Int J Clin Exp Pathol 2016;9(7):7338-7344
www.ijcep.com /ISSN:1936-2625/IJCEP0026026

Original Article

Upregulation of FGF19 in lung adenocarcinoma and predicts poor prognosis

Yuan Cui[1], Jian Liu[2], Yi-Fei Liu[3], Jun-Hua Liu[1]

Departments of [1]Thoracic Surgery, [2]Oncology, [3]Pathology, Affiliated Hospital of Nantong University, Nantong, Jiangsu, China

Received February 15, 2016; Accepted May 20, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: Fibroblast growth factor 19 (FGF19) belong to the FGF19 subfamily which is widely distributed in human tissues. FGF19 plays a crucial role in regulating various cellular processes. Recently, the researchers found that the aberrant expression of FGF19 in tumor development and progression. However, to our limited knowledge, whether FGF19 functions in lung adenocarcinoma remains unknown. In our research, we evaluated the expression of FGF19 in LAC tissues and corresponding normal tissues by Immunohistochemistry and western blot. Moreover, we also demonstrated the FGF19 expression was positively associated with Ki-67, which is a cell proliferation index. Statistics analysis showed FGF19 expression was related with some clinicopathologic parameters and high expression of FGF19 predicted poor prognosis of LAC patients. These findings suggested that up-regulation of FGF19 might potentially be an effective therapeutic approach for LAC.

Keywords: FGF19, LAC, prognosis

Intruduction

Lung cancer has been the leading cause of cancer deaths world-wide [1]. Among all lung cancer cases, non-small-cell lung cancer (NSCLC) accounts for about 80-85%, which including adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma and large cell carcinoma, with adenocarcinoma being the most common type [2]. Current treatment options include surgical resection, platinum-based doublet chemotherapy and radiation therapy alone or in combination. However, the prognosis for lung cancer patients still remains poor with an overall 5-year survival rate of only 15% [3], which is largely due to the fact that the majority of lung cancer patients are diagnosed at advanced and later stages. Therefore, it is urgent to identify new molecular target of lung cancer, which will benefit both diagnosis and treatment of the disease.

The human fibroblast growth factor (FGF) family is composed of 22 structurally related polypeptides, which can be roughly classified into three major groups [4]. Fibroblast growth factor 19 (FGF19) belong to the FGF19 subfamily which also includes FGF15, FGF21 and FGF23. This subfamily is widely distributed in human tissues [5]. FGF19 plays a crucial role in regulating various cellular processes, such as glucose, lipid, and vitamin D metabolisms, as well as bile acid synthesis [6-8]. Several studies have shown the aberrant expression of FGF19 in tumor development and progression it is involved in cell proliferation, differentiation, and motility. For example, LR et al reported that FGF 19 inhibited growth of colon tumor xenografts in vivo and in FGF19 transgenic mice, it effectively prevented hepatocellular carcinomas [9], and Miura et al found that FGF19 increase the proliferation and invasion capabilities of human hepatocellular carcinoma cell lines [10]. Hyeon et al indicated that FGF19 might be an effective predictor of early recurrence and poor prognosis of hepatocellular carcinoma [11]. In invasive breast ductal carcinoma, the expression of FGF19 protein was dramatically increased and correlated with worse prognosis [12]. And FGF19 promoted ovarian cancer proliferation and invasion by AKT-MAPK signaling pathway [13]. Collectively, these findings implied that

Table 1. Expression of FGF19 in 140 human lung adenocarcinoma carcinoma tissues

Clinicopathological parameters	Total	FGF19 Expression		P
		Low	High	
Gender	140			0.738
Male	69	31	38	
Female	71	34	37	
Age (year)				0.089
< 60	60	33	27	
≥ 60	80	32	48	
Tumor size (cm)				< 0.01*
< 3	60	49	11	
≥ 3	80	26	64	
Smoking				0.424
No	108	48	60	
Yes	32	17	15	
Lymph node metastasis				0.02*
No	88	50	38	
Yes	52	15	37	
TNM clinical stage				< 0.01*
I	93	59	34	
II	30	4	26	
III	17	2	15	
Pathology grade				< 0.01*
I	26	21	5	
II	96	40	56	
III	18	4	14	
Ki-67 expression				< 0.01*
Low	78	52	26	
High	62	13	49	

*Statistical analyses were performed by the Pearson χ^2 test. $P < 0.05$ was considered significant.

