KIF14 Messenger RNA Expression Is Independently Prognostic for Outcome in Lung Cancer

Timothy W. Corson,[1,4] Chang Qi Zhu,[1] Suzanne K. Lau,[1,5] Frances A. Shepherd,[2,3,6] Ming-Sound Tsao,[1,2,5,7] and Brenda L. Gallie[1,4,5,8]

Abstract

Purpose: The mitotic kinesin *KIF14* is overexpressed in multiple cancers including lung cancer. Therefore, we investigated *KIF14* expression in association with clinical variables and the effect of KIF14 on *in vitro* colony formation in non – small-cell lung carcinoma.

Experimental Design: RNA was extracted from 129 untreated, resected tumors and *KIF14* expression was quantified by real-time reverse transcription-PCR. Associations with clinical variables were determined by standard statistical methods. KIF14 expression was knocked down by small interfering RNA in H1299 and HeLa cells; proliferation and growth in soft agar were assayed.

Results: Squamous cell carcinoma had the highest *KIF14* level, followed by large-cell undifferentiated carcinoma, then adenocarcinoma ($P = 0.002$). *KIF14* level decreased with differentiation ($P = 0.01$) but was not associated with pathologic stage, T or N stage, or sex. When dichotomized about the median, *KIF14* overexpression significantly decreased disease-free survival (Kaplan-Meier log-rank, $P = 0.01$) and trended toward decreasing overall survival ($P = 0.08$). In a univariate Cox proportional hazard regression, increasing *KIF14* expression decreased disease-free survival [$P = 0.01$; hazard ratio, 1.44 (95% confidence interval, 1.09-1.91)]. In a multivariate Cox regression, including stage, differentiation, histology, and tumor purity as covariates, *KIF14* overexpression remained an independent prognostic factor for disease-free survival [$P = 0.01$; hazard ratio, 1.45 (95% confidence interval, 1.09-1.94)]. Knockdown of *KIF14* in non – small-cell lung carcinoma and cervical carcinoma cell lines decreased proliferation and colony formation in soft agar.

Conclusions: *KIF14* expression is independently prognostic for disease-free survival in lung cancer and knockdown decreases tumorigenicity *in vitro*, showing that it is a clinically relevant oncogene and an exciting therapeutic target for further study.

Lung cancer remains the most common cancer mortality worldwide, with 5-year survival in the United States of only 15% (1). Although effective surgical and chemotherapeutic interventions exist, the high fatality rates emphasize the need for early detection, useful genetic diagnostic and prognostic tools, and molecular targets for next-generation therapeutics (2). Hence, we and others have endeavored to identify clinically significant gene expression signatures of lung tumors (3 – 8).

Cancer progression initiates with genomic change, secondarily reflected in gene expression changes. By searching for genes with altered genomic copy number in cancer, we recently identified the mitotic kinesin *KIF14* as a candidate oncogene in multiple cancers, including lung tumors (9). We applied a DNA-based genomic approach in retinoblastoma and breast cancer to narrow the region of genomic gain at 1q31-q32 in these cancers. This region has also been reported to be gained or amplified in 22% of 430 lung tumors currently in the Progenetix cytogenetic abnormalities in human cancer database (10); a minimal region of gain at 1q32 containing *KIF14* was recently defined in lung cancer (8). Gain of this region has also recently been shown to associate with the progression from premalignant to malignant lesions and with metastasis in squamous cell lung carcinoma (11). We found that *KIF14* was a gene in the minimal region of gain that was dramatically overexpressed in breast cancer and medulloblastoma cell lines and primary retinoblastoma (9). The *KIF14* locus itself also showed genomic gain or amplification in the majority of retinoblastoma, breast cancer cell lines, and hepatocellular carcinoma (12).

We showed that 10 of 22 primary lung tumors displayed 3- to 34-fold increased *KIF14* mRNA expression over matched

Authors' Affiliations: [1]Division of Applied Molecular Oncology and [2]Clinical Studies Resource Centre, Ontario Cancer Institute/Princess Margaret Hospital, University Health Network; [3]Division of Medical Oncology and Hematology, Department of Medicine, University Health Network; and Departments of [4]Molecular and Medical Genetics, [5]Medical Biophysics, [6]Medicine, [7]Laboratory Medicine and Pathobiology, and [8]Ophthalmology, University of Toronto, Toronto, Ontario, Canada

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Requests for reprints: Brenda L. Gallie, Division of Applied Molecular Oncology, Room 8-415, Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9. Phone: 416-946-2324; Fax: 416-946-4619; E-mail: gallie@attglobal.net.

