Associations of IFT20 and GM130 Protein Expression with Clinicopathological Features and Survival of Patients with Lung Adenocarcinoma

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Abstract

Background: Lung cancer is the leading cause of malignancy-related mortality and lung adenocarcinoma accounts for about 40% of lung malignancies. The aim of this study was to investigate the associations of IFT20 and GM130 protein expression with clinicopathological features and survival in patients with lung adenocarcinoma.

Methods: The expressions of IFT20 and GM130 protein in cancerous and matched adjacent lung tissues of 235 patients with lung adenocarcinoma were assessed by tissue microarray and immunohistochemistry. Associations of IFT20 and GM130 protein expression with clinicopathological features of patients were analyzed by multivariate logistic regression models. The survival analysis of patients was performed by a Cox proportional hazards regression model and the median overall survival (OS) was analyzed by Kaplan-Meier survival analyses.

Results: With adjustment for multiple potential confounders, each one point increase in IFT20 protein staining intensity score was significantly associated with 32% and 29% reduced ORs for TNM stage in II~III and lymphatic metastasis of patients, respectively ($P<0.05$). And each one point increase in GM130 staining intensity scores was associated with a significant reduction in the risk of poor differentiation and tumors size $>7\text{cm}$ by 29% and 38% for lung adenocarcinoma patients, respectively ($P<0.05$). The median OS was 3.62 years among patients in the third quartile of GM130 positive cells rate, which was significantly longer than those patients of 2.56 years in the lowest quartile. For patients with tumor size $>7\text{cm}$ or distant metastasis, enhanced GM130 expression was significantly decreased the risk of death by 51% and 61%, respectively ($P<0.05$).

Conclusion: IFT20 and GM130 expressions were negatively associated with differentiated types and size of tumor, TNM stage and lymphatic metastasis. GM130 expression has a protective influence on OS for lung adenocarcinoma, especially patients with tumor size $>7\text{cm}$ or distant metastasis.

Introduction

Lung cancer is the leading cause of malignancy-related mortality, resulting in more deaths than breast, prostate, colorectal and brain cancers [1]. Lung adenocarcinoma is the most common histological subtype in non-small cell lung cancer (NSCLC), which accounts for about 40% of lung malignancies [2]. The average 5-year survival rate of lung adenocarcinoma is only 18%, mainly due to invasion and metastasis of tumor cells, diagnosed at advanced stages or lack of effective treatment strategies [3]. The tumor invasion and metastasis have a significant impact on the quality of life and overall survival (OS) of patients with lung adenocarcinoma [4]. Thus, early detection of tumor invasion and metastasis is crucial to improve the survival rate of patients with lung adenocarcinoma.

Primary cilia are antenna-like organelles protruding from the surface of most eukaryotic cells that participate in cell proliferation, differentiation and migration [5]. Some studies have reported that the number of primary cilia markedly decreased in the transformation of many tumor cells, including breast, pancreas, ovarian, and kidney cancer cells, whereas it was significantly higher in cancerous tissues of lung adenocarcinoma, colon adenocarcinoma and follicular lymphoma than that in normal control tissues [6]. Primary cilia require a series of intraflagellar transport (IFT) protein complexes involved in the transportation of ciliary signaling proteins as well as the process of cellular protrusion and resorption to assemble and maintain their structure [7]. IFT20, the smallest IFT protein, localizes at the cis-Golgi apparatus that regulates the assembly and maintenance of primary cilia by sorting of ciliary cargo from the cis-Golgi to the base of the cilium [8, 9]. Down-regulated expression of IFT20 mRNA or protein could not only inhibit ciliary assembly and decrease the quantity of primary cilia in mammalian cells, but also reduce invasion and metastasis of colorectal cancer and osteosarcoma cells [8–10].

Recent studies indicated that tumor's invasion and metastasis often require cell polarization and migration [10]. IFT20 protein combining with GM130-AKAP450 complex could regulate cellular microtubules nucleation, which contributes to Golgi ribbon formation involved in cell migration and invasion [10, 11]. As a cis-Golgi matrix protein, GM130 is essential for maintaining Golgi structure and transporting proteins that participates in the process of cell polarization and migration [11, 12]. Expression of GM130 mRNA or protein was significantly lower in tissues of colorectal adenocarcinoma and breast cancer than that in matched normal tissues, and the depletion of GM130 mRNA enhanced the invasiveness of breast cancer cells [13, 14]. Thus, IFT20 and GM130 proteins might play crucial roles in the invasion and metastasis of various malignant tumors, but their roles in the tumorigenesis and development of lung adenocarcinoma remains unclear so far.

Hence, we determined the expression of IFT20 and GM130 proteins in cancerous tissues and adjacent tissues of patients with lung adenocarcinoma by tissue microarray and immunohistochemistry to investigate their potential roles in the development of lung adenocarcinoma and the relationship between their expressions and survival of patients with lung adenocarcinoma.