FGF19 could promote aggressive tumor progression of many cancer types. However, to our limited knowledge, whether FGF19 functions in lung adenocarcinoma (LAC) remains unknown. In the present study, we aimed to investigate FGF19 expression and its prognosis role FGF19 in lung adenocarcinoma. Our research implicated that the level of FGF19 was increased and might serve as a prognostic factor of LAC.

Materials and methods

Patients and tissue samples

A total of 140 LAC specimens were obtained from the Affiliated Hospital of Nantong University from 2009 to 2013. None of the patients who underwent lung resection was treated with preoperative and postoperative systemic chemotherapy. All patients were followed up for 1-66 months. Permission to use the tissue sections for research purposes was obtained and approved by the Ethics Committee of Affiliated Hospital of Nantong University and written informed consent was obtained from every patient. The main clinical and pathologic variables were shown in **Table 1**. After surgical removal, a portion of paired tissue samples were snap-frozen in liquid nitrogen and then maintained at -80°C until use for protein extraction, and another portion of paired tissue samples were immediately fixed in formalin and embedded in wax for immunohistochemistry. The main clinicopathological variables of the patients are shown in **Table 1**.

Western blot analysis and antibodies

Frozen lung tissues and harvested cells were carried out for immunoblot analysis. Tissue and cell protein were promptly homogenized in a homogenization buffer containing 1 M Tris HCl pH 7.5, 1% Triton X-100, 1% Nonidet p-40 (NP-40), 10% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 0.5 M EDTA, leupeptin 10 μg/ml, aprotinin 10 μg/ml, and 1 mM PMSF, then centrifuged at 12,000×g for 30 minutes to collect the supernatant liquid. Protein concentrations were determined with a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). The supernatant diluted in 2×SDS loading buffer and boiled for 15 min. The proteins were separated with SDS polyacrylamidegel electrophoresis (SDS-PAGE) and transferred to polyvinyllidine difluoride filter (PVDF) membranes (Millipore, Bed ford, MA). The membranes were blocked with 5% dried skim milk in TBST (20 mM Tris, 150 mM NaCl, and 0.05% Tween-20). After 2 h at room temperature, the membranes were incubated overnight with anti-FGF19 (1:800; Santa Cruz Biotechnology); GAPDH (1:1000; Sigma). Blots were washed three times in TBST buffer, followed by incubation with the appropriate horseradish peroxidase-linked secondary antibodies. The band density was measured with a computer-assisted image analysis system (Adobe Systems, San Jose, CA). The relative protein levels were calculated based on GAPDH as the loading control. The experiments were carried out on three separate occasions.