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normal samples. The patients in this very small, preliminary cohort with increased *KIF14* levels showed a trend ($P = 0.08$) toward decreased survival time (9). We have since shown that *KIF14* mRNA expression increases with grade in breast cancer, seeming to be a novel proliferation marker, and in univariate analyses is prognostic for both disease-free and overall outcome in this cancer (13).

Despite its growing importance as a candidate oncogene, relatively little is known about the cellular function of KIF14. It was cloned in 1994 (14) and was readily identified as a member of the kinesin superfamily, which are molecular motors that use the hydrolysis of ATP to power movement along microtubules (15). Only recently has KIF14 been functionally analyzed. In a study knocking down mitotic kinesins in HeLa cells, decreased KIF14 expression caused misalignment of chromosomes at the metaphase plate, with a subsequent delay in mitosis (16). However, more recently, two studies have localized KIF14 to the central spindle in HeLa cells, where it is essential for the final phase of cytokinesis, rather than chromosome alignment (17, 18). KIF14 interacts with protein regulator of cytokinesis 1 and citron kinase, displaying a central organizing role at the midbody in cytokinesis (18). Knockdown of KIF14 leads to binucleate cells (17, 18), with consequent polyploidy and apoptosis (17). KIF14 thus seems to be an important, previously overlooked part of the machinery that allows efficient cell division. Moreover, as an ATPase, KIF14 is a very promising anticancer drug target.

We report here for the first time the clinical significance of *KIF14* mRNA expression in a large cohort of non–small-cell lung carcinoma (NSCLC) patients and its effect on tumor cell colony formation *in vitro*. These results further implicate *KIF14* as an oncogene that is potentially a powerful prognostic marker and attractive therapeutic target in lung cancer.

Materials and Methods

Clinical samples. Lung tumor samples were obtained from patients who had undergone lung cancer resection as their primary mode of therapy without preoperative radiation or chemotherapy, as previously described (3). Tissue specimens were banked with informed consent, and the University Health Network Research Ethics Board approved the study. Percentage tumor purity in sections adjacent to regions used for RNA extraction was estimated during routine histopathologic analysis.

RNA extraction, reverse transcription, and real-time reverse transcription-PCR. Total RNA was extracted from primary tumor material by the guanidinium isothiocyanate-phenol-chloroform method and 5-μg RNA was reverse transcribed using TaqMan reverse transcription reagents and random hexamer primers (Applied Biosystems, Inc.) as described (3).

First-strand reverse transcription products (5 μL) were added to 12.5-μL reactions with 1× TaqMan Universal PCR Master Mix including AmpErase UNG (Applied Biosystems) and 1× TaqMan Gene Expression Assay primer-probe mix (Applied Biosystems) for either *KIF14* (Hs00978216_m1) or *TBP* (TATA-box binding protein), a housekeeping gene used as an endogenous control (Hs99999910_m1). These primers span exons. Triplicate reactions for each gene for each sample were prepared in 384-well plates and PCR was done using a PRISM 7900HT system (Applied Biosystems). Cycling conditions were 2 min at 50°C to activate UNG, 10 min at 95°C to activate the polymerase, and 40 cycles of 15 s at 95°C plus 1 min at 60°C. SDS 2.1 software (Applied Biosystems) was used to calculate ΔC_t relative expression values, normalized to *TBP*. Fold increase was calculated relative to a transformed lung epithelial cell line because matched normal tissue was not available for all samples; the choice of calibration sample does not influence statistical analyses of these data. Validation of equivalent amplification efficiency between *KIF14* and *TBP* was confirmed in pilot experiments by amplification of a dilution series.

To validate the TaqMan results and assess the relationship between *KIF14* and citron kinase (*CIT*) expression, a subset of samples ($n = 94$) was also analyzed by real-time quantitative PCR amplification using the SYBR Green assay in the PRISM 7900HT (Applied Biosystems). Each 10-μL reaction contained a 10-ng equivalent of cDNA. The reactions were activated at 95°C for 3 min followed by 40 cycles of 95°C (15 s), 65°C (15 s), and 72°C (20 s). The transcript number per nanogram of cDNA was obtained using standard curves generated with a pool of 10 nontumor lung genomic DNAs. Expression data were normalized to the geometric mean of four housekeeping genes (Table 1) to control for variability in expression levels. Primers were designed with Primer Express v 2.0 (Applied Biosystems) and sequences are provided in Table 1.