Materials And Methods

Study population

A total of 235 patients with lung adenocarcinoma were collected from one hospital in Wuhan city in China from March 2010 to September 2015 and followed up to June 2018. All the patients underwent lung cancer surgery and were finally diagnosed with lung adenocarcinoma by pathological biopsy. This study was approved by the Ethics and Human Subject Committees of Tongji hospital at Huazhong University of Science and Technology. All participants enrolled in this study signed written informed consent for participation, storage and use of surgically-removed cancer and adjacent tissues. And all methods of this study were carried out in accordance with relevant guidelines and regulations.

Data and tissue samples collection

We collected basic information such as age, gender, smoking status, smoking amount, and clinicopathological features including differentiated types of tumor cells, tumor size, tumor node metastasis (TNM) stage, lymphatic or distant metastasis, cell proliferation index (Ki67) and chemoradiotherapy. The differentiated types of lung adenocarcinoma were classified into micropapillary and solid, acinar and papillary, and lepidic types, which are identified as poorly, moderately and well differentiated tumors according to the criteria established by World Health Organization [15]. The TNM stage of patients with lung adenocarcinoma was diagnosed based on the 7th edition (2009) of the American Joint Committee on Cancer for Lung Cancer [16]. And the cancerous tissue and matched adjacent tissue of each patient were collected for histological analysis.

Tissue chips preparation

Small pieces of tissue samples were fixed in 4% paraformaldehyde for 24 h, and then dehydrated, paraffin-embedded, sliced and stained with hematoxylin and eosin (HE), which were examined by microscope to determine the sampling location of the chip. After heating the paraffin block, the target samples taken out by a sampler were inserted into the prepared paraffin block receptor hole in sequence. The samples were merged at 60~65°C for 20 min using tissue chip fusion instrument (BP0100, Biossci Company). The melted block was then put into a wax mold, embedded in paraffin and cut into 4 µm thick sections.

Immunohistochemistry (IHC)

The sections were deparaffinized, rehydrated, rinsed with distilled water, and repaired at high temperature and pressure for 1~2 min. After cooled to room temperature and washed with Tris-buffer solution (TBS) three times, the sections were incubated with fresh 3% hydrogen peroxide at room temperature for 20 min and blocked with 10% normal goat serum for 20 min. The sections were then incubated with primary antibodies (IFT20, 1:200, proteintech, 13615-1-AP; GM130, 1:200, abcam, ab52649) overnight at 4°C. After incubated at room temperature for 15 min, the sections were washed by TBS three times and then incubated with secondary antibodies (50 µl DAKO) for another 25 min at room temperature. Then the sections were washed three times, stained with diaminobenzidine and counterstained by hematoxylin.

The expressions of positive staining IFT20 and GM130 protein were indicated by the mean optical density (IOD/area), the rate and intensity of positive staining cells. The mean IOD/area and the rate of positively staining cells in five representative views (original objective 400×) from each section were analyzed by Image J software version 1.2.4 (the National Institutes of Health, NIH free software, Bethesda, MD, USA). The mean IOD/area of these views represented the relative expression of positive staining IFT20 and GM130 protein, and the rate of positive stained cells was equal to positive cells number/total cells number×100%. The staining intensity of positive cells of each section was denoted by staining intensity score (0= no staining; 1= weak staining; 2= intermediate staining; 3= strong staining), which was independently evaluated by five professionally trained persons without knowledge of the clinicopathological data of patients. The final score was determined as the consistent results made by three or more persons.

Statistical analysis

Continuous variables were divided into normal and non-normal distribution, which were compared by Student's t test and Wilcoxon rank sum test, respectively. Categorical variables were analyzed by chi-square test or Fisher's exact test. Associations of IFT20 and GM130 protein expressions with clinicopathological features of lung adenocarcinoma patients were assessed by multivariate logistic regression models, adjusting for multiple potential confounders including age, gender, smoking status, smoking amount. The survival analysis of patients with lung adenocarcinoma after lung cancer surgery was performed by a Cox proportional hazards regression model and the median OS was analyzed by Kaplan-Meier survival analyses. All statistical analyses were carried out with SPSS version 26.0 (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered statistically significant.

Results

The basic characteristics of all participants

The basic characteristics of all patients with lung adenocarcinoma were summarized in Table 1, including 56 survivors and 179 death cases by the end of follow-up. The average age was 53.86 ± 8.64 years for surviving patients, which were significantly younger than that of dead patients at age of 57.33 ± 9.98 years ($P < 0.05$). The OS of surviving patients after lung cancer surgery was significantly longer (4.70 ± 1.22 years) than that of dead patients (2.26 ± 1.40 years). The proportions of poorly differentiated tumors, T3~T4 stages, N2~N3 stages, M1 stage, TNM stage II~IV, lymphatic and distant metastasis in survivors were significantly lower than those in deaths ($P < 0.05$). Furthermore, the size of tumor in survivors (3.83 ± 1.87 cm) was statistically smaller when compared with dead patients (4.79 ± 2.28 cm) ($P = 0.001$). However, there were no significant differences in gender, smoking status, smoking amount, Ki67, chemoradiotherapy between survivors and death cases ($P > 0.05$).