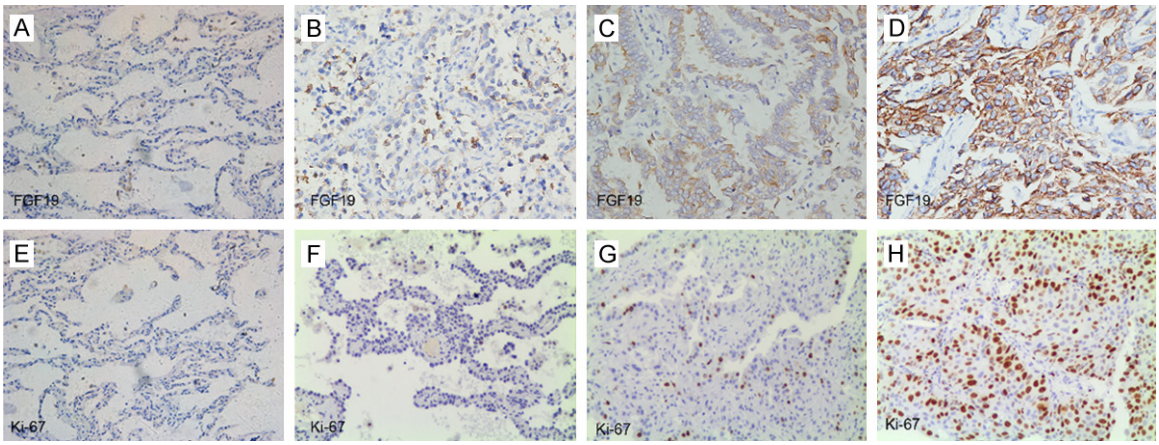

Figure 1. Immunohistochemical stain of FGF19 and ki-67 in LAC tissues. Paraffin-embedded tissue sections were stained with antibodies against FGF19 and Ki-67 and counterstained with hematoxylin in normal lung tissue (A, E), Pathology grade I LAC tissues (B, F), Pathology grade II LAC tissues (C, G), and Pathology grade III LAC tissues (D, H). Details of the experiment were described in "Materials and methods".

Immunohistochemistry (IHC)

The sections were deparaffinized with a graded ethanol series, and endogenous peroxidase activity was blocked by soaking in 3% hydrogen peroxide for 30 min. And then, the sections were processed in 10 mmol/l citrate buffer (pH = 6.0) and heated to 121°C in an autoclave for 20 min to retrieve the antigen. After rinsing in PBS (pH = 7.2), PBS containing 10% goat serum was added for 1 h at room temperature to block any nonspecific reactions. The sections were then incubated with anti-FGF19 antibody (diluted 1:200) and anti-Ki-67 antibody (diluted 1:400) for 2 h at room temperature. Negative control slides were also processed in parallel using a non-specific immunoglobulin in IgG (diluted 1:100; Santa Cruz Biotechnology) at the same concentration as the primary antibody. All slides were processed using the peroxidase-anti-peroxidase method (DAKO, Hamburg, Germany). After rinsing in water, the peroxidase sections were counterstained with hematoxylin, dehydrated, and cover-slipped.

Immunohistochemical evaluation

Two observers who were blinded to clinical and follow-up data evaluated staining results independently with a multihead microscope and co-observed for a consensus when they were divergent. Five high-power (200× magnification) fields were chosen randomly for each section, and 500 cells were counted per field. For statistical analysis of FGF19 stain, each slide was evaluated using a semiquantitative scoring system for both the intensity of the stain and the percentage of positive malignant cells. The intensity of FGF19 nuclear staining was scored as 0 (no staining), 1 (weak), or 2 (marked). The percentage of cells were scored as 1 (1-25% positive), 2 (26-50%), 3 (51-75%), and 4 (76-100%). Then, we multiplied the two scores and classified them into two groups: PCBP low expression, (-) with a score < 4 or overexpression (+) with a score ≥ 4. As for statistical analysis of Ki-67 stain, a cut-off value of 50% or more positively stained nuclei in five high-power fields was used to define Ki-67 staining: low expression group (< 50%) and high expression group (≥ 50%)

Statistical analysis

All computations were carried out using SPSS version 16.0 software. The association between FGF19 expression and clinicopathological features were analyzed using the χ^2 test. The correlation between FGF19 and Ki67 expression in LAC was further evaluated by Spearman rank correlation test. For analysis of survival data, Kaplan-Meier curves were constructed, and the log-rank test was performed. Multivariate analysis was performed using Cox's proportional hazards model. The *P* value was based on the two-sided statistical analysis, and *P* Values of less than 0.05 were considered statistically significant.