RNA interference, proliferation, and soft-agar colony formation assays. H1299 NSCLC cells (a kind gift from the laboratory of Dr. Sam Benchimol, Ontario Cancer Institute, Totonto, Canada) and HeLa cervical carcinoma cells were grown in H21 minimal essential medium supplemented with penicillin-streptomycin and 10% fetal bovine serum at 37°C, 5% CO_2. Cells in six-well plates were transfected with 100 pmol of small interfering RNA (siRNA) or buffer (mock) using 5 μL of Lipofectamine 2000 (Invitrogen) in OptiMEM (Invitrogen). Published siRNAs targeting KIF14 (18) and luciferase (GL2; ref. 19) were synthesized by Eurogentec, whereas two other siRNAs (SI00462336 and SI00462343) targeting different regions of the *KIF14* transcript were obtained from Qiagen. After 24 h of growth, 100 cells were seeded in wells of a 96-well plate and counted at 24-h intervals using CyQUANT NF fluorescence (Invitrogen) on a Typhoon 9410 imaging system running ImageQuaNT TL software (version 2005, Amersham Biosciences). A further 250 cells were seeded in 3.5-cm dishes in 0.3% noble agar prepared in growth medium, atop a "plug" of 0.6% noble agar. After 2 weeks of growth, all colonies visible under the 0.8× objective of a dissecting microscope were scored. Efficacy of KIF14 knockdown was assessed by immunoblot with an anti-KIF14 antibody (K1760, U.S. Biological), normalized to β-tubulin (T4026, Sigma) or β-actin (AC-15, Sigma). Horseradish peroxidase–labeled secondary antibodies (H1299 experiments) were detected using ECL+ (Amersham) chemifluorescence on the Typhoon imager, whereas alkaline phosphatase–labeled secondary antibodies (HeLa experiments) were visualized with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate

Table 1. Primer sequences used in SYBR Green real-time quantitative reverse transcription-PCR, written 5′ to 3′

Gene*	Forward primer	Reverse primer
KIF14	TGGTGAAATGGCCTG-TACAAGT	GGCAACCAGTTAACCCTTTGAG
CIT	AGCTCCGTGTGACC-ACCAA	TGATTGATGCTAATAAGC-TTTTGACA
ACTB	TCCTAAAAGCCACCCC-ACTTCT	GGGAGAGGACTGGGCCATT
BAT1	CGGTATCAGCAGTTT-AAAGATTTTCA	TGCCTCGGCCAAATAGGTT
B2M	GAGTGCTGTCTCCAT-GTTTGATGT	AAGTTGCCAGCCCT-CCTAGAG
TBP	GGGCATTATTTGTGCA-CTGAGA	TAGCAGCACGGTATGA-GCAACT

*Full gene names: *KIF14*, kinesin family member 14; *CIT*, citron (rho-interacting, serine/threonine kinase 21); *ACTB*, actin, β; *BAT1*, HLA-B associated transcript 1; *B2M*, β_2-microglobulin; *TBP*, TATA-box binding protein.