Table 1
The basic characteristics of all patients with lung adenocarcinoma

Variables	All patients(n=235)	Survivors(n=56)	Deaths(n=179)	χ^2	P value
Age (years, Mean $\pm$ SD)	56.50 $\pm$ 9.82	53.86 $\pm$ 8.64	57.33 $\pm$ 9.98		0.020
Gender, (Female, n, %)	98 (41.70)	24 (42.86)	74 (41.34)	0.040	0.841
Smoking status (n, %)				0.131	0.936
Current smoking	72 (30.64)	18 (32.14)	54 (30.17)		
Never smoking	135 (57.45)	31 (55.36)	104 (58.10)		
Former smoking	28 (11.91)	7 (12.50)	21 (11.73)		
Smoking amount, pack-year (Mean $\pm$ SD)	15.80 $\pm$ 23.97	16.56 $\pm$ 22.51	15.56 $\pm$ 24.47		0.856
Differentiated types of tumors (n, %)				9.486	0.009
Poorly	71 (30.21)	10 (17.86)	61 (34.08)		
Moderately	124 (52.77)	30 (53.57)	94 (52.51)		
Well	40 (17.02)	16 (28.57)	24 (13.41)		
Ki67				3.666	0.056
<50%	189 (80.43)	50 (89.29)	139 (77.65)		
$\geq$ 50%	46 (19.57)	6 (10.71)	40 (22.35)		
Tumor size(cm)	4.57 $\pm$ 2.22	3.83 $\pm$ 1.87	4.79 $\pm$ 2.28		0.001
T stages (n, %)				5.210	0.022
T1~T2	155 (65.96)	44 (78.57)	111 (62.01)		
T3~T4	80 (34.04)	12 (21.43)	68 (37.99)		
N stages (n, %)				7.258	0.007
N0~N1	90 (38.30)	30 (53.57)	60 (33.52)		
N2~N3	145 (61.70)	26 (46.43)	119 (66.48)		
M stages (n, %)				3.962	0.047
M0	188 (80.00)	50 (89.29)	138 (77.09)		
M1	47 (20.00)	6 (10.71)	41 (22.91)		
TNM stages (n, %)				16.648	0.001
I stage	52 (22.13)	23 (41.07)	29 (16.20)		
II stage	37 (15.74)	6 (10.71)	31 (17.32)		
III stage	99 (42.13)	21 (37.50)	78 (43.58)		
IV stage	47 (20.00)	6 (10.71)	41 (22.91)		
Chemoradiotherapy (yes, n, %)	97 (41.28)	27 (48.21)	70 (39.11)	1.460	0.227
Lymphatic metastasis (yes, n, %)	145 (61.70)	26 (46.43)	119 (66.48)	7.258	0.007
Distant metastasis (yes, n, %)	47 (20.00)	6 (10.71)	41 (22.91)	3.962	0.047
Alive duration (years)	2.82 (1.37,4.06)	4.66 (3.92,5.63)	2.11 (1.07,3.30)		<0.001
Notes: Smoking amount (pack-years) for each smoker was calculated as packs of cigarettes per day multiplied by years of smoking.					

Expressions of IFT20 and GM130 protein in lung sections of patients with lung adenocarcinoma

As shown in Figure 1A and B, the expressions of IFT20 and GM130 protein were both positively expressed in cancerous and matched adjacent tissues of lung adenocarcinoma. In Table 2, compared with matched adjacent tissues, the expression of IFT20 protein (IOD/area, rate of positive staining cells and staining intensity scores) in cancerous tissues was significantly increased whereas the IOD/area of GM130 protein was statistically decreased ($P<0.001$). Difference in the rate of GM130 positive staining cells between cancerous and matched adjacent tissues of surviving patients was significantly higher than those of deaths ($P<0.05$). However, there was no significant difference in IFT20 expression between survivors and death cases ($P>0.05$).

Table 2
Expressions of IFT20 and GM130 protein in cancerous and matched adjacent tissues of patients with lung adenocarcinoma

Protein	Variables	All patients(n=235)	Survivors(n=56)	Deaths(n=179)
IFT20	IOD/area			
	Cancerous tissue	0.201 (0.175, 0.230)*	0.201 (0.169, 0.221)	0.200 (0.176, 0.231)
	Adjacent tissue	0.177 (0.158, 0.198)	0.170 (0.157, 0.196)	0.180 (0.159, 0.199)
	D value	0.021 (-0.006, 0.052)	0.019 (-0.001, 0.052)	0.021 (-0.006, 0.052)
	Rate of positive cells			
	Cancerous tissue	31.05 (20.14, 42.09)*	28.70 (19.61, 39.76)	31.14 (20.51, 42.93)
	Adjacent tissue	17.10 (12.01, 24.42)	14.65 (11.45, 22.85)	18.27 (12.45, 24.42)
	D value	12.47 (3.10, 22.83)	11.82 (0.91, 24.83)	12.53 (3.52, 21.96)
	Staining intensity scores			
	Cancerous tissue	3.0 (2.0, 3.0)*	3.0 (2.0, 3.0)	3.0 (2.0, 3.0)
	Adjacent tissue	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)
	D value	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)
GM130	IOD/area			
	Cancerous tissue	0.095 (0.079, 0.109)*	0.098 (0.082, 0.116)	0.094 (0.079, 0.107)
	Adjacent tissue	0.110 (0.095, 0.123)	0.112 (0.097, 0.125)	0.109 (0.094, 0.123)
	D value	-0.014 (-0.031, 0.005)	-0.013 (-0.036, 0.012)	-0.014 (-0.030, 0.004)
	Rate of positive cells			
	Cancerous tissue	9.37 (3.03, 19.00)	12.52 (3.61, 22.06)	7.47 (2.86, 18.78)
	Adjacent tissue	10.21 (6.29, 13.61)	9.74 (6.23, 11.99)	10.41 (6.29, 15.08)
	D value	-0.16 (-6.36, 7.68)	2.06 (-2.61, 10.56)	-1.64 (-7.46, 7.64)#
	Staining intensity scores			
	Cancerous tissue	2.0 (1.0, 2.0)*	2.0 (1.0, 3.0)	2.0 (1.0, 2.0)
	Adjacent tissue	1.0 (0.0, 1.0)	1.0 (0.0, 1.0)	1.0 (0.0, 1.0)
	D value	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)

