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Overexpression of KIF23 predicts clinical outcome in primary lung cancer patients

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ABSTRACT

Objective: High-level expression of kinesin family member 23 (KIF23), a member of microtubule-dependent molecular motors that transport organelles within cells and move chromosomes during cell division, has been observed in a variety of human malignancies. The aims of the present study were to observe the expression of KIF23 in lung cancer, examine the role of KIF23 in lung cancer cell growth and/or survival by small interfering RNA experiments, and explore its clinicopathologic significance and evaluate KIF23 expression as a prognostic marker.

Materials and methods: Quantitative reverse transcription-polymerase chain reaction analysis was performed to detect the expression of KIF23 mRNA using metastatic lymph nodes from patients with advanced lung cancer obtained by endobronchial ultrasonography-guided transbronchial needle aspiration (EBUS-TBNA) and primary lung tumors through surgical sample. The role of KIF23 in cancer cell growth was examined by small interfering RNA experiments. A total of 339 lung cancers were analyzed immunohistochemically on tissue microarrays to examine the expression of KIF23 protein and its clinicopathologic significance.

Results: KIF23 transcript was found to be overexpressed in the great majority of metastatic lymph nodes from advanced lung cancers and primary lung tumors. Inhibiting KIF23 expression effectively suppressed lung cancer cell growth. High-level KIF23 expression was observed in 67.8% of the 339 cases. Lung adenocarcinoma patients with tumors displaying a high-level of KIF23 expression was also identified as an independent prognostic factor by multivariate analysis ($P = 0.0064$).

Conclusion: KIF23 not only provides additional prognostic information for surgical treatment of lung cancer, but may also be a novel therapeutic target for these patients.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. In particular, the 5-year survival for patients with regional lymph node spread shows very poor prognosis [2]. The lack of major improvements in the survival rate for lung cancer despite advances in surgery, chemotherapy, and radiotherapy has driven a search for new strategies aimed at improving lung cancer management. To improve the survival rate, it is important to examine or analyze metastatic lymph node samples taken from advanced lung

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cancer patients, especially using minimally invasive techniques like endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) [3–5].

We have been attempting to isolate potential molecular targets for diagnosis and/or treatment of lung cancer by analyzing expression profiles of our previous performed microarray [5] and various types of database: NCBI-Gene® [6], GeneCards® [7], GenomeRNAi® [8], and CTDatabase® [9]. Throughout these screenings, real-time PCR analysis was performed against 122 possible candidate genes using tumor samples obtained by EBUS-TBNA. To investigate the functional role of each gene in the tumor cell growth/survival, RNA interference (RNAi) experiments were performed using several lung cancer cell lines. We have also performed tumor tissue microarray analysis of clinical lung cancer specimens to verify the biological and clinicopathologic significance of the respective gene products [10–12].

KIF23, a human homolog of mouse Kif23, is a member of kinesin motor protein involved in the regulation of cytokinesis [13,14]. Inhibiting KIF23 in HeLa cells induces formation of binucleated/multinucleated cells because of a cytokinesis defect [15,16]. KIF23 also plays an important role in the bundling and transport of microtubules (MTs) to explicit intracellular locations in different cells at specific time points [17]. Considering KIF23 acts as both a regulator of cytokinesis and a motor enzyme of microtubules, and that KIF23 is highly expressed only in lung cancer compared with normal lung tissues or other human vital organs [18], KIF23 could be a promising molecular target gene for the treatment of lung cancer.