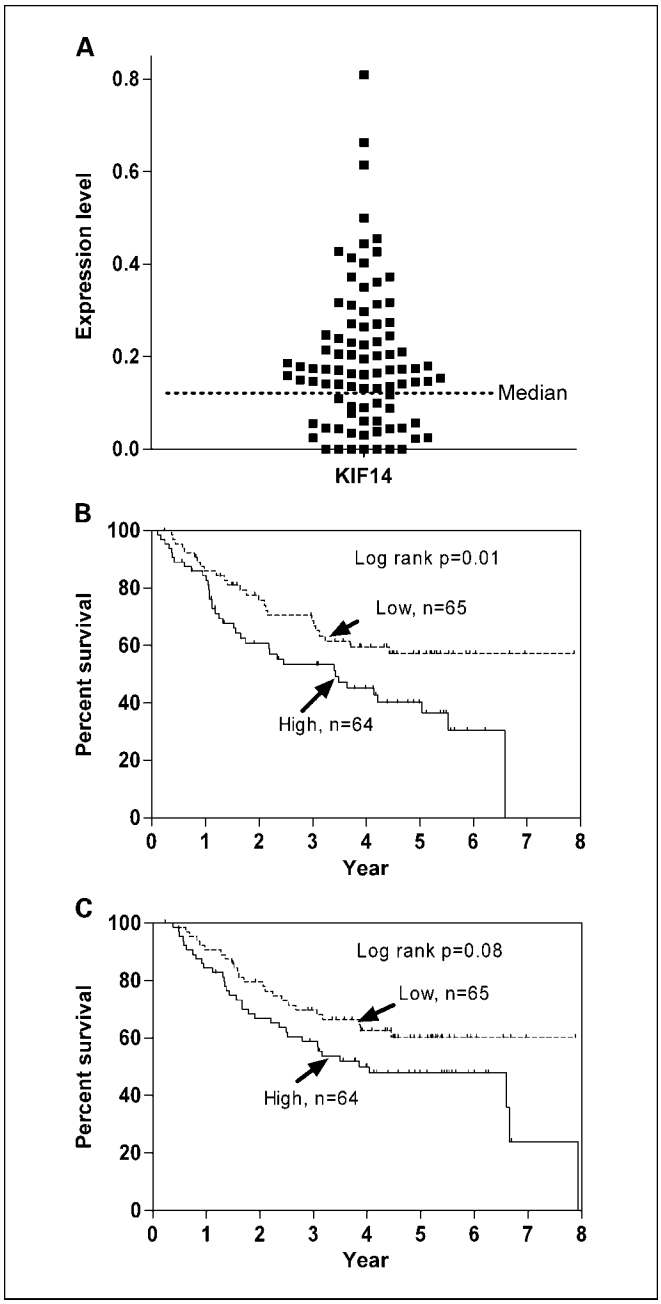

Fig. 1. *KIF14* mRNA overexpression is prognostic for disease-free and overall survival in NSCLC. *A*, relative *KIF14* expression distribution, showing dichotomization around the median. Some data points overlap and are not visible. *B* and *C*, survival plots for disease-free (*B*) and overall survival (*C*). *P* values shown are for Kaplan-Meier log-rank survival analyses. In univariate and multivariate Cox regressions, *KIF14* expression was a significant predictor of disease-free survival (see text).

(Roche Applied Science). Relative KIF14 band intensity was calculated using ImageQuaNT TL software.

Statistical analyses. Relative *KIF14* expression was treated as a continuous variable in all analyses. The associations between *KIF14* expression and demographic and clinical data were analyzed by the Wilcoxon two-sample test or by the Kruskal-Wallis test (for more than two groups) where appropriate. Spearman's r was used to assess correlations. Disease-free survival (from the date of surgery to date of recurrence) and overall survival (from date of surgery to date of death) were used to describe the survival function and Cox proportional hazard regression was used for the univariate and multivariate analyses. In addition, for graphical display purposes, *KIF14* expression was dichotomized at the median, which is the most conservative cutoff point for categorizing a continuous variable (20) and which also divides the bimodal distribution of *KIF14* expression neatly (Fig. 1A). Survival patterns of these two subgroups were compared using the Cox proportional hazards regression model and Kaplan-Meier log-rank test. These statistical analyses were done with SAS v.9.1 for Microsoft Windows. The Kruskal-Wallis test (SPSS v. 13 for MacOS) was used to test for differences in colony number between siRNA treatments. $P \leq 0.05$ was considered significant in all tests.

Results

To ascertain whether *KIF14* expression is a prognostic factor in NSCLC, we examined its mRNA expression by real-time reverse transcription-PCR in 129 tumors (Fig. 1A). The results of this TaqMan-based analysis were highly correlated ($r = 0.36$, $P = 0.004$) to an independent analysis of absolute *KIF14* mRNA levels in a subset of these tumors, validating this method.

The expression of *KIF14* was highest in squamous cell carcinoma, followed by large-cell undifferentiated carcinoma and adenocarcinoma (Table 2; $P = 0.002$). *KIF14* expression was inversely related to the pathologic differentiation of the

Table 2. *KIF14* expression stratified by demographic and clinical variables

Variable	*n*	Expression (fold; mean ± SD)	*P*
Age	129		0.28*
Gender [†]			
Male	68	0.55 ± 0.83	0.84 [‡]
Female	51	0.50 ± 0.63	
Histology			
AD	83	0.47 ± 0.82	0.002 [§]
LCUC	7	0.51 ± 0.61	
SQ	39	0.65 ± 0.50	
Differentiation [‖]			
Well differentiated	34	0.34 ± 0.63	0.01 [§]
Moderately differentiated	42	0.52 ± 0.49	
Poorly differentiated	52	0.66 ± 0.91	
Stage			
I	90	0.47 ± 0.60	0.29 [§]
II	27	0.57 ± 0.53	
III	12	0.87 ± 1.52	
T stage			
T_1	49	0.60 ± 0.91	0.60 [§]
T_2	79	0.48 ± 0.58	
T_3	1	0.78	
N stage			
N_0	90	0.47 ± 0.60	0.29 [§]
N_1	27	0.57 ± 0.53	
N_2	12	0.87 ± 1.52	
KRAS			
Wild type	90	0.61 ± 0.83	0.09 [‡]
Mutant	39	0.34 ± 0.35	

Abbreviations: AD, adenocarcinoma; LCUC, large-cell undifferentiated carcinoma; SQ, squamous cell carcinoma.
*Spearman $r = 0.097$.
[†]Ten missing cases.
[‡]Wilcoxon two-sample test.
[§]Kruskal-Wallis test (see Materials and Methods).
[‖]One missing case.