Notes: D value is equal to the difference value of protein expression between cancerous tissue and adjacent tissue; * $P<0.05$, comparison between cancerous and adjacent tissues in all patients; # $P<0.05$, comparison between survivors and deaths.

Association of IFT20 and GM130 protein expression with clinicopathological features of patients with lung adenocarcinoma

With adjustment for multiple potential confounders, each one point increase in the difference of IFT20 protein staining intensity score between cancerous and matched adjacent tissues was significantly associated with a 32% and 29% reduced ORs for TNM stage in II~IV and lymphatic metastasis of patients, respectively. The adjusted OR (95%CI) of IFT20 positive staining cells rate for lung adenocarcinoma patients with lymphatic metastasis in the highest quartile was 0.44 (0.20, 0.93) in comparison with that in the lowest quartile. Although there was no association of IFT20 protein expression with differentiated types, size and distant metastasis of tumors, each one point increase in difference of GM130 staining intensity scores was correlated with a significant reduction in the risk of poorly differentiated types and tumors size>7cm by 29% and 38%, respectively. In categorical analysis, there was also statistically negative relationship of GM130 protein expression (IOD/area and rate of positive cells) with the risk of poorly differentiated tumors, tumor size >7cm and TNM stage in II~IV. Additionally, elevated rate of GM130 positive cells was significantly associated with decreased risk of poorly differentiated types of tumors in a dose-dependent manner ($P_{\text{trend}}<0.05$). However, no significant relationships were observed between GM130 expression (IOD/area, positive cells rate and staining intensity scores) and other clinicopathological features including lymphatic and distant metastasis of patients with lung adenocarcinoma ($P>0.05$). (Shown in Table 3)

Table 3
Association of IFT20 and GM130 protein expression with clinicopathological features of patients with lung adenocarcinoma

