A Targeted RNAi Screen of the Breast Cancer Genome Identifies KIF14 and TLN1 as Genes That Modulate Docetaxel Chemosensitivity in Triple-Negative Breast Cancer

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Abstract

Purpose: To identify biomarkers within the breast cancer genome that may predict chemosensitivity in breast cancer.

Experimental Design: We conducted an RNA interference (RNAi) screen within the breast cancer genome for genes whose loss-of-function enhanced docetaxel chemosensitivity in an estrogen receptor-negative, progesterone receptor-negative, and Her2-negative (ER−, PR−, and Her2−, respectively) breast cancer cell line, MDA-MB-231. Top candidates were tested for their ability to modulate chemosensitivity in 8 breast cancer cell lines and to show in vivo chemosensitivity in a mouse xenograft model.

Results: From ranking chemosensitivity of 328 short hairpin RNA (shRNA) MDA-MB-231 cell lines (targeting 133 genes with known somatic mutations in breast cancer), we focused on the top two genes, kinesin family member 14 (KIF14) and talin 1 (TLN1). KIF14 and TLN1 loss-of-function significantly enhanced chemosensitivity in four triple-negative breast cancer (TNBC) cell lines (MDA-MB-231, HCC38, HCC1937, and Hs478T) but not in three hormone receptor-positive cell lines (MCF7, T47D, and HCC1428) or normal human mammary epithelial cells (HMEC). Decreased expression of KIF14, but not TLN1, also enhanced docetaxel sensitivity in a Her2-amplified breast cancer cell line, SUM190PT. Higher KIF14 and TLN1 expressions are found in TNBCs compared with the other clinical subtypes. Mammary fat pad xenografts of KIF14- and TLN1-deficient MDA-MB-231 cells revealed reduced tumor mass compared with control MDA-MB-231 cells after chemotherapy. KIF14 expression is also prognostic of relapse-free and overall survival in representative breast cancer expression arrays.

Conclusion: KIF14 and TLN1 are modulators of response to docetaxel and potential therapeutic targets in TNBC. Clin Cancer Res; 19(8); 2061–70. ©2013 AACR.

Introduction

Expression array analyses in breast cancer have revealed multiple subtypes of breast cancer, each with distinct clinical prognosis and response to treatments (1–4). Every tumor acquires a complex combination of somatic mutations that contribute to the cancer phenotype. Large-scale sequencing of multiple cancers has reported thousands of genes that have low frequency mutation rates in cancer (5–9). This poses a tremendous challenge for finding novel therapeutic targets and identifying patient subgroups that may benefit from specific treatment regimens. Furthermore, besides sequence mutations, there are numerous chromosomal alterations, copy number variations, miRNA dysregulations, and epigenetic events that are frequently found in human cancers (10–12). Successful therapy depends on the identification of critical genes in the oncogenic network where pharmacologic inhibition can result in death of cancer cells while sparing normal cells. Clinical trials in breast cancer so far have often shown that the most effective treatment is when chemotherapy is combined with targeted therapies rather than chemotherapy or targeted therapies alone (13–15).

We used a combinatorial approach using RNA interference (RNAi; short hairpin RNA; shRNA) against a cohort of candidate breast cancer genes identified via whole-genome cancer sequencing along with docetaxel to identify gene targets whose loss-of-function would augment chemosensitivity. We conducted the chemosensitivity screen against a well-characterized estrogen receptor–negative, progesterone receptor–negative, and Her2-negative (ER−PR−Her2−), “triple-negative,” claudin-low breast cancer cell line, MDA-MB-231, as it represents the clinical subtype that has...
Translational Relevance

Use of chemotherapies is often limited by side effects and intrinsic or acquired resistance of the tumor. Given the vast heterogeneity of genetic aberrations found in breast cancer, biomarkers that may predict response or resistance to certain chemotherapy can be used not only to identify patients appropriate for certain treatment regimens, but also to define patient populations who may benefit from additional targeted therapies. Recent acceleration of cancer genome sequencing efforts has outpaced downstream molecular studies focused on the identification of robust biomarkers and cancer drivers. Genes with known somatic mutations, compared with the entire genome, are more likely to include biomarkers that are not only prognostic but also encompass key genes that drive or maintain the malignant phenotype. A chemosensitivity screen using RNA interference (RNAi) against this subset of genes is a functional way to identify biomarkers whose expressions are important from a treatment response point of view.

