Activation of KIF4A as a Prognostic Biomarker and Therapeutic Target for Lung Cancer

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Abstract

Purpose and Experimental Design: To identify molecules that might be useful as diagnostic/prognostic biomarkers and as targets for the development of new molecular therapies, we screened genes that were highly transactivated in a large proportion of 101 lung cancers by means of a cDNA microarray representing 27,648 genes. We found a gene encoding KIF4A, a kinesin family member 4A, as one of such candidates. Tumor tissue microarray was applied to examine the expression of KIF4A protein and its clinicopathologic significance in archival non–small cell lung cancer (NSCLC) samples from 357 patients. A role of KIF4A in cancer cell growth and/or survival was examined by small interfering RNA experiments. Cellular invasive activity of KIF4A on mammalian cells was examined using Matrigel assays.

Results: Immunohistochemical staining detected positive KIF4A staining in 127 (36%) of 357 NSCLCs and 19 (66%) of 29 small-cell lung cancers examined. Positive immunostaining of KIF4A protein was associated with male gender (P = 0.0287), nonadenocarcinoma histology (P = 0.0097), and shorter survival for patients with NSCLC (P = 0.0005), and multivariate analysis confirmed its independent prognostic value (P = 0.0012). Treatment of lung cancer cells with small interfering RNAs for KIF4A suppressed growth of the cancer cells. Furthermore, we found that induction of exogenous expression of KIF4A conferred cellular invasive activity on mammalian cells.

Conclusions: These data strongly implied that targeting the KIF4A molecule might hold a promise for the development of anticancer drugs and cancer vaccines as well as a prognostic biomarker in clinic.

Lung cancer is one of the most common and fatal cancers in the world (1). A number of genetic alterations associated with development and progression of lung cancer have been reported; however, its molecular mechanisms still largely remain unclear (2). Two major histologically distinct types of lung cancer, non–small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) have different pathophysiological and clinical features that suggest differences in the mechanisms of their carcinogenesis. NSCLC accounts for nearly 80% of lung cancers, whereas SCLC accounts for 20% of them and is categorized as neuroendocrine tumors of the lung with certain morphologic, ultrastructural, and immunohistochemical characteristics (3, 4). In spite of applying surgical techniques combined with various treatment modalities such as radiotherapy and chemotherapy, the overall 5-year survival rate of lung cancer is still low at ~15% (5). Patients with SCLC respond favorably to the first-line multiagent chemotherapy; however, they often relapse in a short time. Hence, the only 20% of patients with limited-stage disease can be cured with combined modality therapy and <5% of those with extensive disease are able to achieve 5-year survival after the initial diagnosis (6, 7). Therefore, new therapeutic strategies focusing on SCLC as well as NSCLC such as molecular-targeted agents are eagerly awaited.

The genome-wide cDNA microarray analysis covering complete or near-complete set of genes enabled us to obtain comprehensive gene expression profiles and to compare the gene expression levels with clinicopathologic and biological information of cancers (8–14). This kind of approach is also useful to identify unknown molecules involved in the carcinogenic pathway. Through the gene expression profile analysis of 15 SCLCs and 86 NSCLCs coupled with purification of cancer cell population by laser microdissection on a cDNA microarray consisting of 27,648 genes, and their comparison with the expression profile data of 31 normal human tissues (27 adult and 4 fetal organs; refs. 15, 16), we identified a number of potential molecular targets for diagnosis, treatment,
KIF4A Activation in Lung Carcinogenesis

and/or choice of therapy (8–12). To verify the biological and clinicopathologic significance of the respective gene products, we have also been performing high-throughput screening of loss-of-function effects by means of the RNA interference technique as well as tumor tissue microarray analysis of clinical lung cancer materials (17–30). This systematic approach revealed that kinesin family member 4A (KIF4A) was frequently overexpressed in the great majority of lung cancers and was essential to growth or progression of lung cancers.

Kinesin superfamily proteins, such as KIF4A, are microtubule-based motor proteins that generate directional movement along microtubules. KIFs are key players or central proteins in the intracellular transport system, which is essential for cellular function and morphology, including cell division (31). The kinesin superfamily is also the first large protein family in mammals whose constituents have been completely identified and confirmed both in vivo and in vitro (32). A large portion of human KIF4A is associated with the nuclear matrix during the interphase, whereas a small portion of them is found in the cytoplasm. During mitosis, it is associated with chromosomes throughout the entire process (33). A previous microarray study disclosed the elevated expression of KIF4A mRNA in human cervical cancer (34), although no report has clarified the significance of KIF4A transactivation in human cancer progression. A previous study and its potential as a therapeutic target.