Table 3. Effects of *KIF14* expression and other prognostic variables on disease-free and overall survival in univariate and multivariate Cox proportional hazards regression analyses

	Disease-free survival		Overall survival	
	P	**HR (95% CI)**	***P***	**HR (95% CI)**
Univariate				
KIF14	**0.01**	1.44 (1.09-1.91)	*0.10*	1.33 (0.95-1.85)
Multivariate				
KIF14	**0.01**	1.45 (1.09-1.94)	0.14	1.31 (0.91-1.87)
Stage	**0.001**	1.78 (1.26-2.53)	**0.002**	1.78 (1.25-2.55)
Differentiation	0.39	1.16 (0.83-1.62)	*0.07*	1.39 (0.97-2.00)
Histology	0.77	1.05 (0.76-1.45)	0.98	1.01 (0.72-1.41)
Tumor purity	0.21	1.00 (1.00-1.02)	0.65	1.00 (0.99-1.02)

NOTE: Significant *P* values are marked in boldface, whereas trends are italicized.
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

tumor (P = 0.01). In contrast, *KIF14* expression was not associated with pathologic stage (P = 0.29), T stage (P = 0.60) or N stage (P = 0.29), patient age (r = 0.097, P = 0.28), or gender (P = 0.84). There was a trend toward higher *KIF14* expression in tumors with wild-type *KRAS* (P = 0.09). Because KIF14 interacts with citron kinase (18) and citron kinase mRNA expression is prognostic for disease-free survival in lung cancer (3), we examined the relationship between mRNA expression levels of these two genes in a subset (n = 94) of tumors. There was a weak negative correlation between *KIF14* and *CIT* expression (r = -0.13, P = 0.2).

When dichotomized at the median expression level (Fig. 1A), *KIF14* overexpression was associated with significantly decreased disease-free survival (median survival time, 2.04 versus 3.70 years) in a Kaplan-Meier log-rank analysis (Fig. 1B; P = 0.01) and showed a trend toward decreasing overall survival (Fig. 1C; P = 0.08; median survival time, 3.09 versus 4.00 years). In univariate Cox proportional hazard regression survival analyses, increasing *KIF14* expression was also associated with significantly decreased disease-free survival (Table 3; P = 0.01) and showed a trend toward decreased overall survival (Table 3; P = 0.10).

In multivariate Cox regressions including stage, differentiation, histology, and tumor purity as covariates, *KIF14* remained an independent prognostic factor for disease-free survival (Table 3) but not for overall survival (Table 3).

Because *KIF14* overexpression is associated with poor prognosis in patients, we sought to evaluate if KIF14 is important for tumorigenicity *in vitro*, as a prelude to further investigations of this protein as a therapeutic target in NSCLC. Three different siRNA molecules targeting KIF14 transfected into H1299 NSCLC cells showed ~90% knockdown of KIF14 protein levels (Fig. 2A). Two of the three siRNAs caused a subtle but reproducible decrease in proliferation (Fig. 2B), and