protein	D Value	Differentiated types		Tumor size		TNM stage		Lymphatic metastasis		Distant me
		Poorly/moderately to well	OR(95%CI)	>7cm/ ≤7cm	OR(95%CI)	II~Ⅲ/I	OR(95%CI)	Yes/no	OR(95%CI)	Yes/no
IFT20	IOD/area	71/164	0.96 (0.72, 1.28)	25/210	0.71 (0.46, 1.09)	183/52	0.84 (0.62, 1.16)	145/90	0.82 (0.63, 1.08)	47/188
	Q1(<-0.006)	20/38	reference	8/50	reference	49/9	reference	37/21	reference	13/45
	Q2(-0.006~0.0207)	14/45	0.57 (0.25, 1.29)	8/51	0.92 (0.30, 2.82)	49/10	0.82 (0.31, 2.23)	40/19	1.13 (0.52, 2.45)	12/47
	Q3(0.0207~0.052)	18/42	0.85 (0.39, 1.87)	5/55	0.60 (0.17, 2.05)	42/18	0.44 (0.17, 1.04)	36/24	0.81 (0.38, 1.72)	9/51
	Q4(>0.052)	19/39	0.97 (0.44, 2.12)	4/54	0.39 (0.10, 1.47)	43/15	0.50 (0.20, 1.28)	32/26	0.65 (0.30, 1.38)	13/45
	P_{trend}	0.922		0.131		0.080		0.186		0.823
	Rate of positive cells	71/164	1.00 (0.98, 1.01)	25/210	1.00 (0.97, 1.02)	183/52	0.99 (0.97, 1.01)	145/90	0.99 (0.97, 1.01)	47/188
	Q1(<3.096)	17/41	reference	5/53	reference	49/9	reference	40/18	reference	15/43
	Q2(3.096~12.466)	20/40	1.27 (0.58, 2.79)	6/54	1.17 (0.32, 4.27)	46/14	0.58 (0.23, 1.48)	35/25	0.63 (0.30, 1.34)	11/49
	Q3(12.466~22.828)	21/37	1.41 (0.64, 3.10)	10/48	2.39 (0.73, 7.86)	47/12	0.67 (0.26, 1.77)	41/17	1.09 (0.49, 2.40)	10/48
	Q4(>22.828)	13/46	0.72 (0.31, 1.67)	4/55	0.76 (0.18, 3.16)	41/17	0.40 (0.16, 1.01)	29/30	0.44 (0.20, 0.93)	11/48
	P_{trend}	0.414		0.912		0.086		0.07		0.331
	Staining intensity scores	71/164	0.99 (0.74, 1.34)	25/210	0.66 (0.44, 1.01)	183/52	0.68 (0.47, 0.96)	145/90	0.71 (0.53, 0.94)	47/188
GM130	IOD/area	71/164	0.97 (0.73, 1.29)	25/210	0.82 (0.56, 1.20)	183/52	1.10 (0.82, 1.48)	145/90	0.97 (0.74, 1.27)	47/188
	Q1(<-0.031)	23/36	reference	11/48	reference	48/11	reference	39/20	reference	7/52
	Q2(-0.031~-0.014)	15/45	0.52 (0.24, 1.14)	3/57	0.14 (0.03, 0.60)	46/14	0.67 (0.27, 1.66)	38/22	0.81 (0.37, 1.74)	13/47
	Q3(-0.014~0.005)	20/37	0.85 (0.40, 1.80)	3/54	0.23 (0.06, 0.92)	43/14	0.75 (0.30, 1.88)	35/22	0.82 (0.38, 1.77)	12/45
	Q4(>0.005)	13/46	0.44 (0.20, 0.99)	8/51	0.44 (0.15, 1.33)	46/13	0.70 (0.28, 1.78)	33/26	0.57 (0.27, 1.23)	15/44
	P_{trend}	0.102		0.204		0.508		0.226		0.17
	Rate of positive cells	71/164	0.97 (0.95, 0.99)	25/210	0.97 (0.94, 1.01)	183/52	1.00 (0.98, 1.03)	145/90	0.99 (0.98, 1.02)	47/188
	Q1(<-6.377)	23/36	reference	10/49	reference	48/10	reference	38/21	reference	12/47
	Q2(-6.377~-0.164)	22/36	1.01 (0.48, 2.15)	4/54	0.28 (0.08, 1.03)	48/12	0.78 (0.31, 1.99)	40/18	1.23 (0.57, 2.65)	13/45
	Q3(-0.164~7.683)	13/47	0.42 (0.19, 0.95)	5/55	0.33 (0.10, 1.08)	39/20	0.38 (0.16, 0.92)	29/31	0.52 (0.25, 1.08)	9/51
	Q4(>7.683)	13/45	0.48 (0.21, 1.09)	6/52	0.45 (0.14, 1.45)	48/10	0.95 (0.36, 2.51)	38/20	1.05 (0.49, 2.25)	13/45
	P_{trend}	0.024		0.294		0.897		0.073		0.948
	Staining intensity scores	71/164	0.71 (0.55, 0.91)	25/210	0.62 (0.41, 0.93)	183/52	0.95 (0.72, 1.26)	145/90	0.79 (0.62, 1.01)	47/188

Influence of IFT20 and GM130 protein expression on the survival of patients with lung adenocarcinoma

Cox proportional hazards regression model was conducted to evaluate the influence of IFT20 and GM130 protein expression on OS of patients with lung adenocarcinoma after surgery. As shown in Figure 2, there were no effects of IFT20 and GM130 protein expressions on the survival of patients with lung adenocarcinoma. Some clinicopathological features such as poorly differentiated type, TNM stage II~IV and distant metastasis had strong influences on increased death risk, with the HRs of 2.29, 1.74 and 1.70, respectively, whereas chemoradiotherapy could significantly reduce the death risk by 34% for

patients with lung adenocarcinoma ($P<0.05$). In Figure 3, The OS was significantly reduced among patients with poorly differentiated type, TNM stage II~IV, distant metastasis and without chemoradiotherapy ($P<0.05$). Furthermore, the median OS of patients in the third quartile of GM130 positive cells rate was 3.62 years, which was significantly longer than those patients of 2.56 years in the lowest quartile. In stratified analysis, for lung adenocarcinoma patients with tumor size >7cm or distant metastasis, elevated GM130 relative expression (IOD/area) was significantly decreased the risk of death by 51% and 61%, respectively (Table 4).

Table 4
Effects of IFT20 and GM130 protein expression on the survival of patients with lung adenocarcinoma by stratified

protein	Variables	Differentiated types		Tumor size		TNM stage		Lymphatic metastasis	
		Poorly	moderately to well	>7cm	≤7cm	II~IV	I	Yes	No
IFT20	IOD/area	1.22(0.95,1.57)	0.84(0.70,1.01)	0.90(0.50,1.65)	0.99(0.84,1.17)	0.97(0.82,1.14)	0.93(0.59,1.46)	1.03(0.85,1.27)	0.8
	Rate of positive cells	1.01(0.99,1.02)	1.00(0.99,1.01)	1.01(0.98,1.04)	1.00(0.99,1.01)	1.01(0.99,1.02)	0.99(0.96,1.02)	1.00(0.99,1.01)	0.9
	Staining intensity scores	1.06(0.83,1.35)	0.85(0.70,1.03)	0.79(0.49,1.29)	0.92(0.78,1.08)	0.88(0.75,1.04)	1.02(0.62,1.69)	0.91(0.76,1.10)	0.8
GM130	IOD/area	0.94(0.64,1.40)	0.92(0.77,1.10)	0.49(0.27,0.89)	0.99(0.82,1.20)	0.90(0.75,1.10)	1.21(0.76,1.91)	1.04(0.82,1.32)	0.8
	Rate of positive cells	1.00(0.98,1.03)	0.99(0.97,1.01)	1.01(0.94,1.08)	0.99(0.98,1.01)	0.99(0.98,1.01)	1.02(0.99,1.06)	1.00(0.99,1.02)	0.9
	Staining intensity scores	1.02(0.79,1.31)	0.92(0.78,1.10)	1.10(0.55,2.19)	0.92(0.80,1.07)	0.91(0.78,1.06)	1.33(0.87,2.01)	0.94(0.79,1.13)	0.8