We here report the identification of KIF4A to be a promising target as a prognostic biomarker as well as for development of therapeutic agents and cancer vaccines, and also describe possible biological roles of KIF4A protein in progression of lung cancer.

Materials and Methods

Lung cancer cell lines and tissue samples. The human lung cancer cell lines used in this study were as follows: lung adenocarcinomas A427, A549, LC319, and NCI-H1373; lung squamous cell carcinomas (SCC) RERF-LC-AI, SK-MES-1, NCI-H226, NCI-H520, and NCI-H2170; and SCLCs DMS114, DMS273, SBC-3, and SBC-5. All cells were grown in monolayers in appropriate medium supplemented with 10% FCS and/or choice of therapy (8–12).

Immunocytochemistry. Cells were fixed in 4% formaldehyde solution for 30 min at 37°C, and rendered permeable by treatment for 3 min with PBS(−) containing 0.1% Triton X-100. Cells were covered with CAS-BLOCK (2ymed) for 7 min to block nonspecific binding before the primary antibody reaction. Then, the cells were incubated with polyclonal antibody to human KIF4A protein (Abcam). The immunocomplexes were stained with a donkey anti-goat secondary antibody conjugated to Alexa 488 (Molecular Probes) and viewed with a laser confocal microscope (TCS SP2 AOBs: Leica Microsystems).

Western blot analysis. To investigate the significance of KIF4A overexpression in clinical lung cancers, we stained tissue sections using ENVISION+ kit/horseradish peroxidase (DakoCytomation). Anti-KIF4A antibody (Abcam) was added after blocking of endogenous peroxidase and proteins, and each section was incubated with horseradish peroxidase–labeled anti-goat IgG as the secondary antibody. Substrate chromogen was added and the specimens were counterstained with hematoxylin. Tissue microarrays were constructed as published previously, using formalin-fixed NSCLCs (35–37). Tissue areas for sampling were selected based on visual alignment with the corresponding H&E-stained sections on slides. Three, four, or five tissue cores (diameter 0.6 mm; height 3–4 mm) taken from donor-tumor blocks were placed into recipient paraffin blocks using a tissue microarray (Beecher Instruments). A core of normal tissue was punched from each case. Five-micrometer sections of the resulting microarray block were used for immunohistochemical analysis. Positivity for KIF4A was assessed semiquantitatively by three independent investigators without prior knowledge of the clinical follow-up data, each of whom recorded staining intensity as either negative (no appreciable staining in tumor cells) or positive (brown staining appreciable in the nucleus and cytoplasm of tumor cells). Cases were accepted as positive only if reviewers independently defined them as such.

Statistical analysis. Statistical analyses were done using the StatView statistical program (SaS). We used contingency tables to analyze the relationship between KIF4A expression and clinicopathologic variables in NSCLC patients. Tumor-specific survival curves were calculated from the date of surgery to the time of death related to NSCLC, or to the last follow-up observation. Kaplan-Meier curves were calculated for each relevant variable and for KIF4A expression; differences in survival times among patient subgroups were analyzed using the log-rank test. Univariate and multivariate analyses were done with the Cox proportional hazard regression model to determine associations between clinicopathologic variables and cancer-related mortality. First, we analyzed associations between death and possible prognostic factors, including age, gender, histologic type, pT classification, pN classification, and smoking history, taking into consideration one factor at a time. Second, multivariate Cox analysis was applied on backward (stepwise) procedures that always forced KIF4A expression into the
model, along with any and all variables that satisfied an entry level of \( P < 0.05 \). As the model continued to add factors, independent factors did not exceed an exit level of \( P < 0.05 \).

