Expression of KGF mRNA in Human NSCLC and Its Role in the Development of Lung Cancer

Ling Liu, Jiping Da*, Shumin Zhao

Chui Yangliu Hospital, Peking, China, 100021
* General Hospital of Air Force, PLA, Peking, China, 100086

Abstract **Objective** To investigate the expression of KGF messenger ribonucleic acid in human non-small cell cancer (NSCLC) and to study the role of KGF in the development of NSCLC. **Methods** With in situ hybridization (ISH) the expression of KGF mRNA in 50 cases of NSCLC was detected. Also, it was compared with normal tissue. **Results** On ISH slides, positive KGF mRNA was mainly shown as fusiform stain in plasma of fibroblast and smooth muscle cell of blood vessel in mesenchymal of NSCLC, meanwhile, some parenchyma cell plasma was also stained. The positive rate of KGF mRNA in tumor (86%) is statistically higher than that in normal tissue(24%) ($p<0.05$). **Conclusions** KGF mRNA is highly expressed in NSCLC. Through a paracrine or an autocrine way, KGF is possibly involved in the development of NSCLC.

Key Words keratinocyte growth factor (KGF); non-small cell lung cancer(NSCLC); in situ hybridization (ISH)

Among growth factors related to the development of carcinoma epidermal, the growth factor (EGF) was once a highlighting candidate, which can bind on EGFR (C-erbB1) on the surface of epithelial cell through an autocrine way and thus promote the progress of epithelial original carcinoma[1]. However, recent studies shown that the stimulating effects of keratinocyte growth factor (KGF), produced by fibroblast in stroma, on DNA synthesis in epithelial cell are 2-10 times greater than those of any known epidermal mitogens, including transforming growth factor (TGFa), epidermal growth factor (EGF), acidic fibroblast growth factor (aFGF), and basic fibroblast growth factor (bFGF)[2]. Meanwhile, a positive relationship between KGF and lots of epithelial original carcinomas such as esophageal cancer, oral mucosa, carcinoma of prostate, carcinoma of bladder, adrenal gland carcinoma etc was noticed. But, except for very few animal trials, we found no research on how about its relationship with pulmonary carcinoma. In our study we detected the expression of KGF mRNA in 50 cases of NSCLC through in situ hybridization (ISH), and based on this, we tried to discuss the role of KGF in the development of lung cancer, especially of NSCLC, which is a kind of epithelial original carcinoma.

MATERIALS AND METHODS

Clinical materials

Objects 50 paraffin embedded samples of NSCLC obtained from operation in Air Force General Hospital, PLA from 1998 to 2002 were randomly picked out for investigation, and all case with integrated medical data and without any radiation or chemotherapeutics prior to surgery. All cases of NSCLC were reviewed and classified according to latest classification standard for tumor issued by World Health Organization in 2001. Among 50 cases there were 35 male and 15 female, the ages under 50 years old were 19 cases, between 51 and 69 years old 29 cases, 2 cases over 70 years old, and average 56.26 years old. According to International Pulmonary Carcinoma Staging Classification Standard there were 8 cases for stage I, 10 cases for stage II, 32 cases for stage III, 0 cases for stage IV respectively. There were 28 cases with smoking habit and 22 cases without smoking habit (or give up smoking for more than 5 years). There were 14 cases with tumor diameter less than or equal to 3cm, and 36 cases more than 3 cm. And there were 37 cases with lymph node metastasis and 13 cases without lymph node metastasis. Normal lung was selected as control group.

Reagents The In situ hybridization kit for KGFmRNA and digoxin tagged KGFmRNA probe were purchased from Wu Han Boshide Biotechnology Company Limited. The sequence of oligonucleotide probe for human KGFmRNA is 5´-GTTGG CAGGA TCCAT GTCAG TATCC ATTTG-3'.

In situ hybridization

The experiment was strictly carried out according to the direction of Boshide Biotechnology Company Limited. Briefly, 6um paraffin tissue section was deparaffinized and hydrated. Sections were then incubated in 10 mmol/L Tris-HCL (pH 8) for 5 minutes, and rinsed in distilled, deionized water. Endogenous peroxidase was inactivated by 0.3% (w/v) hydrogen peroxide in methanol for 10 minutes at room temperature. Sections were then washed with phosphate-buffered saline (PBS) for 5 minutes. Fresh attrnuant pepsin (two drops in 1ml 3% citromalic acid) was added on slides and digested for 10 minutes at 37℃ to exposure messenger ribonucleic acid. 20ul pre-hybridization fluid was added on each slide and incubated at 37℃ in electrothermal homeothermia incubator for 3 hours. Then washed slides with distilled water. Hybridization was carried out with 20 ul hybridization solution added on each slide and incubated at 42℃ in electrothermal homeothermia incubator for overnight. Washing after hybridization. Blocking solution was added and incubated at 37℃ for 30 minutes. Didn´t wash. Biotin-conjugated antidigoxigenin antibody was added and incubated at 37℃ for 60 minutes. Washed with 0.5M PBS. SABC was added and incubated at 37℃ for 20 minutes. Washed with 0.5M PBS again. Added with biotin-conjugated peroxidase. Washed with 0.5M PBS again. Stained with DAB. Re-stained with lignins. Dehydrated, cleared and mounted.