2. Materials and methods

2.1. Lung cancer clinical samples and tissue samples

Thirty samples taken from metastatic lymph nodes from lung cancers, including twelve lung adenocarcinomas (ADCs), eight lung squamous cell carcinomas (SqCCs), five lung large cell carcinomas (LCCs), and five SCLCs, and four samples without malignancy were obtained via EBUS-TBNA from patients with written informed consent at Toronto General Hospital (Toronto, ON, Canada) (study number: 11-0109-CE). cDNAs from lung cancer tissues were obtained from patients who underwent surgery at the Toronto General Hospital. A total of 339 NSCLC tissue samples on tissue microarray were obtained from patients who underwent surgery at Hokkaido University and its affiliated hospitals with the patients' informed consents [10–12]. Patients were consecutive Japanese patients with lung cancer who underwent surgical lobectomy or pneumonectomy in the Department of Thoracic Surgery of these institutes. Detailed clinical and pathological information was collected retrospectively for all patients. Follow-up lasted through 30 October, 2009, with a median follow-up period of 60.5 months for living patients. Median age of patients at the time of diagnosis was 63.8 years (range, 37–87 years) and 64.9% of patients were men (for more detailed information see Table 1). The primary end point was overall survival as measured from the date of surgery to the time of death of the patients. Disease-free survival rates were calculated as the period from surgery until the date of disease relapse. All specimens were fixed in formalin and embedded in paraffin wax. Representative blocks were selected (based primarily on greatest dimensions of each tumor), and serial 4-μm-thick sections were examined by immunohistochemistry. Histological diagnoses of tumors was based on the 4th Edition of World Health Organization Classification [19]. All tumors were staged according to the pathological tumor/node/metastasis (pTNM) classification (7th edition) of the International Union against Cancer [20]. All tumors were histologically reviewed by an experienced pathologist (M.S.).

Table 1
Associations between KIF23 expressions and clinicopathological features in patients with lung cancer[a] ($n = 339$).

Variables	No. of cases	KIF23 expression		P[b]
		KIF23-H($n = 230$)	KIF23-L($n = 109$)	
Age (years)				
<60	110	75	35	0.9270
≥60	229	155	74	
Gender				
Male	220	153	67	0.3625
Female	119	77	42	
pT status				
pT1	125	82	43	0.4985
pT2-4	214	148	66	
pN status				
pN0	251	166	85	0.2546
pN1-2	88	64	24	
Pleural invasion				
pl0	208	149	59	0.0599
pl1-3	131	81	50	
Histological classification				
ADC	227	154	73	0.9977[d]
SqCC	83	63	20	
LCC	18	10	8	
Other[c]	11	3	8	
Smoking history				
Smoker	227	152	75	0.6189
Never-smoker	112	78	34	

ADC, adenocarcinoma; SqCC, squamous cell carcinoma; LCC, large cell carcinoma.
[a] TNM classification system of the International Union Against Cancer (7th edition) [20] and TNM classification (4th edition) [19].
[b] P value was analyzed by high-level KIF23 (KIF23-H) vs. low-level KIF23 (KIF23-L) expression using the χ^2 test.
[c] Other including adenosquamous carcinoma, mucoepidermoid carcinoma, carcinosarcoma, small cell carcinoma, and atypical carcinoid.
[d] P value was analyzed by ADC vs. non-ADC from the χ^2 test.

2.2. Lung cancer cell lines

The human lung cancer cell lines used in this study were as follows: lung ADC DFC1032, NCI-H2228, NCI-H1975, NCI-H3255, NCI-H4006, NCI-H1650, NCI-H2122, NCI-H2009, NCI-H2030, NCI-H1819, NCI-H23, NCI-H2405, NCI-H1437, A549, HCC827, and HCC2935; lung adenosquamous carcinoma (ASC) NCI-H647; lung SqCC MGH7; lung LCC NCI-H460 and NCI-H661; and SCLC SBC-5. All cancer cells were grown in monolayers in appropriate medium supplemented with 10% FBS and were maintained at 37 °C in atmospheres of humidified air with 5% CO_2.

2.3. Specimen handling and EBUS-TBNA sample preparation

EBUS-TBNA was performed in the same fashion as described previously [24,25]. After confirmation of adequate sampling for cytological evaluation, an additional pass was performed for the preservation of RNA. The aspirate was mixed with Allprotect Tissue Reagent® (Qiagen, Valencia, CA) following the manufacturer's instructions and stored at −80 °C. The QIAzol Lysis Reagent (Qiagen) and one 5-mm stainless steel Bead (Qiagen) were added before homogenizing with a TissueLyser Adapter Set (Qiagen) for 2 min at 20 Hz [5,21]. Total RNA was then purified using a miRNeasy Mini Kit (Qiagen). The amount and purity were measured using a spectrophotometer (NanoDrop; Thermo Scientific, Wilmington, DE).

2.4. Quantitative RT-PCR analysis

The cDNA was synthesized from 2 μg total RNA using QuantiTect® Reverse Transcription Kit (Qiagen). The primers