensity and percentage of dyed cells were also included in interpretation. Negative quality control sections were first evaluated to remain unstained before evaluation for immunostaining on every case. A four-level classification was applied into interpretation, including negative (0), no staining or <5% dying; weakly positive (1+), 5~25% tumor cells stained; moderately positive (2+), 25~50% tumor cells stained; and strongly positive (3+), >50% tumor cells stained.

Statistical analysis

We used independent χ^2-test to compare the frequency of clinicopathological characters between HER2/C-Met high expression and low expression groups, and a correlation analysis was also made between HER2 and C-Met protein results. The statistical analyses were conducted using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA), and statistical significance was set as $P < 0.05$.

Results

Patient characteristics

A total of 1479 primary lung adenocarcinoma patients were available for analysis, including 658 men (44.5%) and 821 women (55.5%), with the average and median age of 59 years. Other clinicopathological features are listed in Table 1, including pathological stage, lymph node metastasis, and predominant histological subtypes.

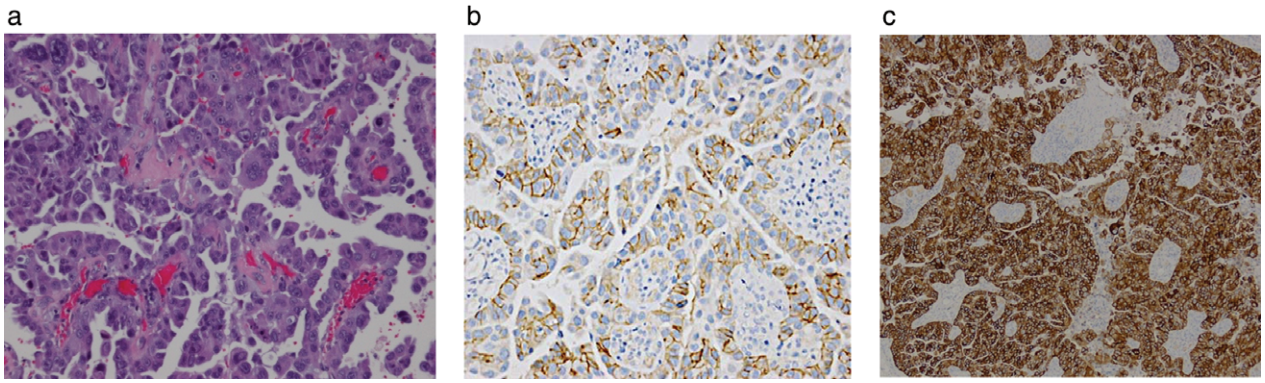

Figure 1 (**a**–**c**) A case of lung adenocarcinoma (hematoxylin–eosin staining, original magnification 20×), showed positive staining both in human epidermal growth receptor 2 (2+) and mesenchymal–epithelial transition factor protein (3+) (immunohistochemistry staining, the Benchmark XT stainer, original magnification 20×).

HER2 protein expression and clinically-related features

HER2 protein was expressed in tumor cell membrane, and there was no expression or weak positive expression in normal lung tissues. The expression rate of HER2 protein was negative (27.8%; 411/1479), 1+ (51.9%; 768/1479), 2+ (18.9%; 279/1479), and 3+ (1.4%; 21/1479); according to the dichotomy classification, 0~1+ was defined as negative or low expression (79.7%; 1179/1479); and 2~3+ was defined as positive/high expression (20.3%; 300/1479). The expression of HER2 was statistically correlated with sex, tumor stage, lymph node metastasis, and histological subtypes ($P < 0.05$), but not correlated with age ($P > 0.05$) (Table 2).

C-Met protein expression and clinically-related features

C-Met protein was found positive in cytoplasm of tumor cells. The expression rate of C-Met protein was negative (4.3%; 64/1479), 1+ (23.2%; 343/1479), 2+ (50.0%; 739/1479), 3+ (22.5%; 333/1479). According to the dichotomy classification, 0~1+ is defined as negative or low expression, which is 27.5% (407/1479); and 2~3+ was defined as positive/high expression, which is 72.5% (1072/1479). The expression of C-Met protein was significantly correlated with sex ($P < 0.05$), but not with age, tumor stage, lymph node metastasis, and histological type ($P > 0.05$) (Table 3).

Correlation between HER2 protein and C-Met proteins

Table 4 shows that HER2 was significantly correlated with C-Met protein, and a positive correlation was found within immunostaining intensity ($P = 2.707 \times 10^{-21}$).