RNA interference assay. We had previously established a vector-based RNA interference system, psiH1BX3.0, that was designed to synthesize small interfering RNAs (siRNA) in mammalian cells (17–27, 29, 30). Ten micromgs of siRNA expression vector were transfected using 30 μL of LipofectAMINE 2000 (Invitrogen) into lung cancer cell lines SBC-3, SBC-5, LC319, and A549. The transfected cells were cultured for 7 days in the presence of appropriate concentrations of geneticin (G418); the number of colonies was counted by Giemsa staining; and viability of cells was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay at 7 days after the treatment. Briefly, cell counting kit-8 solution (Dojindo) was added to each dish at a concentration of 1/10 volume, and the plates were incubated at 37°C for additional 2 h. Absorbance was then measured at 490 nm, and at 630 nm as a reference, with a Microplate Reader 550 (Bio-Rad). To confirm suppression of KIF4A mRNA expression, semiquantitative RT-PCR experiments were carried out with the following synthesized KIF4A specific primers according to the standard protocol. The target sequences of the synthesized oligonucleotides for RNA interference were as follows: control 1 (luciferase/LLC: Photinus pyralis luciferase gene), 5’-CGTACGCGGAATACTTCGA-3’; control 2 (scramble/SCR: chloroplast Euglena gracilis gene coding for SS and 16S rRNAs), 5’-CCGGCGCTTTGTAGGATTCG-3’; siRNA-KIF4A-1, 5’-GAAGAATTTGGTCGATTGA-3’; siRNA-KIF4A-2, 5’-GATGGGCG-CACTCAAGAC-3’.

Matrigel invasion assay. We cloned the entire coding sequence into the appropriate site of p3XFLAG-CMV-10 plasmid vector (Sigma). COS-7 and NH13T3 cells transfected either with plasmids expressing KIF4A or with mock plasmids were grown to near confluence in DMEM containing 10% FCS. The cells were harvested by trypsinization, washed in DMEM without addition of serum or protease inhibitor, and suspended in DMEM at 5 × 10⁵/mL. Before preparing the cell suspension, the dried layer of Matrigel matrix (Becton Dickinson Labware) was rehydrated with DMEM for 2 h at room temperature. DMEM (0.75 mL) containing 10% FCS was added to each lower chamber in a 24-well Matrigel invasion chambers, and 0.5 mL (2.5 × 10⁵ cells) of cell suspension were added to each insert of the upper chamber. The plates of inserts were incubated for 22 h at 37°C. After incubation, the chambers were processed; cells invading through the Matrigel were fixed and stained with Giemsa as directed by the supplier (Becton Dickinson Labware).

Results

Expression of KIF4A in lung cancers and normal tissues. Using a cDNA microarray to screen for genes that were highly transactivated in a large proportion of lung cancers, we identified KIF4A gene as a good candidate. This gene showed a 5-fold or higher level of expression in the majority of SCLC cases and in ~40% of NSCLCs we examined. Subsequently, we confirmed its transactivation by semiquantitative RT-PCR experiments in all of eight SCLC cases and in 5 of 10 NSCLC cases (Fig. 1A). We further confirmed a high level of KIF4A in 10 of 12 lung cancer cell lines by semiquantitative RT-PCR and Western blot analyses using anti-KIF4A antibody (Fig. 1B, top and bottom). To determine the subcellular localization of endogenous KIF4A in lung cancer cells, we did immunocytochemical analysis using anti-KIF4A polyclonal antibodies; KIF4A protein was localized in the cytoplasm and nucleus of DMS273 cells (Fig. 1C).

Northern blot analysis using KIF4A cDNA as a probe identified a 5.0-kb transcript specifically in testis among the 23 normal human tissues examined (Fig. 1D). Furthermore, we compared KIF4A protein expressions in five normal tissues (liver, heart, kidney, lung, and testis) with those in lung cancers using anti-KIF4A polyclonal antibodies by immunohistochemistry. KIF4A expressed abundantly in testis (mainly in cytoplasm and/or nucleus of primary spermatocytes) and lung cancers; however, its expression was hardly detectable in the remaining four normal tissues (Fig. 2A).

