Nek4 Status Differentially Alters Sensitivity to Distinct Microtubule Poisons

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Abstract

Microtubule poisons are widely used in cancer treatment, but the factors determining the relative efficacy of different drugs in this class remain obscure. In this study, we identified the NIMA kinase Nek4 in a genetic screen for mediators of the response to Taxol, a chemotherapeutic agent that stabilizes microtubules. After Taxol treatment, Nek4 promoted microtubule outgrowth, whereas Nek4 deficiency impaired G2-M arrest and decreased formation of mitotic-like asters. In contrast, Nek4 deficiency sensitized cells to vincristine, which destabilizes microtubules. Therefore, Nek4 deficiency may either antagonize or agonize the effects of microtubule poisons, depending on how they affect microtubule polymerization. Of note, Nek4 gene maps to a commonly deleted locus in non-small cell lung cancer. Thus, Nek4 deletion in this disease may rationalize the use of particular types of microtubule poisons for lung cancer therapy. Cancer Res 70(3); 1033–41. ©2010 AACR.

Introduction

Microtubules are highly dynamic tubulin polymers crucial for the proper execution of numerous cellular processes. They play key roles in mitosis, promoting both mitotic spindle formation as well as the subsequent segregation of replicated DNA. Consequently, microtubules are an attractive anticancer target, as disruption of mitosis in highly proliferative cancer cells often results in cell death (1–5). Indeed, several classes of microtubule-disrupting drugs are currently used in clinical settings. Notably, the taxanes and the Vinca alkaloids are frontline therapies in the treatment of ovarian, breast, lung, and certain hematopoietic malignancies. Unfortunately, acquired and intrinsic drug resistance significantly limits the efficacy of these agents (6–9).

One of the most widely studied mechanisms of tumor cell survival following chemotherapy is multidrug resistance (MDR), a phenotype involving decreased drug accumulation resulting from increased drug efflux (10, 11). However, many tumors with inactive MDR still display resistance to microtubule poisons. Thus, multifactorial or alternative mechanisms of resistance must exist. Indeed, several resistance-causing alterations at the drug-target interface have previously been described for tubulin, including genetic mutations, isotype selection, post-translational modification, and altered regulation (12). Further, modifications in downstream signal transduction have also been suggested to contribute to microtubule poison resistance (13–15). Still, major genetic factors underlying the efficacy of microtubule-targeting drugs, as well as the rationale for using one microtubule poison versus another, remain unclear.

In an effort to better understand the genetic basis of chemotherapeutic response to specific microtubule drugs, we did an in vitro RNA interference (RNAi)–based screen for mediators of the response to Taxol, a commonly used microtubule-stabilizing taxane. This screen identified Nek4, a gene with unknown function belonging to a family of mitotic kinases termed NIMA-related kinases. Functional studies involving Nek4 showed that it has a role in microtubule regulation and that altered expression of this protein not only affected chemotherapeutic response but also conferred differential sensitivity to select microtubule-disrupting drugs. Interestingly, Nek4 is frequently deleted in lung cancer, and Nek4 levels in several human cell lines correlated with differential sensitivity to microtubule poisons.

Materials and Methods

Cell culture and chemicals. Eμ-myc mouse B-cell lymphomas were cultured in B-cell medium [45% DMEM/45% IMDM/10% fetal bovine serum (FBS), supplemented with 2 mmol/L L-glutamine and 5 mmol/L β-mercaptoethanol]. Mouse and human lung adenocarcinoma cells were cultured in standard DMEM/FBS and RPMI 1640/FBS media, respectively. Chemotherapeutic agents were purchased from LC Laboratories (Taxol) and Calbiochem (doxorubicin, vincristine, cisplatin, and 5-flourouracil) and used at the indicated concentrations. For in vivo studies, vincristine (0.9% NaCl solution) and Taxol (ethanol/cremophor/NaCl) were dissolved immediately before injection.