Discussion

Molecular targeted therapy of lung adenocarcinoma is the representative of current cancer precise medicine. With the decrease of the cost of gene sequencing and data analysis technology, the search for abnormal gene targets for cancer tissue genomics and proteomics has become the mainstream of targeted drug research and development.[10] At the

Table 1 Clinicopathological features of 1479 lung adenocarcinomas

Clinicopathological features	Case number (n = 1479)	Percentage (%)
Age	58.85 ± 9.56	
Gender		
Male	658	44.5
Female	821	55.5
Pathological stage		
0	8	0.5
I	879	59.4
II	168	11.4
III	346	23.4
IV	20	1.4
Unknown	58	3.9
Lymph node metastasis		
Yes	452	30.6
No	949	64.1
Unknown	78	5.3
Predominant subtypes		
Lepidic	157	10.6
Papillary	228	15.4
Acinar	843	57.0
Solid	177	12.0
Mucinous adenocarcinoma	37	2.5
Micropapillary	13	0.9
AIS	8	0.5
MIA	9	0.6
Enteric adenocarcinoma	7	0.5

AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma.

Table 2 Correlation between human epidermal growth receptor 2 expression and clininopathological features

	HER2 protein			
Clinicopathological features	Low expression (%)	High expression (%)	χ^2	*P*-value
Age	57.85 ± 8.5	59.55 ± 8.56	1.129	0.259
Gender				
Male	501 (33.9)	157 (10.6)	7.616	0.006
Female	673 (45.5)	148 (10.0)		
Pathological stage				
0	8 (0.6)	0	10.357	0.035
I	720 (50.7)	159 (11.2)		
II	126 (8.9)	42 (3.0)		
III	262 (18.4)	84 (5.9)		
IV	16 (1.1)	4 (0.3)		
Lymph node metastasis				
Yes	339 (24.2)	113 (8.1)	8.932	0.003
No	777 (55.5)	172 (12.3)		
Predominant subtypes				
Lepidic	140 (9.5)	17 (1.1)	12.22	0.032
Papillary	184 (12.4)	44 (3.0)		
Acinar	663 (44.8)	180 (12.2)		
Solid	140 (9.5)	37 (2.5)		
Mucinous Adenocarcinoma	28 (1.9)	9 (0.6)		
Special type†	26 (1.8)	11 (0.7)		

†For statistical convenience, a special group was created including those subtype lower than 15 cases, including a total of 37 cases, which were 13 cases of micropapillary adenocarcinomas, 8 cases of adenocarcinoma in situ, 9 cases of minimally invasive adenocarcinoma, and 7 cases of enteric adenocarcinomas.

Table 3 Correlation between mesenchymal–epithelial transition factor expression and clininopathological features

	C-Met protein			
Clinicopathological features	Low expression (%)	High expression (%)	χ^2	*P*-value
Age	59.63 ± 9.51	58.77 ± 9.57	–1.541	0.124
Gender				
Male	200 (13.5)	458 (31.0)	6.620	0.010
Female	202 (13.7)	619 (41.8)		
Pathological stage				
0	3 (0.2)	5 (0.4)	8.362	0.079
I	224 (16.0)	647 (46.1)		
II	58 (4.1)	107 (7.6)		
III	101 (7.1)	239 (17.0)		
IV	8 (0.6)	12 (0.9)		
Lymph node metastasis				
Yes	246 (17.7)	697 (50.3)	2.949	0.086
No	136 (9.8)	308 (22.2)		
Predominant subtypes				
Lepidic	34 (2.3)	128 (8.3)	9.543	0.089
Papillary	65 (4.4)	163 (11.0)		
Acinar	228 (15.4)	615 (41.6)		
Solid	56 (3.8)	121 (8.2)		
Mucinous adenocarcinoma	10 (0.7)	27 (1.8)		
Special type†	17 (1.1)	20 (1.4)		

†For statistical convenience, a special group was created including those subtypes lower than 15 cases, including a total of 37 cases, which were 13 cases of micropapillary adenocarcinomas, 8 cases of adenocarcinoma in situ, 9 cases of minimally invasive adenocarcinoma, and 7 cases of enteric adenocarcinomas.

same time, the application of machine learning in multiple dimension analysis for correlation of different types of proteins also showed great power in the design process of targeting drugs.[11,12] HER2 and C-Met protein are closely related with the EGFR receptor tyrosine kinase, and HER2 protein shows a high similarity with EGFR. In the present