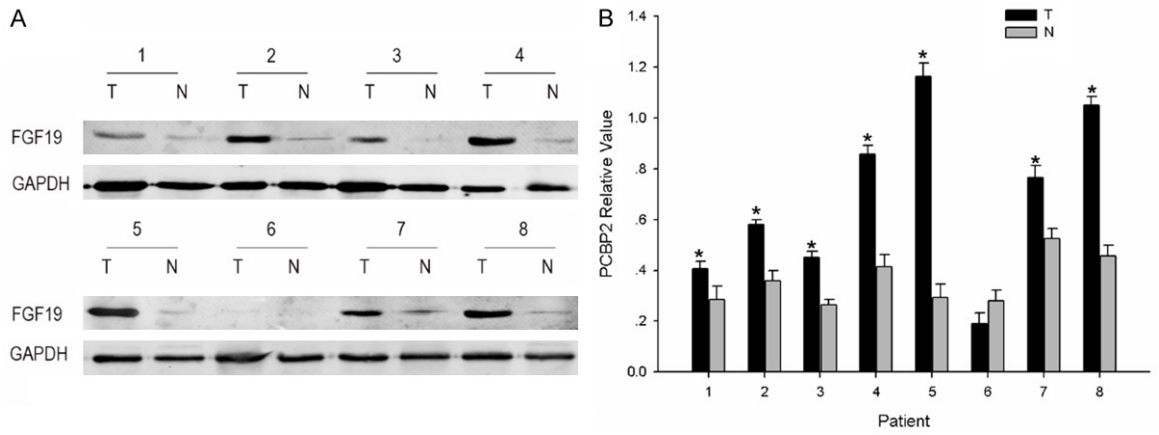

Figure 2. FGF19 was over-expressed in human LAC tumor tissues. A: Western blot was performed to detect expression of FGF19 and GAPDH in eight representative paired LAC tumor tissues (T) and adjacent non-tumor tissues (N). GAPDH was used as a control for protein load and integrity. B: The band intensity of FGF19 and GAPDH was quantified and FGF19 protein level was normalized to the GAPDH protein and plotted. The data were mean ± SD of three independent experiments (*P < 0.01, compared with adjacent non-tumor tissues).

Results

FGF19 expression was up-regulated in LAC tumor tissues

To evaluate the expression of FGF19 and its clinical significance in LAC, immunohistochemistry assay was performed to determine the expression of FGF19 and Ki-67, which is a cell proliferation index, in 140 paraffinn-embedded LAC samples. Both of their expression were up-regulated in LAC tumor tissues compared with non-tumor tissues, which showed rare or almost none expression. In addition, poorer differentiated LAC tissues showed much significant higher expression of both FGF19 and Ki-67 (**Figure 1**). Next, we evaluated the expression of FGF19, as well as GAPDH, which was used as a loading control, by Western blot analysis in 8 paired fresh LAC tumor tissues and adjacent non-tumor tissues. As expected, 7 out of the 8 paired LAC tissues (except sample 6) showed much higher FGF19 expression in tumor tissues than adjacent non-tumor tissues (**Figure 2A** and **2B**). Therefore, FGF19 might be a candidate regulator for LAC progression.

Correlation of FGF19 expression with clinicopathologic variables in LAC

To confirm the correlation of FGF19 and Ki-67 expression, Spearman's correlation test was next performed by evaluating the percentage of positive malignant cells. Significant positive correlation was found between the expression status of FGF19 and Ki67 (**Figure 3A**, $P < 0.05$, $r = 0.813$). In addition, we further evaluated the association of FGF19 expression with clinicopathologic variables including Ki-67 by Pearson χ^2 test. The level of FGF19 and Ki-67 expression was divided into high group and low group according to the cutoff value stated in the aforementioned methods. The data were summarized in **Table 1**. The results were consistent with the previous Spearman correlation test, significant positive correlations were found between the expression level of FGF19 and Ki-67 (**Table 1**, $P < 0.01$). And in agreement with the results shown in **Figure 1**, the expression level of FGF19 was associated with pathology grade and clinical stage (**Table 1**, $P < 0.01$). Moreover, there was significant positive correlation between the expression level of FGF19 with tumor size and lymph node metastasis (**Table 1**, $P < 0.05$).