the worst prognosis (16, 17). We used docetaxel as it is one of the most common chemotherapies given for breast cancer. Although response rates are high to taxanes, toxicities including neuropathy and myelosuppression often preclude use of these drugs at high doses or for prolonged periods of time. Identification of novel targets that would enhance docetaxel chemosensitivity and enable lower effective dosages may allow patients a better quality of life and perhaps improved prognosis.

Materials and Methods

Cells

MDA-MB-231, HCC38, Hs578T, and MCF7 cells were kindly provided by M. White (Department of Cell Biology, University of Texas Southwestern Medical School, Dallas, TX), T47D and HCC1428 cells were kindly provided by G. Pearson (Department of Pharmacology, Simmons Comprehensive Cancer Center, Dallas, TX). HME2424 cells were a gift from D. Euhus and were originally immortalized by retroviral infection with human telomerase reverse transcriptase (hTERT) by D. Euhus (Department of Surgery, Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX). The 2800delAA of BRCA1 in HME2424 was sequence verified. SUM190PT cells were purchased from Asterand. HCC1937 cells were originally derived by A. Gazdar (University of Texas Southwestern Medical Center, Dallas, TX) and are available from American Type Culture Collection (ATCC) Cell Systems. Human mammary epithelial cells (HMEC; HME1) were originally immortalized by retroviral infection with hTERT by J.W. Shay (University of Texas Southwestern Medical Center, Dallas, TX) and are available from ATCC Cell Systems (Gaithsburg, MD). HME50 cells were originally derived by J.W. Shay from the noncancerous breast tissue of a female diagnosed with Li-Fraumeni syndrome as previously described (18). The missense p53 mutation (M133T) in HME50 was sequence verified. All cancer cell lines were cultured in basal medium supplemented with 10% fetal calf serum. All benign cells were cultured in serum-free conditions as described elsewhere (19).

Chemicals

Doxorubicin and docetaxel were obtained from Sigma. PP2 and SB203580 were obtained from Tocris Bioscience.

Expression array analysis and statistics

Five publicly available breast cancer expression datasets (3, 20, 21) were separately normalized then pooled for analysis (n = 946). These datasets were chosen for having clinical annotations that include ER status, axillary lymph node involvement, relapse-free and/or overall survival (OS) information. Data were downloaded from Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) with accession numbers GSE3494 (20), GSE5532 (21), GSE1456 (22), GSE7390 (23), and from Nederlands Kanker Instituut (http://bioinformaticstki.nl/data.php; ref 3). Neoadjuvant data were accessed with GSE25066 (24). Probes were mapped using Entrez identifiers and Human Genome Organization gene symbols and then averaged for gene level analysis. Missing values were imputed using nearest neighbor averaging. All data analysis was conducted using tools in R/Bioconductor. Datasets were merged and standardized by scaling the columns then the rows and checked using principal component analysis (PCA). Visualization of the PCA method for normalization is provided in Supplementary Fig. S1. Univariate and multivariate Cox proportional hazard models were used to analyze prognostic capacity of KIF14 and TLN1, with and without clinical feature, to relapse-free and OS. Using PCA, patients were split into 2 groups by the median value of the first component. Survival curves for these groups were evaluated by Kaplan–Meier estimators with log-rank P values reported. The Cancer Genome Atlas (TCGA) breast cancer expression data and clinical annotations were downloaded from the TCGA website (https://tcga-data.nci.nih.gov/tcga/), and expression data with clinical annotations on 52 breast cancer cell lines (25) were downloaded from http://cancer.lbl.gov/breastcancer/data.php. For the TCGA dataset and cell line data, the median expression of KIF14 was used to split each dataset into 2 cohorts to examine expression patterns in samples that belong to the 4 clinical subtypes of breast cancer: triple-negative, ER+ and/or PR+ Her2-, ER−PR−HER2+, or ER+ and/or PR+ HER2+. Bootstrapting was confirmed by Kolmogorov–Smirnov test (D = 0.317; P < 0.0001).