Judgement of results

The results were observed and judged by two clinical-pathology specialists in a manner of double blindness. On ISH slides, positive KGF mRNA was mainly shown as fusiform stain in cytoplasm of fibroblast and blood vessel smooth muscle cell in mesenchymal of NSCLC, while some parenchyma cells were also stained in cytoplasm. Positive cells were counted in 5 HPF. Those more than 25% cells were stained and the stain degree was higher than background was determined as positive results, otherwise as negative.

Staistical analysis

Chi-square test of R×C table was performed to determine difference between groups, with alpha(a) set at the usual 0.05. Software SPSS10.0 professional version was adopted for staistical analysis.

RESULTS

As mentioned above, the positive KGF mRNA was mainly shown as fusiform stain in cytoplasm of fibroblast and blood vessel smooth muscle cell in mesenchymal of NSCLC, while some parenchyma cells were also stained in cytoplasma (as shown in Fig.1~2). We found the positive KGF mRNA in 43 cases with positive rate 86% and the positive rate of KGF mRNA in tumor is significantly higher than that in normal tissue (24%) ($p<0.05$). When the relationship between KGF expression and other clinical factors was investigated we found no correlation between KGF and clinical factors such as age, sex, smoking, tumor diameter, TNM stages and even pathological type. But a positive correlation between KGF expression and lymph node metastasis ($p<0.05$) and a converse correlation with differentiation degree ($p<0.05$) were noticed. The results is shown in table 1.

DISCUSSION

KGF was initially discovered by Rubin et al[4] as an epithelial mitogen which was produced by M426 lung fibroblasts. The purified protein was a monomer with an apparent molecular weight of 26–28KD. The subsequent gene cloning and sequencing of the cDNA revealed that it belongs to the FGF family, so it was named FGF-7. Unlike other members of this family, KGF, which is produced by mesenchyme cells such as fibroblast, blood vessel endothelial cell, smooth muscle cell, γδT lymphocyte etc, seems to be specific for epithelial cells through a paracrine way[5,6]. KGF is involved in extensive physiological and pathological actions including epithelia repairing in trauma, hair growth[6,7], radiation protection[8], gender epithelia differentiation [9], epithelia repairing in inflammatory bowel disease [10], embryonic lung morphogensis and synthesis of surfactant etc[11]. Besides, it was reported that KGF was also involved in the development of lots of carcinoma including gastrointestinal tract carcinoma, oral mucosa carcinoma, carcinoma of prostate and carcinoma of bladder etc[12~16]. Both Iida[17] and Ishii

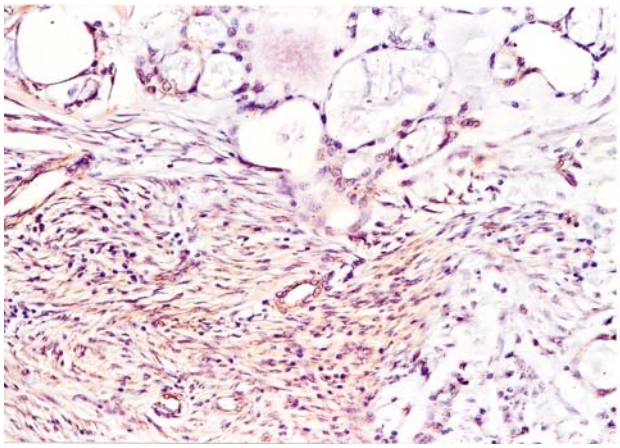

Fig.1 ISH for KGF mRNA in adenocarcinoma (200×), showing fusiform stain in plasma of fibroblast and blood vessel smooth muscle cell in mesenchymal as positive result.

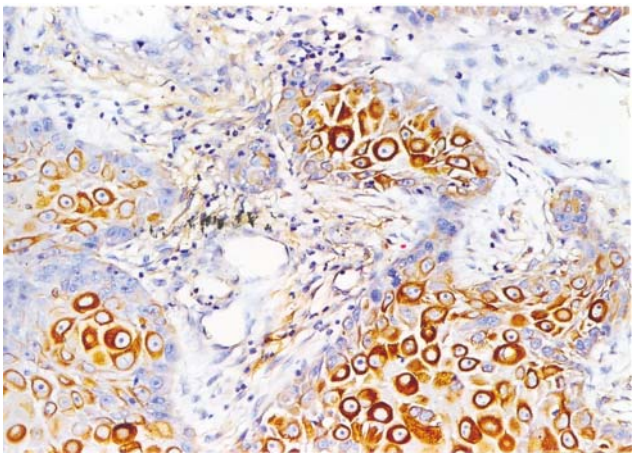

Fig.2 ISH for KGF mRNA in squamous carcinoma (200×), showing buffy stain in plasma of tumor cell as a positive result