Higher expression of FGF19 predicted poor prognosis of LAC patients

Pearson χ^2 test was performed to analyze the association of clinicopathological variables including gender, age, TNM stage, pathology grade and FGF19 expression with clinical prognosis. Of these 140 patients with available complete follow-up data, only 25 of 75 (33.3%) in the group of high FGF19 expression were alive versus 48 of 65 (73.8%) in the group of

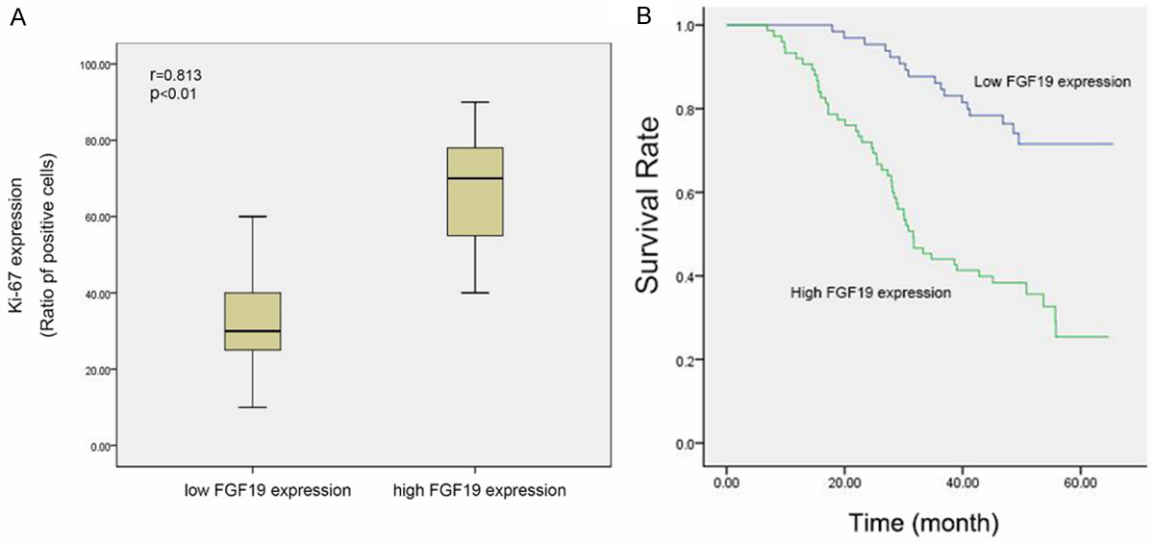

Figure 3. A: Correlation between FGF19 and Ki-67 expression in LAC samples. The correlation between FGF19 and Ki67 expression in LAC was further evaluated by Spearman rank correlation test, which was shown as Box-plot ($P < 0.01$). B: Kaplan-Meier survival curves for high FGF19 expression versus low FGF19 expression in 140 patients of LAC showed a highly significant separation ($P = 0.001$, log rank test).

low FGF19 expression (**Table 2**). Besides FGF19 expression (**Table 2**, $P < 0.01$), there were also significant correlations between tumor size (**Table 2**, $P < 0.01$), lymph node metastasis (**Table 2**, $P < 0.01$), TNM stage (**Table 2**, $P < 0.01$), Pathology grade (**Table 2**, $P < 0.01$) and that of clinical prognosis. Moreover, multivariate analysis using the Cox's proportional hazards model showed that FGF19 expression (**Table 3**, $P = 0.037$), as well as gender (**Table 3**, $P = 0.004$), TNM stage (**Table 3**, $P = 0.038$), and Pathology grade (**Table 3**, $P = 0.001$), was an independent prognostic indicator for patients' overall survival. In addition, Kaplan-Meier survival analysis was used to calculate the impact of FGF19 expression level on patients' survival time (**Figure 3B**). The survival curves indicated that high expression of FGF19 significantly associated with decreased overall survival ($P = 0.001$).

Discussion

Adenocarcinoma (AC) and squamous cell carcinoma (SqCC) are the predominant histological types among NSCLC. More and more evidences show that AC and SqCC respond differently to therapy [14]. Biological differences may lead to differences in progression and response to therapies [15]. Nowadays, studies have reported lung adenocarcinoma tends to grow and spread faster than lung squamous cell carcinoma and accounts for almost half of all lung cancers. However, the pathogenesis of lung adenocarcinoma remains unclear. Therefore, it is urgent to investigate the molecular mechanism of carcinogenesis of lung adenocarcinoma.