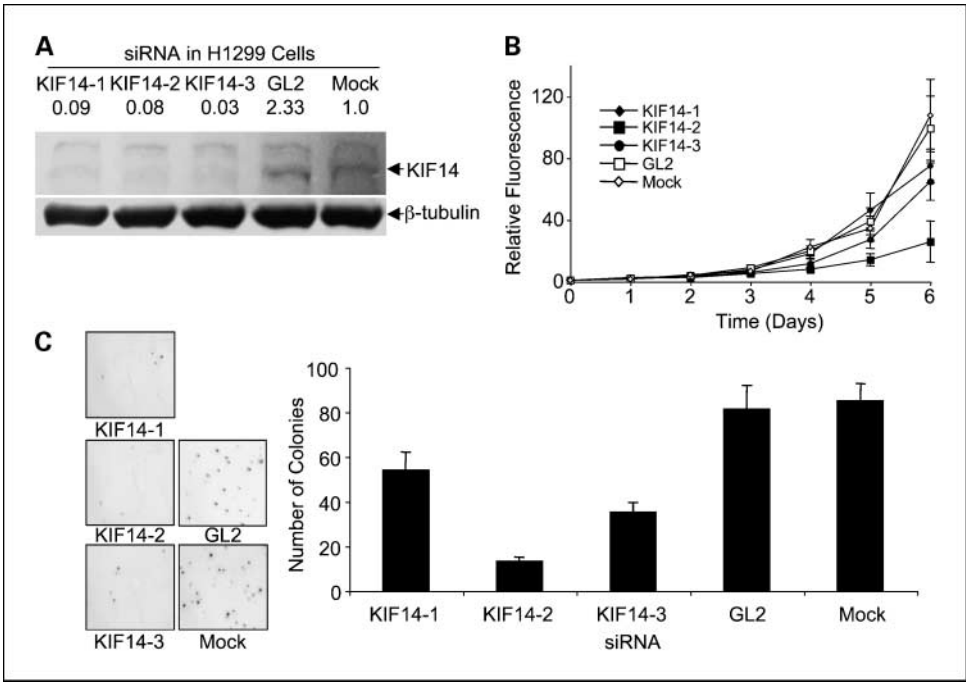

Fig. 2. Knockdown of KIF14 inhibits *in vitro* tumorigenicity in H1299 NSCLC cells. *A*, siRNAs decrease KIF14 compared with control (GL2) or mock transfection. Protein relative to mock, normalized to β-tubulin. *B*, decreased KIF14 (*filled symbols*) decreases proliferation over GL2 (*open squares*) or mock (*open diamonds*). CyQUANT NF fluorescence relative to starting (*points,* mean of six wells; *bars,* SD). *C*, decreased KIF14 decreases soft agar colony formation ability. Left, colonies in soft agar. Right, colony counts (*columns,* mean of triplicate plates; *bars,* SD); P = 0.012. Representative results from three experiments.

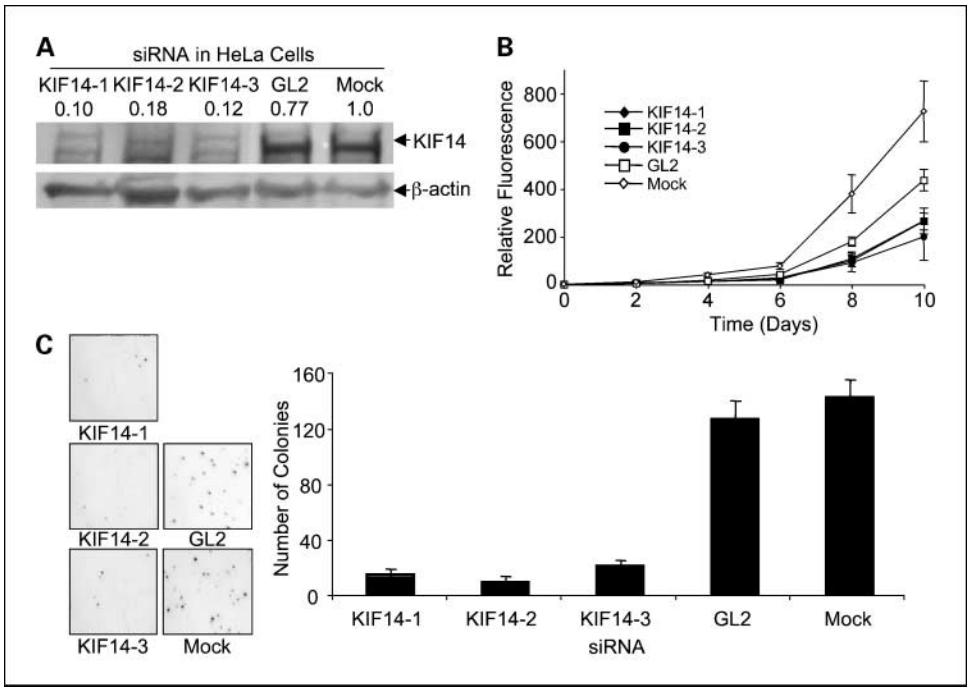

Fig. 3. Knockdown of KIF14 inhibits *in vitro* tumorigenicity in HeLa cervical carcinoma cells. *A*, siRNAs decrease KIF14 compared with control (GL2) or mock transfection. Protein relative to mock, normalized to β-actin. *B*, decreased KIF14 (*filled symbols*) decreases proliferation over GL2 (*open squares*) or mock (*open diamonds*). CyQUANT NF fluorescence relative to starting (*points,* mean of three wells; *bars,* SD). *C*, decreased KIF14 decreases soft agar colony formation ability. Left, colonies in soft agar. Right, colony counts (*columns,* mean of triplicate plates; *bars,* SD); $P = 0.016$. Representative results from three experiments.

all three markedly ($P = 0.012$) decreased the ability of these cells to form colonies in soft agar (Fig. 2C). Although the degree of knockdown was not quite as high in HeLa cells (Fig. 3A), even more dramatic effects on proliferation (Fig. 3B) and colony formation ($P = 0.016$; Fig. 3C) were observed in this cell line.