Retroviral constructs. Short hairpin RNA (shRNA) constructs were designed and cloned as previously described (16). Sequences (5′-3′) targeted by shRNAs are as follows: shNek4-1 (Mm), GGAGAATCGTTGAAGTCTTAA; shNek4-2...
instruments and deconvolution software. imaged and analyzed using Applied Precision DeltaVision bulges and centrosomes, respectively. Stained coverslips were cell sorting analysis. in a sodium citrate/PI buffer before fluorescence-activated (488) secondary antibody. Stained cells were then costained histone H3 (pH3)/PI (cell cycle) assays, cells were fixed in from GFP analysis. Live cell sorting was done using GFP co-incorporation (0.05 mg/mL), and dead cells were excluded cytometers. Cell death was detected by propidium iodide (PI) (described below). shRNA identities in enriched pools were subsequently determined as previously described (16).

Western blotting, immunofluorescence, and quantitative reverse transcription-PCR. For Western blotting and quantitative reverse transcription-PCR (RT-PCR), protein or total RNA was isolated after retroviral infection and puromycin selection. Quantitative RT-PCR was done using SYBR Green on a Bio-Rad thermal cycler. Nek4 mRNA levels were normalized to relative glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA abundance. Primer sequences are available on request. For Western blotting, cell lysates were prepared in lysis buffer [1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 10 mmol/L Tris-HCl (pH 8.0), 140 mmol/L NaCl] for 10 min, cleared for 15 min at 14,000 rpm, and then mixed with 5× SDS sample buffer. Proteins were then run on a 10% SDS-PAGE gel, transferred to polyvinylidene difluoride (Millipore), and detected with the following antibodies: anti-Nek4 (1:250; Hahn Laboratory, Dana-Farber Cancer Institute) and anti-GAPDH (1:10,000; Santa Cruz Biotechnology). Cells for immunofluorescence were grown and treated on poly-L-lysine–coated coverslips, fixed with 100% methanol for 5 min at −20°C, and stored for later use. Anti-α-tubulin (Y11/2; 1:2,000; Abcam) and anti-γ-tubulin (1200; Sigma) were used along with Alexa secondary antibodies (Molecular Probes) to visualize microtubules and centrosomes, respectively. Stained coverslips were imaged and analyzed using Applied Precision DeltaVision instruments and deconvolution software.

Flow cytometry and cell cycle experiments. All assays were done using Becton Dickinson FACScan or MoFlo flow cytometers. Cell death was detected by propidium iodide (PI) incorporation (0.05 mg/mL), and dead cells were excluded from GFP analysis. Live cell sorting was done using GFP co-expression as a marker of cell transduction. For phospho-histone H3 (pH3)/PI (cell cycle) assays, cells were fixed in 70% ethanol and then stained using an anti-pH3 antibody (1:2,500; Santa Cruz Biotechnology) followed by an Alexa (488) secondary antibody. Stained cells were then costained in a sodium citrate/PI buffer before fluorescence-activated cell sorting analysis.

GFP competition and viability assays. For competition assays, lymphoma cells were partially transduced with the indicated shRNA constructs, treated with chemotherapeutic agents (Taxol at 4, 6, and 8 nmol/L; doxorubicin at 10 ng/mL; cisplatin at 7.5 ng/mL; 5-fluorouracil (5-FU) at 40 ng/mL; and vincristine at 1.5 nmol/L), and monitored by flow cytometry for changes in the percentage of GFP+ cells. For viability assays, cells were plated subconfluently in 96-well plates, treated with drug (lung adenocarcinoma, colo669, H460, and H1395 cells were treated with 5 μmol/L Taxol or 5 μmol/L vincristine and SKI) cells were treated with 0.04, 0.2, 1, 5, 25, and 125 μmol/L Taxol or vincristine), and analyzed 48 h after treatment using CellTiter-Glo reagent (Promega) on an Applied Biosystems microplate luminometer.