FGF19 is located at chromosomal region 11q13. It has a range of cellular functions including proliferation, differentiation and angiogenesis, and their ectopic overexpression has been discovered in many types of cancers. In prostate cancer, FGF19 overexpression might be associated with biochemical recurrence by promoting cell proliferation and epithelial-mesenchymal transition [16], and FGF19 promoted the growth, invasion, adhesion and colony formation of prostate cells by indirect Activates MAP Kinase and AKT Pathways [17]. FGF19 Reduces colon tumor growth which depends on the tyrosine phosphorylation of β-catenin and causes loss of E-cadherin binding to β-catenin [18]. Recently Zhang et al reported the expression the level of FGF19 protein in thyroid cancer tissues was significantly higher than that in normal tissues, it might be involved in the malignant behaviors of thyroid cancer [19]. Therefore, dysregulation of FGF19 is closely linked to various human tumors. However, the role of FGF19 in LAC is still unclear. In this study, we first evaluated the

Table 2. Survival status and clinicopathological parameters in LAC speciments

Clinicopathological parameters	Total	Survival status		P
		Dead	Alive	
Gender	140			0.011
Male	69	41	28	
Female	71	26	45	
Age (year)				0.529
< 60	60	29	31	
≥60	80	38	42	
Tumor size (cm)				
< 3	60	19	41	
≥ 3	80	48	32	< 0.01*
Smoking				0.318
No	108	49	59	
Yes	32	18	14	
Lymph node metastasis				< 0.01*
No	88	32	56	
Yes	52	35	17	
TNM clinical stage				< 0.01*
I	93	32	61	
III	17	14	3	
Pathology grade				< 0.01
I	26	6	20	
II	96	45	51	
III	18	4	14	
FGF19				< 0.01*
Low	65	17	48	
High	75	50	25	
Ki-67				< 0.01*
Low	78	15	63	
High	62	52	10	

*Statistical analyses were performed by the Pearson χ^2 test. P < 0.05 was considered significant.

Table 3. Contribution of various potential prognostic factors to survival by Cox regression analysis on 140 human lung adenocarcinoma carcinoma

	Hazard ratio	P	95.0% Confidence interval
Age	0.814	0.504	0.507-1.396
Gender	2.175	0.004*	1.286-3.677
Tumor size	0.829	0.551	0.447-1.537
TNM clinical stage	1.484	0.038*	1.021-2.157
Lymph node metastasis	0.982	0.948	0.559-1.723
Pathology grade	2.285	0.001*	1.374-3.802
Smoking	0.892	0.700	0.500-1.592
FGF19 expression	2.096	0.037*	1.047-4.194

Statistical analyses were performed by the Cox regression analysis. *P < 0.05 was considered significant.

expression of FGF19 in human lung adenocarcinoma by Western blot and immunohistochemistry assays. It demonstrated that FGF19 was overexpressed in human LAC compared with the normal lung tissues. In addition, multivariate analysis revealed that FGF19 was an independent prognosis marker for LAC. And high expression of FGF19 was significantly associated with poor prognosis of LAC patients. Taken together, these findings supported our hypothesis that FGF19 might play an important role in promotion of LAC progression.

In summary, the present study shows that FGF19 is overexpressed in LAC and correlates with its advanced stage and poor prognosis. All these results support a novel role for FGF19 in LAC progression and provide a potential novel molecular target for detection and treatment of LAC.

Acknowledgements

This study was supported by the project of “Six talent peaks project in Jiangsu Province”, the scheme for Hospital Affiliated to Nantong University talents (no. WSN-059).

Disclosure of conflict of interest

None.