Discussion

In this study, we found that the proportions of poorly differentiated tumors, TNM stage in II~IV, lymphatic and distant metastasis were significantly lower in survivors than those in deaths for patients with lung adenocarcinoma. And Cox proportional-hazards model confirmed that age, poorly differentiated types, TNM stage II~IV and distant metastasis were significant risk factors for prognostic survival time of patients with lung adenocarcinoma. A large amount of evidence has revealed that the prognosis of lung adenocarcinoma is negatively correlated with the histologic subtypes in early stage, where micropapillary and solid subtypes classified as poor differentiation seems to be the most adverse prognostic factor [17, 18]. Supporting by Kalogeraki and his colleagues' study, apoptosis and cell proliferation increase with the decreased grade of differentiation in lung adenocarcinoma [19]. In addition, loss of cell polarization is associated with invasive adenocarcinoma of lung [20]. Primary cilium, a sensory appendage on the cell surface has a strong role in cell cycle control, differentiation and polarity that is correlated with prognosis of adenocarcinoma [21].

IFT20 is the smallest subunit of IFT complex, which is crucial for the formation of primary cilium within a cell [8]. Our results showed that the expression of IFT20 protein was significantly higher in cancerous tissues than that in matched adjacent tissues in patients with lung adenocarcinoma. Thus, primary cilia frequency was significantly elevated in adenocarcinoma of lung when compared with normal tissues, which may be partially caused by increased IFT20 expression in cancer cells [6]. However, we found a significantly negative association of IFT20 staining intensity score with clinicopathological features like TNM stage and lymphatic metastasis of patients with lung adenocarcinoma. It is suggested that IFT20 might be a promoter of tumorigenesis, whereas it functions as a negative regulator in the progression of invasion and metastasis of lung adenocarcinoma cells. These seemingly paradoxical effects of IFT20 could be explained by a dual role of primary cilia mediated Hh signaling activation and suppression in oncogenesis and tumor progression [22]. Consistent with our results, Yang et al reported that the level of IFT20 protein was negatively correlated with the malignancy of breast cancer cells, in which deletion of IFT20 promoted epithelial-mesenchymal transitions (EMT) and enhanced the migration of breast cancer cells by decreasing F-actin associated protein Transgelin-2 (TAGLN2) expression [23]. Overexpression of TAGLN2 could suppress lung metastasis in a mouse model of breast cancer [24]. A previous publication has identified that TAGLN2 was upregulated in patients with lung adenocarcinoma, most of whom were in the early stage of lung adenocarcinoma [25]. It is thus possible that negative relationship of IFT20 expression with TNM stage and lymphatic metastasis due to its role in regulating elevated synthesis of TAGLN2 in early steps of carcinogenesis, a hypothesis that will need to be explored in detail in the future.

Compared with matched adjacent tissues of lung adenocarcinoma, we found that GM130 expression was significantly decreased in cancerous tissues. Additionally, the median OS was significantly longer among patients in the third quartile of GM130 positive cells rate when compared with the lowest quartile. In agreement with our findings, GM130 expression was progressively down-regulated in colorectal and breast cancer patients, increasing cellular velocity and invasiveness of breast cancer cells [13, 26]. The cis-Golgi matrix protein, GM130 plays a vital role in maintaining Golgi structure and transporting proteins which participates in the regulation of cell directional migration and polarity [11, 12]. Loss of cell polarity is a typical feature of EMT that promotes epithelial tumorigenesis and metastasis [27]. Depletion of GM130 resulted in a reduction of E-cadherin expression in epithelial cells, which is considered to be an indicator of a loss in cell polarity and epithelial identity [13]. It has been confirmed that a reduction in membranous E-cadherin/catenin complex expression

was strongly associated with poor differentiation, distant metastasis and post-operative recurrence of patient with lung adenocarcinoma [28, 29]. Therefore, the role of GM130 in regulation E-cadherin expression of epithelial cells may be used to explain our results of negative association between CM130 expression and the risk of poor differentiation, tumor diameter and shortened OS of patients with lung adenocarcinoma, which would be interesting to study further.