In vivo mouse experiments. Syngeneic C57BL6/J female recipient mice were i.v. injected (via tail vein) with 4 million lymphoma cells and monitored until palpable tumors formed (~14 days). On tumor presentation, mice were administered either 25 mg/kg Taxol or 1.0 mg/kg vincristine (short-term enrichment studies) or 1.5 mg/kg vincristine (long-term survival studies) i.p. and monitored until the indicated time points, at which time mice were sacrificed and tumor material was collected, if necessary.

Microtubule polymerization assay. Cells plated on poly-L-lysine–coated coverslips were treated with 0.5 μmol/L nocodazole for 30 min at 37°C/5% CO2. Coverslips were briefly washed with PBS and allowed to recover for the indicated periods of time in nocodazole-free medium at room temperature. Coverslips were then fixed using ice-cold methanol (5 min, −20°C) and stored in 4% bovine serum albumin, 0.1% Triton X-100, and 0.05% sodium azide for subsequent immunofluorescent detection of α-tubulin and γ-tubulin. Microtubule length measurements were done on representative images (five fields per sample) using OpenLab5 (Improvision) software.

Results

RNAi screen for modulators of Taxol-induced cell death. We used cells from a well-established preclinical model of Burkitt’s lymphoma, the Eµ-myc mouse, to screen a library of shRNAs (the Cancer 1000) for genes that promote the activity of the microtubule poison Taxol. These shRNA-encoding vectors also expressed GFP to facilitate easy identification of transduced cells (17). shRNA pools were introduced into lymphoma cells by retroviral transduction, such that 20% to 30% of the target cells were infected, and were subsequently treated with Taxol to enrich for shRNA-containing cells displaying enhanced drug resistance (Fig. 1A). Using GFP-based flow cytometry to monitor the percentage of transduced (GFP-positive) lymphoma cells, we identified several pools that displayed GFP enrichment following treatment with Taxol, indicating the presence of at least one resistance-conferring shRNA within each pool. Deconvolution of these enriched posttreatment shRNA pools was done using a previously described PCR/colony sequencing technique (18). Identification of known modulators of chemotherapeutic response (e.g., p53) using our screening protocol suggested that this approach was sufficiently robust to identify contributors to the cellular response to Taxol.

Nek4 is a modulator of microtubule poison–induced cell death. Examination of our enrichment data revealed a shRNA

Published OnlineFirst January 26, 2010; DOI: 10.1158/0008-5472.CAN-09-2113

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targeting Nek4 (shNek4) as a candidate suppressor of Taxol-induced death. As an initial validation measure, shNek4 was isolated from the library and tested for the ability to promote Taxol resistance as a single construct. As in our general screening strategy, a population of lymphoma cells was partially transduced with the shNek4 construct (coexpressing GFP), treated with Taxol, and analyzed by flow cytometry before and after treatment for changes in overall GFP percentage. In this context, shNek4-infected cells enriched relative to cells receiving a control vector. Additional shRNA constructs were then designed and tested to confirm this initial finding and address potential issues arising from well-documented “off-target” RNAi effects (Fig. 1B; Supplementary Table S1). Importantly, quantitative PCR analysis of Nek4 mRNA levels and Western blotting for Nek4 protein in knockdown cells showed a correlation between the level of Taxol resistance and the extent of target suppression (Fig. 1C).

To determine how Nek4 suppression might promote resistance to Taxol, we first examined whether Nek4 suppression conferred resistance to other functionally distinct chemotherapeutic drugs. Here, we examined the response of shNek4-transduced cells to doxorubicin (a topoisomerase poison), cisplatin (a platinum-based DNA cross-linking agent), 5-FU (an antimetabolite), and vincristine (a microtubule destabilizer). To control for differential drug efficacy, cells were treated with drug doses that resulted in ~90% cell death after 48 h.