Association of KIF4A overexpression with poor prognosis of NSCLC. In SCLC and NSCLC tissues, immunohistochemical analysis with anti-KIF4A polyclonal antibodies showed that KIF4A localized in the cytoplasm and nucleus (Fig. 2B). Interestingly, the invasive border of the tumor adjacent to the noncancerous areas showed the tendency of stronger staining. Using tissue microarrays prepared from 357 NSCLCs and 29 SCLCs, we did immunohistochemical analysis with anti-KIF4A polyclonal antibodies and found positive staining in 127 (36%) of the 357 NSCLC cases and 19 (66%) of the 29 SCLCs (Fig. 2C), whereas no staining was observed in any of corresponding normal lung tissues examined. Of these KIF4A-positive NSCLC cases, 68 were adenocarcinomas (30% of 223), 37 were SCCs (39% of 94 cases), 14 were LCCs (52% of 27 cases), and 8 were adenosquamous cell carcinomas (62% of 13). We then examined correlations of the KIF4A expression in surgically resected NSCLCs with various clinicopathologic variables. The sample size of SCLCs was too small to be evaluated further. Statistical analysis revealed that gender (higher in male; \( P = 0.0287 \) by \( \chi^2 \) test) and histology (higher in nonadenocarcinomas; \( P = 0.0097 \) by \( \chi^2 \) test) were significantly associated with the KIF4A positivity (Table 1). The Kaplan-Meier method indicated significant association between KIF4A status (positive versus negative) in NSCLCs and tumor-specific survival rate (shorter survival periods in KIF4A-positive cases; \( P = 0.0005 \) by the log-rank test; Fig. 2D, left). Positive immunostaining of KIF4A protein was associated with shorter survival for patients with lung adenocarcinoma \( (P = 0.005 \) by the log-rank test), whereas KIF4A expression also tended to be an unfavorable prognostic factor for patients with lung SCC or LCC \( (P = 0.05 \) by the log-rank test; Supplementary Figs. S1, left and right). By univariate analysis, histology (adenocarcinomas versus nonadenocarcinomas), tumor size \( (pT_1 \text{ versus } pT_{2+4}) \), lymph node metastasis \( (pN_0 \text{ versus } pN_{1+3}) \), age \( (<65 \text{ years versus } \geq 65 \text{ years}) \), gender (female versus male), and KIF4A positivity (negative versus positive) were all significantly related to poor tumor-specific survival of NSCLC patients (Table 2). Furthermore, multivariate analysis using the Cox proportional hazard model indicated that \( pT \) stage, \( pN \) stage, age, and positive KIF4A staining were independent prognostic factors for NSCLC (Table 2). To further analyze the prognostic value of KIF4A in more homogeneous populations of patients, we validated the relationship between KIF4A expression and survival by subgroup analysis based on tumor stage and the status of adjuvant treatment. We divided the 357 NSCLC cases into two subgroups with or without the adjuvant therapy: group 1 for node-negative cases \( (pN_0; 212 \text{ patients}) \) who had no adjuvant treatment and group 2 for node-positive cases \( (pN_{1+3}; 145 \text{ patients}) \) who were treated with cisplatin-based adjuvant chemotherapy after surgery. We confirmed that KIF4A expression was significantly associated with poor prognosis in the group 1 patients \( (P = 0.03 \) by the log-rank test; Fig. 2D, middle), as well as with that in the group 2 patients \( (P = 0.003 \) by the log-rank test; Fig. 2D, right).
Fig. 1. KIF4A expression in lung cancers and normal tissues. A, expression of KIF4A in clinical samples of SCLC (top panels) and NSCLC (bottom panels), and normal lung tissues, analyzed by semiquantitative RT-PCR. We prepared appropriate dilutions of each single-stranded cDNA prepared from mRNAs of lung cancer samples, using the level of β-actin (ACTB) expression as a quantitative control. B, expression of KIF4A in lung cancer cell lines, examined by semiquantitative RT-PCR (top panels) and Western blot analyses (bottom panels). IB, immunoblot. Expression of ACTB served as a quantity control. C, subcellular localization of endogenous KIF4A protein in DMS273 cells. KIF4A staining is observed at the cytoplasm and nucleus of the cells. DAPI, 4',6-diamidino-2-phenylindole. D, expression of KIF4A in normal human tissues, detected by Northern blot analysis.
Inhibition of growth of lung cancer cells by siRNA against KIF4A. To assess whether KIF4A is essential for growth or survival of lung cancer cells, we constructed plasmids to express siRNAs against KIF4A (si-KIF4A) as well as control plasmids (siRNAs for luciferase and scramble) and transfected them into SBC-3, SBC-5, and LC319 cells, which strongly expressed KIF4A (Fig. 3; Supplementary Fig. S2). The KIF4A-mRNA levels in cells transfected with si-KIF4A-1 or si-KIF4A-2 were significantly decreased in comparison with cells transfected with either control siRNAs (Fig. 3A; Supplementary Fig. S2). We observed