Screen and cell viability assay

A total of 328 shRNAs in pGIPZ against 133 candidate breast cancer genes (5) were picked from Open Biosystems CSHL. hs shRNAmir 6.13 (Lenti) library (GE Healthcare Life Sciences). Clone IDs for each shRNA used in this study are provided in Supplementary Table S1 and could be used to retrieve target sequence from Open Biosystems. Of note,
5,000 cells of each stably selected clone were seeded on a 96-well plate using a BioMek FX Automation Workstation (Beckman Coulter Inc.). Five replicates of 12 doses of each chemotherapy were arrayed on each 96-well plate. Cell viability was measured 72 hours later with CellTiter-Glo (Promega) as per manufacturer’s protocol. Docetaxel IC₅₀ values were independently calculated for each cell clone with GraphPad Prism 5 (GraphPad Software, Inc.). Nonsilencing shRNA control underwent 13 temporally distinct determinations of IC₅₀ for docetaxel (Supplementary Table S2) for determination of 95% confidence intervals (95% CI) and z scores. For siRNA experiments, 100 nmol/L pooled siRNAs (SMARTpool; Dharmacon) for KIF14, TLN1, CIT, ARRB2, PSTPiP1, PRC1, SVIL, ITGA2B, ITGB3, VCL, and PXN or siControl (D001206; Dharmacon) were transfected into each cell line using RNAiMAX (Invitrogen) as per manufacturer’s instructions. Docetaxel at 1 nmol/L and chemicals (PP2 and SB203580) or dimethyl sulfoxide (DMSO) as control were added 48 hours after siRNA infection and cell viability was determined 72 hours later with CellTiter-Glo (Promega). Data are means from 2 independent experiments carried out in triplicates. Cell viability was also visualized with Crystal Violet stain (Sigma) for confirmation.

Xenografts
Mammary fat pads were cleared from 3-week-old non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice, as previously described (26). One million cells in growth media suspension was injected in 10 µL into a cleared fat pad. Nonsilencing controls and knockdown cells were injected in contralateral mammary fat pads. Four weekly intraperitoneal injections of docetaxel (5 mg/kg) were administered starting at 7 days after xenograft placement. Eight weeks after xenograft placement, primary tumors were dissected out. Tumor volume was calculated as (width² × length) mm³/2. All animal work was approved and conducted as per institutional guidelines.

Flow cytometry
Cells were trypsizined and suspended in PBS to a final cell concentration of 10 million cells/mL. Annexin V Alexa Fluor 647 (Life Technologies) was used as per manufacturer’s instructions. Flow cytometry was conducted on BD FACS Calibur Flow Cytometer (BD) and data analysis was conducted using FlowJo software (Tree Star, Inc.).

Kinase assay
Inhibitor activity against RIP2 was conducted using a radioisotope assay developed and conducted by Signal-Chem. SB203580 and PP2 were provided at 10 mmol/L to SignalChem. Profiling of the 2 compounds were done at 4 concentrations (4, 40, 400, and 4,000 nmol/L) in triplicate to determine the IC₅₀ values.

Plasmids and transfections
pEGFP KIF14 was kindly provided by T. Corson (Department of Ophthalmology, Indiana University, Bloomington, IN; ref. 27). pEGFP-TLN1 was obtained from Addgene (plasmid #26724). Transfections were carried out with Lipofectamine LTX reagent (Invitrogen) as per manufacturer’s protocol.