Figure 1. RNAi screening identifies Nek4 as a regulator of microtubule poison–induced cell death. A, in vitro screening methodology. Lymphoma cells were partially infected with 48 pools of 48 distinct shRNAs, treated with Taxol (4, 6, and 8 nmol/L), and monitored using GFP-based flow cytometry for changes in the relative percentage of shRNA-containing (GFP+) cells. Genomic DNA from enriched pools was subsequently subjected to shRNA-specific PCR and sequenced to determine relative shRNA abundance. B, an in vitro GFP competition assay comparing relative Taxol sensitivity in cells infected with two distinct shRNAs targeting Nek4 (6 nmol/L Taxol, 48 h after treatment; n = 5 for all samples). C, quantitative RT-PCR (n ≥ 3) and Western blot analysis of Nek4 expression in lymphoma cells. D, partially transduced lymphoma cells were separately treated with doxorubicin (Doxo; 10 ng/mL), cisplatin (Cisp; 7.5 ng/mL), 5-FU (40 ng/mL), and vincristine (Vin; 1.5 nmol/L) at similar levels of cytotoxicity (~90% cell death at 48 h). The percentage of GFP+ cells was determined 48 h after treatment (n = 3 for doxorubicin, 5-FU, and cisplatin and n = 5 for vincristine treatments). Bars, SD. P values were determined using a Student’s t test.
death at 48 h. GFP competition assays using the most potent Nek4 shRNA (shNek4-2) revealed no significant change in GFP percentage when treated with doxorubicin, cisplatin, or 5-FU. Unexpectedly, Nek4 suppression sensitized lymphoma cells to treatment with vincristine, as evidenced by a depletion of GFP-positive shNek4-2-transduced cells following drug treatment (Fig. 1D). Given that Taxol and vincristine have opposing effects on microtubule stabilization, yet activate similar downstream checkpoints (19–21), these results suggested that Nek4 might promote or inhibit drug action directly at microtubules, as opposed to acting in a signaling network emanating from microtubule disruption.

Many regulators of microtubule dynamics have observable effects on cell cycle progression. Further, many chemotherapeutic agents preferentially affect actively cycling cells. To address the possibility that Nek4 suppression promotes Taxol resistance by altering cell cycle progression, we determined the population growth rate, cell cycle profile (DNA content analysis), and mitotic index of shNek4-transduced cells. All three shNek4 populations were indistinguishable from control cells in these experiments, suggesting that gross impairment of the cell cycle was not responsible for the Taxol-resistant phenotype (Table 1).

We next sought to determine if Nek4 status had any effect on microtubule poison–induced cell cycle profiles. For these experiments, we used a cell line derived from a previously described LSL-KrasG12D;p53fl/fl lung adenocarcinoma mouse model (22). These cells display a more protracted response to chemotherapy (as opposed to highly chemosensitive Eμ-myc lymphoma cells), allowing us to more clearly define subtle changes in the intermediate events preceding cell death. Importantly, we first confirmed that these cells also display opposing survival profiles in response to microtubule-stabilizing versus microtubule-destabilizing drugs (Fig. 2A). Additionally, consistent with the data observed from untreated lymphoma cells, a comparison of 4N/2N DNA content ratios of untreated control versus shNek4-2 lung adenocarcinoma cells revealed little to no difference in cell cycle distribution (Fig. 2B, first column). However, in the presence of 5 μmol/L Taxol, shNek4-2–transduced lung adenocarcinoma cells displayed a defective G2-M arrest relative to vector-infected controls. Conversely, vincristine treatment yielded a more pronounced accumulation of G2-M–arrested cells in the absence of Nek4 (Fig. 2B). Thus, the cellular status of Nek4 seems to affect the efficacy of microtubule poisons proximal to the drug-target interface.