Table 4 Correlation between human epidermal growth receptor 2 and mesenchymal–epithelial transition factor expression

		C-Met					
		0	1+	2+	3+	χ^2	*p*
HER2	0	25	153	183	50	118.5	2.707×10^{-21}
	1+	24	149	424	171		(<0.001)
	2+	14	37	122	106		
	3+	1	4	10	6		

C-Met, mesenchymal–epithelial transition factor; HER2, human epidermal growth receptor 2.

study, we retrospectively analyzed lung adenocarcinoma cases from the National Cancer Center within 14 months for expression of C-Met and HER2 proteins, and revealed that they were closely correlated with some clinicopathological factors, such as sex, pathological stage, lymph node metastasis, and so on; also, we found there was no significant correlation either with ALK fusion protein or within mutated EGFR cases (data not shown). As far as we know, this is the first large-scale analysis for C-Met and HER2 proteins in lung adenocarcinomas.

HER2/neu (*EBRR-2*) gene is located on chromosome 17q21, encoding a transmembrane protein that shows protein tyrosine kinase activity. It is highly homogeneous with the human epidermal growth factor receptor (*EGFR HER1*) gene, which plays a role in signal transduction. HER2 mutation or overexpression was found in approximately 3% of non-small cell lung cancer. HER2 overexpression increases the sensitivity of lung cancer cells by inhibiting EGFR-TKI two heterologous dimer formation, and may benefit from trastuzumab.[13] C-Met belongs to a tyrosine kinase receptor (receptor tyrosine kinases) superfamily, which is an oncogene for encoding a class of self phosphorylation activity of the transmembrane receptor, located in 7q31, with a size of approximately 110 kb, including 21 exons. It was found that C-Met gene amplification or high expression was one of the important mechanisms of acquired drug resistance against EGFR-TKI for lung adenocarcinoma, and it could benefit from ALK inhibitors.[14,15]

In this study, we found that HER2 protein and C-Met protein were highly expressed in lung adenocarcinoma, and there was a significant correlation between the two proteins, which could help to explain some molecular characteristics of lung adenocarcinoma from the protein level. Wei *et al.* found that the expression of HER2 protein was related to age and the degree of differentiation in the EGFR mutant of advanced lung adenocarcinoma.[8] In a small sample study, it was found that HER2 and C-Met protein could be expressed in lung adenocarcinoma, and showed a significant positive correlation trend.[7] The present study is consistent with the above results. Similar results were found in carcinomas other than lung adenocarcinoma. C-Met has been reported to be associated with HER2 in breast cancer, and it was an adverse prognostic factor independent of HER2 status in patients with lymph node metastasis.[16,17] Approximately 54% of patients with gastric cancer showed the activation of HER1, HER2, HER3, C-Met, or IGF1R, the prognosis was poorer than those with no activation of receptor tyrosine kinases[18] in advanced gastric cancer patients, a significant difference was found between C-Met-positive and -negative patients in differentiation degree, liver metastasis, and alkaline phosphatase levels; and the positive rate of C-Met was found different in HER2-positive and HER2-negative patients (56% vs. 38%, $P < 0.05$). The above findings showed some correlation of *C-Met* and *HER2* genes in gastric adenocarcinoma.[18–20]

Study on the mechanism of tumor molecular biology identified that both *C-Met* and *HER2* genes showed a potential trend of co-expression, and both of them showed a definite correlation with EGFR mutation.[21–23] The *HER2* gene could promote the rapid proliferation and inhibit apoptosis of tumors cells by activating the PI3K–Akt pathway, thereby increasing the metastatic ability of cancer cells.[24] Meanwhile, C-Met could activate Ras–ERK and PI3K–Akt pathways, triggering epithelial mesenchymal transformation, and promoting invasiveness and migration of cancer cells.[25]

In conclusion, based on the large sample from the National Cancer Center, we demonstrated from the protein level that the expression of HER2 and C-Met in lung adenocarcinoma were synergistic and statistically correlated with some clinicopathological features. The limitation of this study is that the selected cases were mostly from patients who underwent curative-intent surgery, and there was not enough long time to evaluate protein expression with EGFR target therapy and survival analysis, which will be the direction for further research.

Acknowledgments

This work was partially supported by the Fundamental Research Funds for the Central Universities, China (3332015060); the basic research funding of Chinese Academy of Medical Sciences (JK2015B08); and China Cancer Foundation Beijing Hopes Marathon Fund Project (LC2015L14).

Disclosure

No authors report any conflict of interest.

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