Table 1 Relationship between KGF expression and clinical factors

Clinical factors	cases	KGF mRNA		*p* value
		Positive	Negative	
Sex				
Male	35	32	3	
Female	15	11	4	1.760
Age				
≤50	19	15	4	
50–69	29	26	3	
>70	2	2	0	0.489
Pathological type				
Squamous carcinoma	25	23	2	
Adenocarcinoma	25	20	5	0.417
Smoking				
Yes	28	25	3	
No	22	18	4	0.684
Tumor diameter				
≤3cm	14	12	2	
>3cm	36	31	5	1.000
TNM Stages				
I	8	7	1	
II	10	10	0	
III	32	26	6	
IV	0	0	0	0.326
Lymph metastasis				
Yes	37	37	0	
No	13	6	7	0.000
Differentiation				
Low	14	8	6	
Mid	24	23	1	
High	12	12	0	0.00
Position				
Tumor tissue	50	43	7	
Normal tissue	50	12	38	10.000

[18] observed that KGFR (FGFR-2, also named K-sam-Ⅱ), a splicing variant of Bek gene on the level of transcription, was expressed on the surface of almost all of epithelia carcinoma, while there was no FGFR-2 expressed on the surface of sarcom cell, which suggests that KGF/KGFR is possibly involved in the development of all epithelial original carcinoma.

To understand how KGF affects the development of pulmonary carcinoma, through ISH we detected the expression of KGFmRNA in 50 cases of NSCLC. As a result we found the positive KGF mRNA in 43 cases with positive rate 86%, and the positive rate of KGF mRNA in tumor is significantly higher than that in normal tissue (24%) ($p<0.05$), which suggest that KGF is in some degree related to the development of pulmonary carcinoma, especially NSCLC. As a kind of primary response gene, in a manner of independent of protein synthesis KGF gene can response to a series of blood serum growth factors including PDGF, EGF and TGF released by platelets after injury, or to cytokines such as TNF-2, IL-1, IL-6 existing in inflammatory situation [6]. For the case of carcinoma, the reason for KGF expression lays in epithelial injury or inflammation in early stage, and in tumor immunity in late stage. Here, it also gives an interpretation for the interaction between parenchyma and interstitial cell in tumor.

In addition to this, we found that positive expression of KGF mRNA was mainly shown as fusiform stain in plasma of fibroblast and blood vessel smooth muscle cell in mesenchymal of NSCLC, meanwhile some parenchyma cells were also stained in plasma, which was different from the report issued by Knerer B that KGF was only expressed in mesenchymal [19] and the same to the result conducted from Partridge M[20] who reported that epithelia carcinoma cells also autocrined KGF as tumor progressed in some stages. This result suggests that except for paracrine way, autocrine way may also exist during period of KGF activating tumor cell. In fact, the research on signal transmission of KGF is in primary stage, and extensive arguments about signal pathway of KGF exist. But most of the people believe that both PKC and mainly Ras dependent tyrosine kinase pathway are involved in signal transmission of KGF. Experiment conducted from Ushma Savla and Chri-Stopher M has shown that both PKC inhibitor calphostin c and Ras dependent tyrosine kinase inhibitor genistein can remove the KGF mediated protection against radiation[8]. And it is inferred that KGF is possibly involved in the development of epithelial original NSCLC by activating protooncogene such as c-myc, ras, jun and fos through signal transmission pathways mentioned above.

With immunohistochemistry method Hideshi Ishii et al[18] found that positive rate of KGFR (K-sam-Ⅱ)in metastasis carcinoma was significantly higher than that in primitive ones, which meant that, during the progression of KGF dependent carcinoma, tumor cells with positive KGFR were positively selected to grow, and that tumor cells responding to KGF had a potency of high proliferation and metastasis ability[18]. The same as Hideshi Ishii, we also found that the KGFmRNA positive rate of simple companying with lymph node metastasis (86%) was significantly higher than that without lymph node metastasis (48%) ($p<0.05$), which means KGF is possibly related to lymph node metastasis and poor prognosis. We still found that the KGFmRNA expression was conversely related with differentiation degree, that is to say, the lower of carcinoma differentiation is, the higher the KGFmRNA positive rate will be ($p<0.05$), which is similar to the result reported by Zhihong Deng et al who observed the relationship between KGF expression and differentiation degree in carcinoma of larynx[21].

In conclusion, KGF mRNA highly expressed in NSCLC. We infer that KGF is possibly involved in the development of NSCLC through a paracine as well as an autocrine way. And the detection of KGF expression is also helpful for the evaluation of differentiation and prognosis of NSCLC.

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