Nek4 is involved in the regulation of microtubules following exposure to microtubule poisons. Exposure to Taxol is known to have profound effects on microtubule organization, namely, the accumulation of mitotic-like asters and formation of abnormal microtubule bundles (23, 24). Because precise microtubule phenotypes are known to vary from cell type to cell type (25), we first examined the effect of Taxol on microtubules in lung adenocarcinoma cells. After a 4-h exposure to Taxol, we found evidence of both mitotic-like asters and microtubule bundles in control cells. Under these conditions, the “aster” phenotype predominated (Fig. 3A; Supplementary Fig. S1). Quantification of this phenotype in Taxol-treated control versus shNek4-transduced cell populations revealed a significant decrease in the percentage of cells harboring these aster-like structures: ∼14% in Nek4 knockdown populations as compared with ∼22% in controls (Fig. 3A and B). Importantly, this effect was not simply dependent on the number of cells available to form asters, as the mitotic index was not significantly different between the two cell populations, either in the presence or in the absence of Taxol or vincristine (Fig. 3B, right).

It has been previously reported that alterations in microtubule dynamics are associated with and can contribute to microtubule poison efficacy (26). Using an established in situ microtubule polymerization assay, we examined whether Nek4 knockdown had any effect on microtubule repolymerization following nocodazole treatment. Cells were transiently exposed to nocodazole to depolymerize existing microtubules; washed with excess medium to initiate repolymerization; and fixed, stained, and imaged at various time points to examine microtubule status. After a 30-min incubation with nocodazole, microtubules were no longer detectable, as determined using α-tubulin immunofluorescence. Whereas control cells showed rapid microtubule polymerization from centrosomes following nocodazole release, defects in microtubule assembly in Nek4 knockdown cells were apparent as early as 1 min following release and clearly observable at the 2-min time point (Fig. 3C and D). This suggests that impaired microtubule polymerization may underlie the differential sensitivity of Nek4 knockdown cells to Taxol and vincristine.

Nek4 knockdown modulates microtubule poison efficacy in vivo. A strength of the Eμ-myc lymphoma model as a

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**Table 1. Cell cycle analysis of shNek4-infected Eμ-myc lymphoma cells**

<table>
<thead>
<tr>
<th></th>
<th>4N/2N ratio (PI)</th>
<th>Mitotic index (pH3⁺)</th>
<th>Doubling time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector</td>
<td>0.51 ± 0.07</td>
<td>3.7 ± 0.4</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>shNek4-1</td>
<td>0.46 ± 0.5</td>
<td>3.5 ± 1.2</td>
<td>8.4 ± 0.8</td>
</tr>
<tr>
<td>shNek4-2</td>
<td>0.45 ± 0.02</td>
<td>3.2 ± 0.4</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>shNek4-3</td>
<td>0.5 ± 0.06</td>
<td>3.9 ± 0.6</td>
<td>8.7 ± 1.0</td>
</tr>
</tbody>
</table>

NOTE: Actively cycling knockdown cell populations were fixed, stained with anti-pH3/PI, and analyzed by flow cytometry to determine the mitotic index (shown as % pH3 cells) and relative DNA content (shown as a ratio of 4N/2N cells). n = 3 independently infected cell populations for each vector ± SD.
preclinical system is the ability to transplant genetically altered tumor cells into syngeneic, immunocompetent recipient mice, where the resulting disease is pathologically indistinguishable from lymphomas arising in germ-line Eμ−myc mice (27). This, along with the ability to specifically silence individual genes, allows for rapid evaluation of putative regulators of chemotherapeutic response in an immunocompetent in vivo setting (Fig. 4A). Using this approach, we transplanted partially transduced control and shNek4-2 knockdown lymphomas into recipient mice and allowed palpable tumors to form (∼14 days). On tumor presentation, mice were administered either 25 mg/kg Taxol or 1.0 mg/kg vincristine for 24 h, at which time tumor material was harvested for analysis by flow cytometry. In agreement with in vitro experiments, acute treatment of control tumors had no effect on the percentage of GFP-positive cells (data not shown). In contrast, the percentage of GFP-positive shNek4-2 cells increased on treatment with Taxol while selectively depleting when exposed to vincristine (Fig. 4B). Further, mice harboring pure population (GFP-sorted) shNek4-2 tumors showed an improved overall response to vincristine, with extended tumor-free and overall survival relative to their control counterparts (Fig. 4C; data not shown).