To the best of our knowledge, this is the first study to investigate the associations of IFT20 and GM130 proteins expressions with clinicopathological features and survival of patients with lung adenocarcinoma. Furthermore, we found positive effect of GM130 protein on survival of specific patients with lung adenocarcinoma by stratified analyses. However, there are two limitations. Firstly, this is a case study in which the number of subjects is relatively small. But each patient's matched adjacent tissue could be considered as a self-control, which can reduce the bias to some extent. Secondly, although both IFT20 and GM130 protein are localized in the Golgi apparatus of cells, their interaction and mechanism on the development of lung adenocarcinoma could not be evaluated, which need to be explored in animal experimental models or case-control studies in the future.

Conclusions

In conclusion, we found the expressions of IFT20 and GM130 protein were negatively associated with clinicopathological features including differentiated types and size of tumor, TNM stage and lymphatic metastasis of lung adenocarcinoma. GM130 protein expression has a protective influence on OS for lung adenocarcinoma, especially patients with tumor size >7cm or distant metastasis. Thus, it is indicated that IFT20 and GM130 protein play crucial roles in the development of lung adenocarcinoma.

Abbreviations

NSCLC
Non-small cell lung cancer
IFT20
Intraflagellar transport protein 20
GM130
Cis-Golgi matrix protein 130
HE
Hematoxylin and eosin
IHC
Immunohistochemistry
IOD/area
Mean optical density
EMT
Epithelial-mesenchymal transitions
TAGLN2
Transgelin-2

Declarations

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Authors' contributions

LL, YC, WL, TZ and LQ contributed to the study conception and design. QY, HL, GF, XK and LQ performed data collection; LL, YS, LZ and TZ performed data analysis; YS, ZW, XL, XK and LQ provided the methodology; LL and TZ wrote the first draft of the manuscript, and LL, YC, WL, TZ and LQ completed the writing-review and editing. All authors have read and approved the final manuscript..

Founding

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Availability of data and materials

The data used to support findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

This study was approved by the Ethics and Human Subject Committees of Tongji hospital at Huazhong University of Science and Technology. All participants enrolled in this study signed written informed consent for participation, storage and use of surgically-removed cancer and adjacent tissues.

Consent for publication

All authors agree to publish.