Address correspondence to: Yi-Fei Liu, Department of Pathology, Affiliated Hospital of Nantong University, 19 Qixiu Road, Nantong 226001, Jiangsu, China. E-mail: bluefiime@qq.com; Jun-Hua Liu, Department of Thoracic Surgery, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China. E-mail: liujunhuascience@163.com

References

[1] Cancer statistics. JAMA 2013; 310: 982.
[2] Ji XD, Li G, Feng YX, Zhao JS, Li JJ, Sun ZJ, Shi S, Deng YZ, Xu JF, Zhu YQ, Koeffler HP, Tong XJ, Xie D. EphB3 is overexpressed in non-small-cell lung cancer and promotes tumor metastasis by enhancing cell survival and migration. Cancer Res 2011; 71: 1156-1166.
[3] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
[4] Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol 2001; 2: REVIEWS3005.
[5] Harmer NJ, Pellegrini L, Chirgadze D, Fernandez-Recio J, Blundell TL. The crystal structure of fibroblast growth factor (FGF) 19 re-

veals novel features of the FGF family and offers a structural basis for its unusual receptor affinity. Biochemistry 2004; 43: 629-640.
[6] Wu X, Li Y. Therapeutic utilities of fibroblast growth factor 19. Expert Opin Ther Targets 2011; 15: 1307-1316.
[7] Schaap FG. Role of fibroblast growth factor 19 in the control of glucose homeostasis. Curr Opin Clin Nutr Metab Care 2012; 15: 386-391.
[8] Jones S. Mini-review: endocrine actions of fibroblast growth factor 19. Mol Pharm 2008; 5: 42-48.
[9] Desnoyers LR, Pai R, Ferrando RE, Hotzel K, Le T, Ross J, Carano R, D'Souza A, Qing J, Mohtashemi I, Ashkenazi A, French DM. Targeting FGF19 inhibits tumor growth in colon cancer xenograft and FGF19 transgenic hepatocellular carcinoma models. Oncogene 2008; 27: 85-97.
[10] Miura S, Mitsuhashi N, Shimizu H, Kimura F, Yoshidome H, Otsuka M, Kato A, Shida T, Okamura D, Miyazaki M. Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. BMC Cancer 2012; 12: 56.
[11] Hyeon J, Ahn S, Lee JJ, Song DH, Park CK. Expression of fibroblast growth factor 19 is associated with recurrence and poor prognosis of hepatocellular carcinoma. Dig Dis Sci 2013; 58: 1916-1922.
[12] Buhmeida A, Dallol A, Merdad A, Al-Maghrabi J, Gari MA, Abu-Elmagd MM, Chaudhary AG, Abuzenadah AM, Nedjadi T, Ermiah E, Al-Thubaity F, Al-Qahtani MH. High fibroblast growth factor 19 (FGF19) expression predicts worse prognosis in invasive ductal carcinoma of breast. Tumour Biol 2014; 35: 2817-2824.
[13] Hu L, Cong L. Fibroblast growth factor 19 is correlated with an unfavorable prognosis and promotes progression by activating fibroblast growth factor receptor 4 in advanced-stage serous ovarian cancer. Oncol Rep 2015; 34: 2683-2691.
[14] Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, Simms L, Shepherd FA. The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. Oncologist 2009; 14: 253-263.
[15] Garraway LA, Sellers WR. Lineage dependency and lineage-survival oncogenes in human cancer. Nat Rev Cancer 2006; 6: 593-602.
[16] Nagamatsu H, Teishima J, Goto K, Shikuma H, Kitano H, Shoji K, Inoue S, Matsubara A. FGF19 promotes progression of prostate cancer. Prostate 2015; 75: 1092-1101.
[17] Feng S, Dakhova O, Creighton CJ, Ittmann M. Endocrine fibroblast growth factor FGF19 promotes prostate cancer progression. Cancer Res 2013; 73: 2551-2562.
[18] Pai R, Dunlap D, Qing J, Mohtashemi I, Hotzel K, French DM. Inhibition of fibroblast growth factor 19 reduces tumor growth by modulating beta-catenin signaling. Cancer Res 2008; 68: 5086-5095.
[19] Zhang X, Wang Z, Tian L, Xie J, Zou G, Jiang F. Increased expression of FGF19 contributes to tumor progression and cell motility of human thyroid cancer. Otolaryngol Head Neck Surg 2016; 154: 52-58.