**Immunohistochemical and clinicopathologic evaluation of KIF4A protein expression in lung cancer tissues.** A, expression of KIF4A in five normal human tissues as well as lung SCC, detected by immunohistochemical staining. Magnification, ×100. Positive staining appeared predominantly in the cytoplasm and nucleus of primary spermatocytes in the testis and lung cancer cells. B, expression of KIF4A in lung SCC, detected by immunohistochemical staining. Magnifications, ×100 (left) and ×200 (right). Invasive border of the tumor adjacent area to the noncancerous area showed the tendency of stronger staining. C, KIF4A expression in SCLCs, lung adenocarcinomas (ADC), and lung SCCs. Its expression is not observed in normal lung. D, association of KIF4A overexpression with poor clinical outcomes for NSCLC patients. Left, Kaplan-Meier analysis of tumor-specific survival in patients with NSCLC according to KIF4A expression. Middle, Kaplan-Meier analysis of tumor-specific survival period among group 1 patients with node-negative (pN<sub>0</sub>) NSCLC according to presence or absence of KIF4A. Right, Kaplan-Meier analysis of tumor-specific survival period among group 2 patients with node-positive (pN<sub>1-3</sub>) tumor according to presence or absence of KIF4A.
significant decreases in the numbers of viable cells (Figs. 3B-C; Supplementary Fig. S2). si-KIF4As revealed no significant effect on cell viability of A549 cells in which KIF4A expression was hardly detectable (Supplementary Fig. S3).

Cellular invasive effect of KIF4A on mammalian cells. As the immunohistochemical analysis on tissue microarray had indicated that lung cancer patients with KIF4A-positive tumors revealed shorter cancer-specific survival periods than those with KIF4A-negative tumors, we did Matrigel invasion assays to determine whether KIF4A might play some role in cellular invasive ability. Invasion of COS-7-KIF4A cells or NIH3T3-KIF4A cells through Matrigel was significantly enhanced, compared with the control cells transfected with mock plasmids, thus independently suggesting that KIF4A could contribute to the highly malignant phenotype of lung cancer cells (Fig. 4A-C).

Discussion

Several molecular-targeting drugs have been developed and proved their efficacy in cancer therapy; however, the proportion of patients showing good response is still limited (38). Therefore, further development of molecular-targeting drugs for cancer is urgently awaited. We have screened the therapeutic target molecules by the following strategy: (a) identifying up-regulated genes in lung cancer by genome-wide cDNA microarray system (8–12); (b) verifying the candidate genes for no, or very low level of, expression in normal tissues by Northern blotting (15, 16); (c) validating clinicopathologic significance of their overexpression by means of tissue microarray containing hundreds of archived lung cancer samples (18–30); and (d) verifying whether the target gene is essential for growth or the survival of cancer cells by RNA interference (Table 1).

### Table 1. Association between KIF4A positivity in NSCLC tissues and patient characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n = 357)</th>
<th>KIF4A positive (n = 127)</th>
<th>KIF4A absent (n = 230)</th>
<th>$\chi^2$</th>
<th>$P$ (positive vs absent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>107</td>
<td>29</td>
<td>78</td>
<td>4.784</td>
<td>0.0287*</td>
</tr>
<tr>
<td>Male</td>
<td>250</td>
<td>98</td>
<td>152</td>
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<td></td>
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<tr>
<td>Age (y)</td>
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<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>177</td>
<td>69</td>
<td>108</td>
<td>1.78</td>
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<tr>
<td>$\geq$65</td>
<td>180</td>
<td>58</td>
<td>122</td>
<td></td>
<td></td>
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<tr>
<td>Histologic type</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ADC</td>
<td>223</td>
<td>68</td>
<td>155</td>
<td>6.692</td>
<td>0.0097*</td>
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<tr>
<td>Non-ADC</td>
<td>134</td>
<td>59</td>
<td>75</td>
<td></td>
<td></td>
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<tr>
<td>pT factor</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>113</td>
<td>36</td>
<td>77</td>
<td>0.996</td>
<td>NS</td>
</tr>
<tr>
<td>T2-4</td>
<td>244</td>
<td>91</td>
<td>153</td>
<td></td>
<td></td>
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<tr>
<td>pN factor</td>
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<td></td>
</tr>
<tr>
<td>N0</td>
<td>212</td>
<td>75</td>
<td>137</td>
<td>0.009</td>
<td>NS</td>
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<tr>
<td>N1-3</td>
<td>145</td>
<td>52</td>
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<tr>
<td>Smoking history</td>
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<tr>
<td>Never smoker</td>
<td>104</td>
<td>32</td>
<td>72</td>
<td>1.478</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker</td>
<td>253</td>
<td>95</td>
<td>158</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ADC, adenocarcinoma; non-ADC, SCC plus large cell carcinoma and adenosquamous cell carcinoma; NS, no significance. *$P < 0.05$ ($\chi^2$ test).

