Hsp27 Inhibition with OGX-427 Sensitizes Non–Small Cell Lung Cancer Cells to Erlotinib and Chemotherapy

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Abstract

Non–small cell lung cancer (NSCLC) is the most frequent cause of death from cancer worldwide. Despite the availability of active chemotherapy regimens and EGFR tyrosine kinase inhibitors, all advanced patients develop recurrent disease after first-line therapy. Although Hsp27 is a stress-induced chaperone that promotes acquired resistance in several cancers, its relationship to treatment resistance in NSCLC has not been defined. Understanding adaptive responses of acquired resistance will help guide new strategies to control NSCLC. Hsp27 levels were evaluated in an HCC827 erlotinib-resistant–derived cell line (HCC-827 Resistant), and sensitivity to erlotinib was examined in Hsp27-overexpressing A549 cells. The role of Hsp27 in both erlotinib and cytotoxic treatment resistance was evaluated in HCC-827 and A549 NSCLC cells using the Hsp27 antisense drug OGX-427. The effect of OGX-427 in combination with erlotinib was also assessed in mice bearing A549 xenografts. Hsp27 is induced by erlotinib and protects NSCLC cells from treatment-induced apoptosis, whereas OGX-427 sensitizes NSCLC cells to erlotinib. Interestingly, increased resistance to erlotinib was observed when Hsp27 was increased either in HCC827 erlotinib-resistant or overexpressing A549 cells. Combining OGX-427 with erlotinib significantly enhanced antitumor effects in vitro and delayed A549 xenograft growth in vivo. OGX-427 also significantly enhanced the activity of cytotoxic drugs used for NSCLC. These data indicate that treatment-induced Hsp27 contributes to the development of resistance, and provides preclinical proof-of-principle that inhibition of stress adaptive pathways mediated by Hsp27 enhances the activity of erlotinib and chemotherapeutics. Mol Cancer Ther; 14(5); 1–10. © 2015 AACR.

Introduction

Lung cancer is the most prevalent cancer and the leading cause of cancer-related death worldwide (1). In North America and Europe, it is the third most common cause of mortality for men and women and is increasingly observed in nonsmokers. Non–small cell lung cancer (NSCLC), with a 5-year survival rate of only 15%, accounts for approximately 80% of all lung cancers. Platinum-based doublets with gemcitabine or pemetrexed remain the mainstream of first-line therapy for advanced NSCLC with modest improvement in survival and quality of life, and are superior to single-agent therapy or three drug combinations (2). EGFR tyrosine kinase inhibitors (TKI), including afatinib, erlotinib, and gefitinib, are also approved and particularly active in patients with EGFR activating mutations or overexpression (3). Despite the active agents, virtually all patients with advanced NSCLC develop recurrent disease after first-line therapy and long-term survival rates remain low in these patients. Understanding adaptive responses that support acquired resistance are needed to develop new strategies to control NSCLC.

The EGFR is a member of the ErbB receptor tyrosine kinase family, a transmembrane protein with an intracellular and a tyrosine kinase domain that self-phosphorylates upon receptor dimerization. Phosphorylation triggers a cascade of intracellular events, including activation of the PI3K/Akt and the MAPK/RAS pathways, leading to cell survival and proliferation (4). Many solid tumors have constitutively activated EGFR, either by overexpression or by mutation, which leads to excessive signaling. Despite good initial responses to EGFR TKIs, most patients develop resistance and eventually die of metastatic disease. Resistance is often associated with the development of EGFR mutations that interfere with binding to the inhibitors to maintain EGFR activity (5), which can be partially overcome by increasing TKI potency or by cotargeting other pathways upregulated during treatment. For example, there is evidence that cotargeting Hsp90, a molecular chaperone known to interact with EGFR, can increase EGFR molecular degradation even in the presence of mutations (6).

Other heat shock chaperones, such as Hsp27 (Hspb1), play a pivotal role in stress responses to treatment. Hsp27 is a stress-induced ATP-independent molecular chaperone that is transcriptionally regulated by HSF-1, HIF-1α, and other factors influenced by cell type and cell context (7, 8). In addition, Hsp27 oligomerizes and self-associates in a phosphorylation-dependent...
manner regulated by AKT and MAPK signaling pathways (9). Hsp27 is upregulated in cisplatin-resistant ovarian cancer cells as well as taxane-resistant prostate cancer cells (10, 11), and shown to be highly expressed and associated with poor prognosis in many cancers. Hsp27 plays a crucial role in protein homeostasis, in stabilizing unfolding proteins, as well as the actin-cytoskeleton. Hsp27 promotes survival by binding to both cytochrome c and caspase-3 to inhibit intrinsic apoptotic pathway activation, and facilitates epithelial–mesenchymal transition and expression of metastatic genes (10, 12–14). Hsp27 is a difficult protein to target because, unlike Hsp90, it lacks an active site or ATP-binding pocket. Second-generation antisense technology enables targeted inhibition of "undruggable" targets like Hsp27 by downregulating gene expression at the mRNA level. The antisense inhibitor of Hsp27, OGX-427 (OncoGenex Pharmaceuticals) potently reduces Hsp27 levels with reported activity in preclinical cancer models (15–17) and tolerability as single agent and in combination with docetaxel chemotherapy in phase I studies. A phase II clinical trial of OGX-427 in castrate-resistant prostate cancer reported single-agent activity, and OGX-427 is currently in multicenter phase II clinical trials in metastatic CRPC, NSCLC, and bladder cancer (ClinicalTrials.gov, NCT01454089, NCT01829113, and NCT01120470).