Quantitative PCR
All samples were prepared from cultured cells in log phase growth. RNA was extracted with RNeasy MiniKit (Qiagen) and cDNA was made with First Strand CDNA Synthesis Kit (Roche) as per manufacturers’ protocols. Quantitative PCR (qPCR) were run in triplicates in Roche LightCycler 480 System with DNA SYBR Green Master Mix (Roche). Reference genes HSP90, HPR1, and GUSB were used in each experiment for normalization. All data were analyzed with Biogazelle qBasePLUS. Primer efficiencies were determined empirically and were between 1.91 and 2.08. Primers for the genes are as follows: GUSB (forward-tctatttggtttgctggatt; reverse-cggttaggaatctccctttta); HSP90 (forward-ccaaagac-cagctgagatca; reverse-tgtggtcctagctccttt); HPR1 (forward-tgcagctgcaacaatgca; reverse-ggctttcccttcagcga); KIF14 (forward-tgtaggaaggggaaatgacatca; reverse-cacacatca-caacagctcc); TLN1 (forward-ccaaagac-gagaggaggtgtat; reverse-tgcgtactgctagatgtc).

Viral transductions and stable selections
For lentivirus production, 1 µg of pGIPZ-shRNA plasmid together with 1 µg of helper plasmids (0.4 µg pMD2G and 0.6 µg psPAX2) were transfected into 293FT cells with Effectene reagent (Qiagen). Viral supernatants were collected 48 hours after transfections and cleared through 0.45-µm filter. Cells were infected with viral supernatants containing 4 µg/mL polybrene (Sigma) and selected with puromycin for 7 days.

Western blot analysis
Total cell lysates were prepared by harvesting cells in Laemml SDS reducing buffer. Protein concentrations were measured using a Pierce BCA protein assay kit (Thermo Scientific), resolved on an 8% to 10% polyacrylamide gel, and transferred to a polyvinylidene fluoride membrane. Antibodies used are as follows: glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Cell Signaling), KIF14 (Bethyl Laboratories), and TLN1 (Millipore). Detection of peroxidase activity from horseradish peroxidase−conjugated antibodies was done with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific). Images were captured with the G:BOX F3 with GeneSys software (SynGene).

Results
Identification of genes whose expressions most correlate with chemosensitivity
We used 328 shRNAs targeting 133 genes with known somatic mutations in breast cancer (ref. 5; Supplementary Table S1) to generate stable gene knockdown in MDA-MB-231 cells. We arrayed each cell clone in quintuplicate on 96-well microtiter plates for 12 doses of docetaxel to detect the effect of each gene knockdown in chemosensitivity as reflected in changes of IC₅₀ from MDA-MB-231 cells

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containing a nonsilencing shRNA (Fig. 1A and Supplementary Table S2). We rank-ordered the shRNA cell lines according to docetaxel chemosensitivity and focused on the top 2 genes whose knockdown on at least 3 separate IC$_{50}$ determinations resulted in significant sensitivity in MDA-MB-231 cells to docetaxel: KIF14 and Talin 1 (TLN1; Fig. 1A and Supplementary Table S2). KIF14 is a microtubule-dependent molecular motor, containing a kinesin motor domain and a forkhead-associated domain. Its function is essential for the final phase of cytokinesis (27, 28). The mechanism of docetaxel chemosensitivity is unclear but may include further disruption to microtubule dynamics. KIF14 overexpression in many cancers including breast, ovarian, and lung cancers has been described and has been tested successfully as an independent prognostic marker (29–31). TLN1 is a focal adhesion complex protein that regulates integrin interactions with the extracellular matrix (ECM). Prostate cancer cell models have suggested TLN1 to be involved in progression to metastasis (32).