Discussion

To our knowledge, we present the first evidence that a kinesin is an independent prognostic factor in any cancer. Previously, *KIF14* (initially known as KIAA0042) has been identified in high-throughput studies as an overexpressed gene in lung cancer cell lines (21) and was part of a 42-gene set indicating poor prognosis in a mixed population of lung tumors (22). It is also one of 91 genes that lie in a minimal region of genomic gain at 1q and are overexpressed in NSCLC (8). *KIF14* was not represented on the microarrays used in our previous prognostic marker screening study (7). Our identification here of *KIF14* mRNA expression as an independent prognostic factor for disease-free survival argues for the prognostic power of this gene alone in NSCLC. We note that our findings will require replication in larger, independent cohorts, but given our prior findings that *KIF14* is a prognostic indicator in breast cancer (13), it is likely that this gene has wide-ranging prognostic power in cancer. That *KIF14* expression is an *independent* prognostic factor for disease-free survival in NSCLC is particularly important and novel, given the paucity of useful prognostic indicators in this cancer.

How KIF14 acts as oncogene is unclear because the long-term cellular effects of KIF14 overexpression remain unknown. It is feasible, however, that high KIF14 levels contribute to uncontrolled proliferation and cancer cell aneuploidy, perhaps by stimulating premature cytokinesis and/or evasion of the spindle checkpoint (23). Overexpression may also enable cells to bypass apoptosis because KIF14 knockdown increases apoptosis at multiple stages of the cell cycle (17). However, no previous studies have examined the effects of KIF14 knockdown on anchorage-independent growth in colony formation assays—the best *in vitro* indicator of tumorigenesis *in vivo* (24).

We show here that reduction in KIF14 expression by siRNA moderately decreases proliferation but strongly decreases the ability of H1299 and HeLa cells to form colonies in soft agar. Together, these *in vitro* findings suggest that reduced KIF14 expression is not simply cytotoxic but specifically affects the tumorigenic cells capable of independent growth, perhaps the stem cells perpetuating the cell line. This makes KIF14 a very appealing target for cancer therapeutic development. It is interesting to note that HeLa cells (Fig. 3A) are more susceptible to colony formation inhibition when KIF14 is knocked down, yet do not display as high a degree of protein knockdown as H1299 cells (Fig. 2A). Both these phenomena may be attributable to the higher absolute KIF14 protein levels in untreated HeLa cells. Compared with H1299, HeLa cells may be more "addicted" to KIF14 overexpression and hence are more sensitive to its loss, even if incomplete. Extending this logic, the most rapidly fatal tumors with highest KIF14 levels might respond best to a future anti-KIF14 therapeutic.

KIF14 interacts with citron kinase, localizing this Rho effector kinase to the central spindle in HeLa cells (18). Intriguingly, citron kinase was one of three microarray-identified genes shown in our previous study to be prognostic for disease-free survival in univariate analyses of a subset of this same cohort of tumors (3). However, whereas *KIF14* overexpression is associated with decreased disease-free survival (this work), for citron kinase, the converse is true (3). This inverse relationship is in keeping with the weak negative correlation we observed between the expression of these two genes, but also suggests that KIF14 has functional interactions in NSCLC cells with other proteins in addition to citron kinase. We speculate that

the expressions of KIF14 and citron kinase are coordinately controlled to deregulate the central spindle and final phase of cytokinesis in lung cancer cells. It will be interesting to evaluate if KIF14 expression is predictive for response to treatment with spindle poisons such as *Vinca* alkaloids and taxanes.

There does not yet exist an anti-KIF14 antibody that will reliably stain formalin-fixed, paraffin-embedded tissues. Thus, we could not confirm KIF14 overexpression at the protein level in our cohort of tumors. Whereas it is possible that KIF14 is not overexpressed at the protein level, or that overexpression is not specific to the tumor cells, our previous findings of low to undetectable *KIF14* mRNA in normal lung samples and in other normal adult tissues (9) suggest that overexpression is a tumor-specific phenomenon. In breast cancer cell lines, *KIF14* mRNA expression agrees closely with protein expression by immunoblot (9). Moreover, in the present study, *KIF14* remained an independent prognostic factor, even when the potential confounding variable of tumor purity was included in multivariate analyses (Table 3). By adopting a real-time reverse transcription-PCR approach, we were able to quickly obtain quantitative information on *KIF14* mRNA level. As long as fresh or frozen tumor tissue is available, this technique lends itself to clinical deployment for providing prognostic information because it is rapid, rigorous, and highly specific.