In this study, we show that Hsp27 is expressed in nearly 70% to 80% of NSCLC patients and that erlotinib or chemotherapy increases levels of both total and phosphorylated Hsp27 via activation of HSF-1. Hsp27 overexpression limited the activity of erlotinib and cytotoxic agents, whereas Hsp27 inhibition with OGX-427 synergistically increased erlotinib-induced apoptosis and inhibition of A549 and HCC827 lung cancer cell growth in vitro and in vivo. These results illustrate that cotargeting treatment-induced adaptive responses mediated by Hsp27 can sensitize lung cancer cells to EGFR TKIs and cytotoxic therapies.

Materials and Methods

Tumor tissue arrays

Six hundred and nine cases of primary NSCLCs with follow-up data diagnosed between 1978 and 2002 were identified from the archives of St Paul’s Hospital, Vancouver, British Columbia. The paraffin-embedded tissue blocks were used to construct a duplicate core Tissue microarray (TMA). After review of the tissue cores, 588 cases, consisting of NSCLC met the criteria for inclusion in this study. Carcinoids, atypical carcinoids, large cell neuroendocrine carcinoma, and metastatic tumors were all excluded. Hematoxylin and eosin (H&E)-stained sections were reviewed and subclassified as follows: 243 adenocarcinoma, 272 squamous cell carcinoma (SCC), 35 large cell carcinoma (LCC), 32 non-small cell carcinoma, and six other (carcinoma, giant cell carcinoma).

IHC

IHC staining was conducted by Ventana autostainer model Discover XT (Ventana Medical System) with enzyme-labeled biotin streptavidin system and solvent-resistant DAB Map kit by using mouse monoclonal HSP27 antibody from Novoceastra now Leica Biosystems Newcastle Ltd and polyclonal phosphor-HSP27 (Ser82) antibody from Cell Signaling Technology. To ensure specificity of hsp27 and phsp27 antibodies, small cell lung carcinoma and benign prostate gland basal cells tissues were included as negative and positive controls, respectively (Supplementary Fig. S1).

Lung cancer cell lines and reagents

A549 (EGFR wild-type but kRAS mutated: G12S) and HCC827 (EGFR mutated: delE746-A750) cells were purchased from the ATCC and authenticated with short tandem repeat (STR) profile analysis in Jan 2013 by Genetic Resources Core Facility at John Hopkins. Cells were maintained in RPMI-1640 media (Invitrogen Life Technologies) containing 5% FBS (Invitrogen Life Technologies). Erlotinib was purchased from LC Laboratories. A549 cell lines overexpressing Hsp27 (A549Hsp27) were developed by stably transfecting cells with an Hsp27 vector by lentivirus transfection as previously described (8). HCC827_Hsp27 resistant cell lines were developed by maintaining HCC827 parental in 500 μM Leptomycin for 3 months. Genomic sequencing was done to confirm cell line identity. Antibodies: anti-HSF-1 from Santa Cruz Biotechnology; anti-Hsp27 from StressGen; anti-phospho-EGFR (Tyr1068), anti-EGFR, anti-cleaved PARP, anti-phospho-Hsp27 (Ser82), anti-cleaved caspase-3, anti-caspase-3, anti-phospho-ERK and anti-phospho-Akt (Ser423) were purchased from Cell Signaling Technology; and anti-β-actin from Sigma-Aldrich. Paclitaxel was purchased from Sigma Chemical Co. Gemcitabine (Lilly Pharmaceuticals), pemetrexed (Lilly Pharmaceuticals), and cis-platinum (Hospira) were obtained from the B.C. Cancer Agency.

ASO transfection

Cells were transfected with antisense as described previously (18, 19). Hsp27 ASO and scrambled (ScrB) control oligonucleotide sequences were manufactured by ISIS Pharmaceuticals (Carlsbad) and supplied by OncoGenex Technologies. The sequence of OGX-427 corresponds to the human Hsp27 translation initiation site (5’-GGACGCGGCGCTCGGTCA-3’). The sequence of heat shock factor 1 (HSF-1) siRNA corresponds to the human HSF-1 site (sc-35611; Santa Cruz Biotechnology). A scrambled siRNA (5’-CACCGCUUAGAACAGUUIUCAU-3’; Dharmacon) was used as a control for RNAi experiments. Transfections were performed by using Oligofectamine (Invitrogen), as previously described (20).