To evaluate for potential off-target effects of the specific shRNAs, we transfected KIF14 and TLN1 cDNAs into shKIF14 and shTLN1 MDA-MB-231 cells, respectively, and found significant decrease in chemosensitivity, indicating a specific role of KIF14 and TLN1 in chemosensitivity (Fig. 1B). qPCRs and Western blot analyses of these clones showed excellent amount of gene knockdown and reconstituted expressions (Fig. 1C and D). To evaluate effects of gene knockdown on chemosensitivity of multiple cell lines, we used siRNAs against KIF14 and TLN1 (Fig. 1E). Among available breast cancer cell lines, we chose 3 additional triple-negative breast cancer (TNBC) cell lines along with MDA-MB-231 representing subtypes of breast cancer with poor prognosis and no molecularly targeted agents available (33) to evaluate for changes in chemoresponsiveness when KIF14 and TLN1 are knocked down. KIF14 and TLN1 knockdown enhanced...
chemosensitivity in all 4 triple-negative cell lines tested to various degrees ($P = 0.0003-0.0054$; Fig. 1E). KIF14 knockdown but not TLN1 knockdown affected docetaxel chemosensitivity in a Her2-amplified cell line, SUM190PT ($P = 0.0014$ and 0.41, respectively). However, KIF14 and TLN1 loss-of-function in ER+ cell lines (MCF7, T47D, and HCC1428) and normal HMECs (HME1) or premalignant cells including HME50 (p53 mutation carrier), and HME2424 (BRCA1 mutation carrier) did not significantly affect cell survival when treated with docetaxel at IC50 for specific cell lines (Fig. 1E). KIF14 and TLN1 expressions seem to be upregulated at least half of our panel of 8 breast cancer cell lines compared with the 3 benign cell lines tested (Fig. 1F).

**KIF14 and TLN1 are overexpressed more often in triple-negative breast cancers than other clinical subtypes**

To evaluate how elevated KIF14 and TLN1 expressions may be significant for breast cancer, we investigated relative gene expressions within known clinical subtypes in a collection of 52 breast cancer cell lines (Supplementary Table S3) previously described to model recurrent genomic and transcriptional characteristics of primary breast tumors (24). Median values for KIF14 and TLN1 expressions were used to divide cases into high- or low-expression groups. Within TNBC cell lines, 66.7% and 75% of the cell lines have relatively high KIF14 or TLN1 expressions, respectively (Fig. 2A). We then examined TCGA (34) to see if KIF14 and TLN1 overexpressions are more common among triple-negative primary breast cancers. TNBCs, even though representing only 15% (71 of 459) of total cases, have a higher proportion of high-KIF14 (91.6%) and TLN1 (62%) expressions relative to the other clinical subtypes (Fig. 2B).

We next tested if KIF14 and TLN1 may be prognostic biomarkers. We chose 5 large, publicly available breast cancer expression array datasets that have adequate clinical annotations including ER status, axillary lymph node involvement, relapse-free, and/or OS data (see Materials and Methods). We then normalized and merged the datasets (see Materials and Methods and Supplementary Fig. S1), creating a combined breast cancer expression array dataset to evaluate if expressions of these genes correlated with prognosis in breast cancer ($n = 946$ for relapse-free survival and $n = 652$ for OS). KIF14 expression is correlated to relapse-free survival and OS outcomes by univariate Cox regression but TLN1 is not ($P = 2.2e-9$ and $3.9e-7$, respectively for KIF14 and $P = 0.45$ and 0.95, respectively for TLN1). Kaplan–Meier analysis of relapse-free and OS outcomes shows significant prognostic value of KIF14 expression (see Fig. 3A), but not of TLN1 expression (log-rank $P = 0.132$; HR, 0.84 for relapse-free and log-rank $P = 0.957$; HR 0.99 for OS; data not shown). To evaluate the added value of using KIF14 expression in addition to key clinicopathologic prognostic features such as ER status and axillary lymph node involvement, we used PCA and Cox regression analysis on the dataset of 919 patients (27 subjects were excluded from our pooled total from statistical analysis due to incomplete clinical annotation) and found that KIF14 expression, when added to ER and node status, improves relapse-free survival prognostic power compared with clinicopathologic features alone ($P = 6.9e-9$ vs. $P = 4.1e-5$, respectively). For patients with OS and clinicopathologic annotations ($n = 493$), KIF14 expression also improves OS prognostic power compared with clinicopathologic features alone ($P = 3.6e-9$ vs. $P = 6.2e-8$, respectively). Bootstrapping was conducted using 1,000 random genes selected from a pool of all genes in the combined dataset ($n = 11,262$) for relapse-free and OS to show the prognostic capacity of KIF14 relative to a random sample, and showed that KIF14 is unlikely to be randomly associated with improved prognostic power (Fig. 3B).