Nek4 status modulates the relative sensitivity of human lung cancer cell lines to microtubule poisons. Microtubule poisons are frontline chemotherapies for the treatment of non–small cell lung cancer (NSCLC). Interestingly, many lung cancers harbor deletions on the short arm of chromosome 3 that include the Nek4 genomic locus. Thus, we reasoned that Nek4 status in NSCLC might contribute to the differential response of lung cancer cell lines to microtubule poisons. Given the complex constellation of mutations undoubtedly present across multiple cell lines, we limited our analysis to the relative sensitivity of cell lines to Taxol versus vincristine. We tested several cell lines: one with a high level of Nek4 protein (colo669) and three (Sklu1, H460, and H1395) with reduced Nek4 levels. Interestingly, we found that colo669 cells had a significantly lower Taxol versus vincristine survival ratio, indicative of relative Taxol sensitivity and/or vincristine resistance (Fig. 5A). Importantly, shRNA-mediated knockdown of Nek4 in colo669 cells changed the response profile of these cells, promoting both Taxol resistance and vincristine sensitivity (Fig. 5B; Supplementary Table S2). Conversely, changes in drug sensitivity were not seen in a cell line expressing low levels of Nek4 (Sklu1). However, overexpression of human Nek4 promoted vincristine resistance and sensitivity to multiple taxanes in Sklu1 cells (Fig. 5C and D). Thus, Nek4 levels in human cancers can significantly affect the relative sensitivity of these tumors to distinct microtubule poisons.

Discussion

The founding member of the NIMA kinase family was first identified in an Aspergillus nidulans screen for mutants that are “never in mitosis” (28, 29). Since then, 11 NIMA kinases have been identified by homology in mammalian cells. Four of these proteins—Nek2, Nek6, Nek7, and Nek9—have been shown to play roles in mitosis, whereas Nek1 and Nek8 are important for cilia function (30–32). Here, we show that Nek4 also plays a role in microtubule homeostasis. Importantly, however, this effect is only seen in the context of microtubule poisons. Multiple explanations could account for this effect. First, other proteins may compensate for Nek4 loss of function during mitosis in untreated cells. Alternatively, partial Nek4 activity may be sufficient to allow for normal mitosis.
Figure 3. Nek4 knockdown cells show altered microtubule phenotypes. A, lung adenocarcinoma cells expressing a Nek4 shRNA or a control vector were treated with 5 μmol/L Taxol for 4 h and then stained with anti-α-tubulin to visualize microtubules. Microtubule asters (red arrowheads) and bundles (yellow arrowheads) were observed under these conditions. B, left, quantification of aster-containing cells in shNek4-infected and vector control–infected cell populations using OpenLab5 software (n = 3 independently treated replicates, six average fields per sample). Right, pH3 staining of drug-treated lung adenocarcinoma cells failed to show any significant change in the mitotic index in the presence or absence of Nek4 (n = 3 for all samples). Bars, SD. C, in a microtubule repolymerization assay, lung adenocarcinoma cells were allowed to repolymerize microtubules following transient (0.5 μmol/L, 30 min) nocodazole treatment. Qualitative differences were apparent at 1 min (third column) and 2 min (fourth column) after nocodazole release. D, quantification of the repolymerization defect in shNek4-2 lung adenocarcinoma cells. Microtubule (MT) lengths are shown at the 1- and 2-min time points, respectively. The average microtubule length in each sample is indicated with a red bar. P values were determined using a Student’s t test.
Although the shRNAs used in this study achieve near-complete knockdown of Nek4 by Western blot, it remains to be seen whether these vectors recapitulate Nek4-null phenotypes. Interestingly, Nek4 deficiency results in resistance to Taxol and sensitivity to vincristine. These data suggest that Nek4 functions at the level of microtubules rather than on common downstream signal transduction pathways emanating from altered microtubule homeostasis. Given that, at high drug doses, Taxol is a microtubule-stabilizing agent and vincristine destabilizes microtubules, our data also suggest that Nek4 may play a role in promoting microtubule polymerization in the presence of the drug. Consistent with this idea, Nek4 deficiency impairs microtubule repolymerization following nocodazole treatment. That a genetic alteration can confer opposite cellular responses to vincristine and Taxol is not novel (33–36). Previous efforts in generating Taxol-resistant cell lines have, in some instances, yielded cells that were vincristine sensitive (34, 35, 37). Notably, these studies highlighted the ability of tubulin mutations to confer differential sensitivity to microtubule poisons. Here, loss of function screening allowed for the identification of a regulator of therapeutic response that may have been overshadowed in these earlier efforts, presumably by more potent modifiers of the cellular response to microtubule poisons. Importantly, the effects of Nek4 manipulation on overall response to microtubule poisons are relatively minor, so further work will be required to determine whether pharmacologic inhibition of Nek4 or associated proteins would have significant clinical benefit.