Conflicts of interest

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020, 70(1):7–30.
2. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong K-K. Non-small-cell lung cancers: a heterogeneous set of diseases. *Nat Rev Cancer*. 2014, 14(8):535–546.
3. Alam H, Li N, Dhar SS, Wu SJ, Lv J, Chen K, Flores ER, Baseler L, Lee MG. HP1 γ Promotes Lung Adenocarcinoma by Downregulating the Transcription-Repressive Regulators NCOR2 and ZBTB7A. *Cancer Res*. 2018, 78(14):3834–3848.
4. Yang M, Sun Y, Sun J, Wang Z, Zhou Y, Yao G, Gu Y, Zhang H, Zhao H. Differentially expressed and survival-related proteins of lung adenocarcinoma with bone metastasis. *Cancer Med*. 2018, 7(4):1081–1092.
5. Legare S, Chabot C, Basik M. SPEN, a new player in primary cilia formation and cell migration in breast cancer. *Breast Cancer Res*. 2017, 19(1):104.
6. Yasar B, Linton K, Slater C, Byers R. Primary cilia are increased in number and demonstrate structural abnormalities in human cancer. *J Clin Pathol*. 2017, 70(7):571–574.
7. Liu H, Kiseleva A, Golemis E. Ciliary signaling in cancer. *Nat Rev Cancer*. 2018, 18(8):511–524.
8. Folliot JA, Tuft RA, Fogarty KE, Pazour GJ. The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol Biol Cell*. 2006, 17(9):3781–3792.
9. Aoki T, Nishita M, Sonoda J, Ikeda T, Kakeji Y, Minami Y. Intraflagellar transport 20 promotes collective cancer cell invasion by regulating polarized organization of Golgi-associated microtubules. *Cancer Sci*. 2019, 110(4):1306–1316.
10. Nishita M, Park SY, Nishio T, Kamizaki K, Wang Z, Tamada K, Takumi T, Hashimoto R, Otani H, Pazour GJ *et al*. Ror2 signaling regulates Golgi structure and transport through IFT20 for tumor invasiveness. *Sci Rep*. 2017, 7(1):1.
11. Sutterlin C, Colanzi A. The Golgi and the centrosome: building a functional partnership. *J Cell Biol*. 2010, 188(5):621–628.
12. Chang SH, Hong SH, Jiang HL, Minai-Tehrani A, Yu KN, Lee JH, Kim JE, Shin JY, Kang B, Park S *et al*. GOLGA2/GM130, cis-Golgi matrix protein, is a novel target of anticancer gene therapy. *Mol Ther*. 2012, 20(11):2052–2063.
13. Baschieri F, Confalonieri S, Bertalot G, Di Fiore PP, Dietmaier W, Leist M, Crespo P, Macara IG, Farhan H. Spatial control of Cdc42 signalling by a GM130-RasGRF complex regulates polarity and tumorigenesis. *Nat Commun*. 2014, 5:4839.
14. Baschieri F, Uetz-von Allmen E, Legler DF, Farhan H. Loss of GM130 in breast cancer cells and its effects on cell migration, invasion and polarity. *Cell Cycle*. 2015, 14(8):1139–1147.
15. Muller-Hermelink HK, Strobel P, Zettl A, Marx A. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart (WHO Classification of Tumours Series). 2004:35-44.
16. Goldstraw P, Crowley J, Chansky K, Giroux D, Groome P, Rami-Porta R, Postmus P, Rusch V, Sobin L. The IASLC Lung Cancer Staging Project: Proposals for the Revision of the TNM Stage Groupings in the Forthcoming (Seventh) Edition of the TNM Classification of Malignant Tumours. *J Thorac Oncol*. 2007, 2(8):706–714.
17. Sica G, Yoshizawa A, Sima CS, Azzoli CG, Downey RJ, Rusch VW, Travis WD, Moreira AL. A grading system of lung adenocarcinomas based on histologic pattern is predictive of disease recurrence in stage I tumors. *Am J Surg Pathol*. 2010, 34(8):1155–1162.
18. Warth A, Muley T, Meister M, Stenzinger A, Thomas M, Schirmacher P, Schnabel PA, Budczies J, Hoffmann H, Weichert W. The novel histologic International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification system of lung adenocarcinoma is a stage-independent predictor of survival. *J Clin Oncol*. 2012, 30(13):1438–1446.
19. Kalogeraki A, Tzardi M, Zoras O, Giannikaki E, Papadakis M, Tamiolakis D, Petraki PE, Diamantis A, Siafakas N, Stathopoulos E. Apoptosis and cell proliferation correlated with tumor grade in patients with lung adenocarcinoma. *In Vivo*. 2010, 24(5):667–670.
20. Machida Y, Ueda Y, Shimasaki M, Sato K, Sagawa M, Katsuda S, Sakuma T. Relationship of aquaporin 1, 3, and 5 expression in lung cancer cells to cellular differentiation, invasive growth, and metastasis potential. *Hum Pathol*. 2011, 42(5):669–678.
21. Emoto K, Masugi Y, Yamazaki K, Effendi K, Tsujikawa H, Tanabe M, Kitagawa Y, Sakamoto M. Presence of primary cilia in cancer cells correlates with prognosis of pancreatic ductal adenocarcinoma. *Hum Pathol*. 2014, 45(4):817–825.
22. Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH, Jr., Dlugosz AA, Reiter JF. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med*. 2009, 15(9):1055–1061.
23. Yang H, Zhang F, Long H, Lin Y, Liao J, Xia H, Huang K. IFT20 Mediates the Transport of Cell Migration Regulators From the Trans-Golgi Network to the Plasma Membrane in Breast Cancer Cells. *Front Cell Dev Biol*. 2021, 9:632198.
24. Yang L, Hong Q, Xu SG, Kuang XY, Di GH, Liu GY, Wu J, Shao ZM, Yu SJ. Downregulation of transgelin 2 promotes breast cancer metastasis by activating the reactive oxygen species/nuclear factor- κ B signaling pathway. *Mol Med Rep*. 2019, 20(5):4045–4258.
25. Rho JH, Roehrl MH, Wang JY. Tissue proteomics reveals differential and compartment-specific expression of the homologs transgelin and transgelin-2 in lung adenocarcinoma and its stroma. *J Proteome Res*. 2009, 8(12):5610–5618.

26. Baschieri F, Uetz-von Allmen E, Legler DF, Farhan H. Loss of GM130 in breast cancer cells and its effects on cell migration, invasion and polarity. *Cell Cycle*. 2015, 14(8):1139–1147.
27. McCaffrey LM, Montalbano J, Mihai C, Macara IG. Loss of the Par3 polarity protein promotes breast tumorigenesis and metastasis. *Cancer Cell*. 2012, 22(5):601–614.
28. Nozawa N, Hashimoto S, Nakashima Y, Matsuo Y, Koga T, Sugio K, Niho Y, Harada M, Sueishi K. Immunohistochemical alpha- and beta-catenin and E-cadherin expression and their clinicopathological significance in human lung adenocarcinoma. *Pathol Res Pract*. 2006, 202(9):639–650.
29. Higashi K, Ueda Y, Shimasaki M, Ishigaki Y, Nakamura Y, Oguchi M, Takegami T, Watanabe N. High FDG uptake on PET is associated with negative cell-to-cell adhesion molecule E-cadherin expression in lung adenocarcinoma. *Ann Nucl Med*. 2017, 31(8):590–595.

Figures

Figure 1

Expressions of IFT20 and GM130 protein in cancerous and matched adjacent tissues of patients with lung adenocarcinoma (*tissue chip, IHC, objective ×400*). A) IFT20 protein. a and b are patients 70, c and d are patients 76, e and f are patients 98, g and h are patients 4. a, c, e and g are cancerous tissues; b, d, f and h are matched adjacent tissues. B) GM130 protein. a and b are patients 76, c and d are patients 107, e and f are patients 4, g and h are patients 60.

Figure 2

Influence of IFT20 and GM130 protein expression on the survival of patients with lung adenocarcinoma

Figure 3

The survival curves of clinicopathological features and IFT20 and GM130 protein expression. * $P < 0.05$, compared with the blue line indicating as reference. When there is one blue line, it means that the survival curves of other groups overlap with the reference.