We further evaluated if KIF14 and TLN1 may be prognostic for patients who received taxane-containing chemotherapy neoadjuvantly. We used the largest publicly available dataset available with survival annotation (24) and found that KIF14 expression is correlated with worse distant relapse-free (DRF) survival but not TLN1 (KIF14: log-rank $P = 0.0028$, HR, 1.8; TLN1: log-rank $P = 0.088$, HR, 1.4; $n = 508$). However, if we only examine the triple-negative subgroup, both KIF14 and TLN1 are prognostic of DRF survival (KIF14: log-rank $P = 0.035$, HR, 1.9; TLN1: log-rank $P = 0.028$, HR, 1.8; $n = 178$). Neither gene is prognostic in the ER+ subgroup (data not shown).

**KIF14 and TLN1 confer survival advantage in vitro and in vivo**

Although MDA-MB-231 cells with KIF14 or TLN1 knockdown do not have appreciable proliferation defects (Fig. 4A), cell survival is significantly less when low dose chemotherapy (IC20) is given (Fig. 4B). Annexin V staining by flow cytometry indicates that apoptosis contributes largely to the difference seen in cell numbers (Supplementary Fig. S2).

To assess chemosensitivity in vivo, xenografts of KIF14 and TLN1 knockdown cells into cleared mammary fat pads of NOD/SCID mice showed dramatic decrease in tumor mass after docetaxel treatments compared with nonsilencing control MDA-MB-231 cells injected into contralateral cleared fat pads (Fig. 4C and D).

**Interaction networks of KIF14 and TLN1 suggest further therapeutic targets**

Protein–protein interaction networks of KIF14 and TLN1 identify multiple genes, including 3 kinases (FAK, CIT, and RIP2) that may participate in functional networks that affect chemosensitivity. Knockdown of RIP2, but not FAK, CIT, or a number of other known protein–protein interaction partners of KIF14 and TLN1, including ARRB2, PSTPIP1, PRC1, SUL, ITGA2B, ITGB3, VCL, and PXN, significantly altered docetaxel chemosensitivity in MDA-MB-231 cells (Fig. 5A). KIF14–RIP2 interaction was detected from a large-scale mapping of human protein–protein interactions by scale mapping of human protein–protein interactions by www.aacrjournals.org Clin Cancer Res; 19(8) April 15, 2013 2065
Knockdown of both RIP2 and KIF14 further chemosensitizes MDA-MB-231 cells, which suggests a genetic interaction between KIF14 and RIP2 (Fig. 5B). RIP2 kinase inhibitors, PP2 and SB203580 (IC50 for RIP2 is 51.36 and 99.79 nmol/L, respectively; Fig. 5C), enhanced chemosensitivity of KIF14-deficient MDA-MB-231 cells to docetaxel (Fig. 5D). RIP2 knockdown or inhibition by PP2 and SB203580 at less than or equal to 10 μmol/L was not toxic to normal HMEC line HME1 (data not shown).

**Discussion**

We aimed to find novel biomarkers of chemosensitivity from the breast cancer genome—a selected group of genes known to have somatic mutations in breast cancer. Annotation of the breast cancer genome to include functional consequence of gene expression on chemosensitivity is important from a treatment perspective. From the screen, we focused on 2 genes, *KIF14* and *TLN1*, whose loss-of-function most significantly increased chemosensitivity of...
MDA-MB-231 cells to docetaxel. KIF14 and TLN1 knockdown clearly enhanced chemosensitivity to docetaxel in 4 triple-negative, mesenchymal/stem-like/basal breast cancer cell lines (MDA-MB-231, HCC1937, HCC38, and Hs578T) but not significantly in ER+ breast cancers, implicating intrinsic differences in survival mechanisms or dependence on specific oncogenic pathways among different breast cancer subtypes. In addition, because KIF14 and TLN1 knockdown did not affect chemosensitivity of normal HMECs, these genes may allow for pharmacologic intervention.