We now identify for the first time that *KIF14* is an independent prognostic factor for disease-free survival in any cancer; this is the largest clinical study of *KIF14* expression to date. We also show that KIF14 is necessary for the transformed state in NSCLC and cervical cancer cell lines. We conclude that *KIF14* is an important oncogene in NSCLC. Independent validation of these clinical findings, examination of *KIF14* expression in other tumor types, and further investigation of the cell biology of KIF14 and its potential as a therapeutic target are clearly warranted.

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References

1. Parkin DM, Whelan SL, Ferlay J, Storm H. Cancer incidence in five continents. Vols. I to VIII. Lyon: IARC; 2005.
2. Meyerson M, Carbone D. Genomic and proteomic profiling of lung cancers: lung cancer classification in the age of targeted therapy. J Clin Oncol 2005;23:3219–26.
3. Blackhall FH, Wigle DA, Jurisica I, et al. Validating the prognostic value of marker genes derived from a non-small cell lung cancer microarray study. Lung Cancer 2004;46:197–204.
4. Beer DG, Kardia SL, Huang CC, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat Med 2002;8:816–24.
5. Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci U S A 2001;98:13790–5.
6. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. Nat Genet 2003;33:49–54.
7. Wigle DA, Jurisica I, Radulovich N, et al. Molecular profiling of non-small cell lung cancer and correlation with disease-free survival. Cancer Res 2002;62:3005–8.
8. Dehan E, Ben-Dor A, Liao W, et al. Chromosomal aberrations and gene expression profiles in non-small cell lung cancer. Lung Cancer 2007;56:175–84.
9. Corson TW, Huang A, Tsao M-S, Gallie BL. *KIF14* is a candidate oncogene in the 1q minimal region of genomic gain in multiple cancers. Oncogene 2005;24:4741–53.
10. Baudis M, Cleary ML. Progenetix.net: an online repository for molecular cytogenetic aberration data. Bioinformatics 2001;17:1228–9.
11. Ma J, Gao M, Lu Y, et al. Gain of 1q25-32, 12q23-24.3, and 17q12-22 facilitates tumorigenesis and progression of human squamous cell lung cancer. J Pathol 2006;210:205–13.
12. Bowles E, Corson TW, Bayani J, et al. Profiling genomic copy number changes in retinoblastoma beyond loss of *RB1*. Genes Chromosomes Cancer 2007;46:118–29.
13. Corson TW, Gallie BL. *KIF14* mRNA expression is a predictor of grade and outcome in breast cancer. Int J Cancer 2006;119:1088–94.
14. Nomura N, Nagase T, Miyajima N, et al. Prediction of the coding sequences of unidentified human genes. II. The coding sequences of 40 new genes (KIAA0041-0080) deduced by analysis of cDNA clones from human cell line KG-1. DNA Res 1994;1:251–62.
15. Miki H, Okada Y, Hirokawa N. Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol 2005;15:467–76.
16. Zhu C, Zhao J, Bibikova M, et al. Functional analysis of human microtubule-based motor proteins, the kinesins and dyneins, in mitosis/cytokinesis using RNA interference (RNAi). Mol Biol Cell 2005;16:3187–99.
17. Carleton M, Mao M, Biery M, et al. RNA interference-mediated silencing of mitotic kinesin KIF14 disrupts cell cycle progression and induces cytokinesis failure. Mol Cell Biol 2006;26:3853–63.
18. Gruneberg U, Neef R, Li X, et al. KIF14 and citron kinase act together to promote efficient cytokinesis. J Cell Biol 2006;172:363–72.
19. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001;411:494–8.
20. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. J Natl Cancer Inst 1994;86:829–35.
21. Difilippantonio S, Chen Y, Pietas A, et al. Gene expression profiles in human non-small and small-cell lung cancers. Eur J Cancer 2003;39:1936–47.
22. Borczuk AC, Shah L, Pearson GD, et al. Molecular signatures in biopsy specimens of lung cancer. Am J Respir Crit Care Med 2004;170:167–74.
23. Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 2005;5:773–85.
24. Clark GJ, Cox AD, Graham SM, Der CJ. Biological assays for Ras transformation. Methods Enzymol 1995;255:395–412.