Interestingly, Nek4 is located in a genomic region that is commonly mutated in lung cancer. Although it is unclear whether Nek4 is relevant to the pathogenesis of lung cancer, these data suggest a connection between the specific alterations that occur during lung cancer development and the

Figure 4. Nek4 suppression alters the response to microtubule poisons in vivo. A, schematic depicting in vivo experimental approaches. Partially transduced (top row) or GFP-sorted (bottom row) lymphoma cells were injected into recipient mice and allowed to develop into palpable lymphomas. Resulting tumors were then treated with Taxol (tax) or vincristine (vin) and then either harvested to examine the percentage of GFP+ cells or monitored for tumor-free and overall survival rates. B, partially transduced shNek4-2 lymphomas were harvested 24 h after drug treatment (25 mg/kg Taxol or 1.0 mg/kg vincristine) and analyzed by flow cytometry for changes in GFP percentage. Taxol treatment resulted in an increase in the percentage of GFP+ cells (compared with preinjection GFP levels), whereas vincristine-treated tumors displayed dramatic selection against Nek4 knockdown. P values were determined using a Student’s t test. C, Kaplan-Meier survival curve depicting overall survival of shNek4-2 or control tumor-bearing mice following treatment with a maximally tolerated dose (1.5 mg/kg) of vincristine (vector, n = 13; shNek4-2, n = 12). P values were determined using a log-rank test.
ultimate response of that cancer to chemotherapy. Notably, the fact that Nek4 deficiency confers sensitivity to microtubule destabilizers suggests that existing therapies can be tailored toward this deficiency. For example, the combination of cisplatin and Taxol is commonly used as a frontline therapy for NSCLC. Our data suggest that a more personalized approach to treating lung cancer, using vincristine rather than Taxol in tumors with 3p deletions, may result in enhanced chemotherapeutic response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank William Hahn for generously providing us with the Nek4 antibody used in these studies, Paul Chang for help with microtubule assays, and members of the Hemann lab for helpful advice and discussions.
Grant Support

M.T. Hemann is a Rita Allen Fellow and the Latham Family Career Development Assistant Professor of Biology and is supported by NIH grant ROI CA128803-01. J. Doles is supported by the MIT Department of Biology training grant.

References

Correction: Online Publication Dates for Cancer Research April 15, 2010 Articles

The following articles in the April 15, 2010 issue of Cancer Research were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:


Dudka AA, Sweet SMM, Heath JK. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. Cancer Res 2010;70:3391–401. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3033.


Published OnlineFirst 05/11/2010.
©2010 American Association for Cancer Research.
doi: 10.1158/0008-5472.CAN-10-1347
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Cancer Res 2010;70:1033-1041. Published OnlineFirst January 26, 2010.