### Table 2. Cox proportional hazards model analysis of prognostic factors in patients with NSCLCs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>Unfavorable/favorable</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIF4A</td>
<td>1.690 (1.254-2.276)</td>
<td>Positive/negative</td>
<td>0.0006*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.572 (1.171-2.112)</td>
<td>$\geq$65/65&gt;</td>
<td>0.0026*</td>
</tr>
<tr>
<td>Gender</td>
<td>1.690 (1.203-2.372)</td>
<td>Male/female</td>
<td>0.0025*</td>
</tr>
<tr>
<td>pT factor</td>
<td>2.708 (1.857-3.951)</td>
<td>$T_2$-$T_4$/T1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>pN factor</td>
<td>2.369 (1.769-3.171)</td>
<td>$N_0$/$N_1$-$N_3$</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Histologic type</td>
<td>1.407 (1.050-1.884)</td>
<td>Non-ADC/ADC</td>
<td>0.0222*</td>
</tr>
<tr>
<td>Smoking history</td>
<td>1.193 (0.862-1.652)</td>
<td>Smoker/never smoker</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIF4A</td>
<td>1.657 (1.221-2.248)</td>
<td>Positive/negative</td>
<td>0.0012*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.754 (1.300-2.365)</td>
<td>$\geq$65/65&gt;</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Gender</td>
<td>1.368 (0.942-1.986)</td>
<td>Male/female</td>
<td>NS</td>
</tr>
<tr>
<td>pT factor</td>
<td>2.130 (1.446-3.138)</td>
<td>$T_2$-$T_4$/T1</td>
<td>0.0001*</td>
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<tr>
<td>pN factor</td>
<td>2.429 (1.800-3.279)</td>
<td>$N_0$/$N_1$-$N_3$</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Histologic type</td>
<td>0.99 (0.717-1.367)</td>
<td>Non-ADC/ADC</td>
<td>NS</td>
</tr>
</tbody>
</table>

*$P < 0.05$. 

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Using this approach, we have shown here that KIF4A is frequently overexpressed in clinical lung cancer samples and cell lines, and that its gene products play indispensable roles in the growth and progression of lung cancer cells.

Kinesins constitute a superfamily of microtubule-based motor proteins with 45 members in mice and humans that represent diverse functions, including the transport of vesicles, organelles, chromosomes, protein complexes, and mRNA (39–42). The inhibition of the mitotic kinesin Eg5 by small molecules such as monastrol is being evaluated as an approach to develop a novel class of antiproliferative drugs for the treatment of malignant tumors (43, 44). The KIF4 subfamily consists of KIF4A, KIF4B, KIF21A, and KIF21B (39). KIF4A is a novel component of the chromosome condensation and segregation machinery functioning in multiple steps of mitotic division and plays essential roles in regulating anaphase spindle dynamics and the completion of cytokinesis (45, 46). KIF4 is also shown to be involved in neuronal survival (40). In this study, the treatment of NSCLC cells with specific siRNA to knockdown KIF4A expression resulted in suppression of cancer cell growth. We also showed additional evidences supporting the significance of this pathway in carcinogenesis; for example, the expression of KIF4A also resulted in the significant promotion of the cellular invasion in in vitro assays. Moreover, clinicopathologic evidence obtained through our tissue microarray experiments indicated that NSCLC patients with KIF4A-positive tumors had shorter cancer-specific survival periods than those with KIF4A-negative tumors. The results obtained by in vitro and in vivo assays strongly suggested that KIF4A is likely to be an important growth factor and might be associated with a highly malignant phenotype of lung cancer cells, although the molecular mechanisms underlying increased KIF4A expression levels in many cancer cells remains to be clarified. Because KIF4A should be classified as one of the typical cancer testis...
antigens, selective inhibition of KIF4A activity by molecular targeted agents could be a promising therapeutic strategy that is expected to have a powerful biological activity against cancer with a minimal risk of adverse events. Moreover, KIF4A antigens may be used as HLA-restricted epitope peptides for cancer vaccines that can induce specific immune responses by cytotoxic T cells against cancer cells with KIF4A expression.

In summary, activation of KIF4A has a specific functional role for growth and/or malignant phenotype of cancer cells. KIF4A overexpression in ressed specimens may be a useful index for application of adjuvant therapy to the patients who are likely to have poor prognosis. In addition, the data strongly imply the possibility of designing new anticancer drugs to specifically target the oncogenic activity of KIF4A for treatment of lung cancer patients.

References

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