Besides their roles in chemosensitivity, KIF14 and TLN1 expressions are found to be upregulated more often within the TNBC subtype than the other clinical subtypes. TNBCs convey a poor prognosis, insensitivity to adjuvant chemotherapy, and resistance to current targeted therapies (37). We have found that KIF14 and TLN1 expressions correlate with docetaxel chemosensitivity in basal and mesenchymal triple-negative cell lines in general better than other cell lines tested. Although a number of biomarkers have been identified for TNBC, none has seemed to be expressed in a significant proportion of TNBC cases or be specifically upregulated in the TNBCs compared with the other clinical subtypes (37–39). We showed that KIF14 expression is prognostic of survival in breast cancer in a large combined dataset (>900 patients with annotations for relapse-free survival and >400 patients for OS, Fig. 3), corroborating previous report of KIF14 as a prognostic biomarker in breast cancer (29). Because we have found that high KIF14 expression is predominantly in TNBC (>90% of TNBC cases within TCGA have high KIF14 expression) and that decreasing KIF14 expression or function would chemosensitize TNBC cells to docetaxel, KIF14 is an attractive target for therapeutic intervention.

Via interrogation of protein–protein interaction partners with KIF14, we further identified RIP2 as a potential novel target for chemosensitization. Additive or synergistic chemosensitization of RIP2 knockdown or RIP2 chemical inhibition in a KIF14-deficient background in MDA-MB-231 cells also suggest a potential therapeutic combination. RIP2 is known to be an important mediator of inflammation (40, 41). Here, we show a previously unrecognized role of RIP2 in breast cancer and as a potential chemosensitizer and interactor with KIF14.

Figure 3. A, survival curves using Kaplan–Meier estimators using KIF14 as a biomarker (dichotomized about the median) for relapse-free and OS with log-rank P values reported. Red denotes high- and black denotes low-KIF14 expressions. B, distribution of P values following bootstrapping with 1,000 random genes as biomarkers for relapse-free and OS when using the genes alone versus genes along with clinical features (ER positivity and axillary lymph node involvement).
In a search for specific druggable oncogenic dependence, we found that inhibition of 2 genes, KIF14 and TLN1, enhances the therapeutic index of docetaxel in TNBC. These results suggest that KIF14 and TLN1 play a role in response to cytotoxic chemotherapy, that decreased KIF14 and TLN1 expression is a prognostic marker for better outcome after cytotoxic chemotherapy, and that inhibition of these genes can sensitize KIF14 and TLN1 overexpressing TNBC cells to therapeutic intervention.

Figure 4. Functional consequences of KIF14 and TLN1 knockdown in MDA-MB-231, Hs478T, and HCC38 cells by (A) normal proliferation rates with no drug treatment, (B) proliferation rates when cells are treated with low dose docetaxel. Crystal violet stains of representative images from day 4 after docetaxel treatment. Data represent average of triplicates ± SEM. Data are representative of at least 2 independent experiments. C, representative mammary fat pad xenografts after treatment with docetaxel. Paired mammary fat pads are from contralateral sides of the same mice. Scale bar, 0.5 cm. D, summarizes average tumor volume ± SD for each group treated with docetaxel, pooled from 2 independent experiments.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: S.M. Singel, L. Lum, J.W. Shay
Development of methodology: S.M. Singel, J.W. Shay
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.M. Singel
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.M. Singel, K. Batten, L. Lum, J.W. Shay
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Figure 5. RNAi of various known protein–protein interaction partners of KIF14 and TLN1 in control MDA-MB-231 cells (A) and stably knocked down KIF14 and TLN1 MDA-MB-231 cells (B) and their effects on cell viability when docetaxel is given at 1 nmol/L for 3 days. Data represent average of triplicates ± SEM. Data are representative of at least 2 independent experiments. C, in vitro RIP2 kinase activity using PP2 and SB203580 as inhibitors. D, relative viability when MDA-MB-231 cells are treated with 1 nmol/L docetaxel with or without IC50 of PP2 and SB203580 (see text) for 3 days. Data represent average of triplicates ± SEM. Data are representative of at least 2 independent experiments.


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