Western blot analysis

Total proteins were extracted using RIPA buffer (50 mmol/L Tris, pH 7.2, 1% NP-40, 0.1% deoxycholate, 0.1% SDS, 100 mmol/L NaCl, Roche complete protease inhibitor cocktail) and centrifuged to remove the insoluble material. Western blot analysis was performed as described previously (19).

Quantitative reverse-transcriptase PCR

RNA extraction and reverse-transcriptase PCR (RT-PCR) were carried out as described previously (21). Real-time monitoring of PCR amplification of cDNA was carried out using the following primer pairs and probes: Hsp27 (Hs03044127_g1), HSF1 (Hs00232134_m1), and GAPDH (Hs03929097_g1; Applied Biosystems) on the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) with a TaqMan Gene Expression Master Mix (Applied Biosystems). Target gene expression was normalized to GAPDH levels in the respective samples as an internal control. The results are representative of at least three independent experiments.

In vitro cell growth assay

Cells were plated in 12-well plates, and transfected with siRNA for 1 day or ASO for 2 days as indicated followed by erlotinib treatment at various concentrations for 48 hours. Cell viability
was then assessed using crystal violet assay as described previously (22). Absorbance was determined with a micro-culture plate reader (Becton Dickinson Labware) at 560 nm. Changes in cell viability were calculated as percentage relative to the vehicletreated cells. Each assay was performed in triplicate. The combination index (CI) was evaluated using CalcuSyn dose–effect analysis software (Biosoft) as described previously (21). CI was calculated at ED50, ED75, and ED90.

Cell-cycle analysis

Cell-cycle populations were analyzed by propidium iodide–staining using flow cytometry as previously described (18).

Animal manipulation and assessment of in vivo tumor growth

For in vivo xenograft studies, 6 × 10^6 A549 cells were inoculated s.c. in the flank of 6- to 8-week-old male athymic nude mice (Harlan Sprague Dawley, Inc.) via a 27-gauge needle under isoflurane anesthesia. When tumors reached 100 mm³, mice were randomly selected for treatment with 50 mg/kg erlotinib (formulated in 0.5% methylcellulose + 0.1% Tween-80) orally administered once daily for 5 days per week and 15 mg/kg OGX-427 or ScrB injected i.p. once daily for 7 days and three times per week thereafter. Each experimental group consisted of 8 mice. Tumor volume measurements were performed once weekly and calculated by the formula length × width × depth × 0.5236. Data points were expressed as average tumor volume ± SEM. All animal procedures were performed according to the guidelines of the Canadian Council on Animal Care and with appropriate institutional certification.

Luciferase assay

HCC827 and A549 cells were transfected with HSE luciferase plasmid and pRL-TK as internal control using lipofectin reagent (Invitrogen) followed by erlotinib treatment for 24 hours at the indicated concentrations. The luciferase activity was measured using a dual-luciferase reporter assay system (Promega) and a microplate luminometer (Tecan). The results are representative of three independent experiments.

Statistical analysis

Differences between the two groups were compared using Student t test and Mann–Whitney U test. All statistical calculations were performed using Statview 5.0 software (Abacus Concepts, Inc.), and P values < 0.05 were considered significant.

Results

Hsp27 and phospho-Hsp27 are highly expressed in human NSCLC

Hsp27 has been reported to be highly and uniformly expressed in several cancers (23–27). To assess the distribution of Hsp27...
and phospho-Hsp27 expression in human NSCLC, we performed immunostaining of human NSCLC spotted on TMA. Positive Hsp27 staining was noted in 176 of 214 SCCs (82.2%), 139 of 195 adenocarcinomas (71.3%), and 21 of 31 LCCs (67.7%; Fig. 1A). Positive phospho-Hsp27 staining was observed in 184 of 212 SCCs (86.8%), 147 of 184 adenocarcinomas (79.9%), and 27 of 31 LCCs (87.1%; Fig. 1B). These data suggest that both Hsp27 and phospho-Hsp27 are frequently expressed in NSCLC.

Erlotinib induces Hsp27 expression in A549 and HCC827 cells via HSF-1 activation

To evaluate the effect of erlotinib on Hsp27 expression and phosphorylation, we used erlotinib-resistant A549 (IC25 = 4.2 μmol/L) and erlotinib-sensitive HCC827 NSCLC cell lines (IC25 = 8 nmol/L). Erlotinib caused a clear decrease of EGFR phosphorylation associated with a time- (Fig. 2A) and dose- (Supplementary Fig. S2)-dependent increase of total Hsp27 and phospho-Hsp27 with a parallel increase of apoptosis as detected by cleaved PARP. Because HSF-1 is a key regulator of Hsp27 expression, we evaluated the effect of erlotinib on HSF-1 activity.

Erlotinib led to increased HSF-1 and Hsp27 both at the protein (Fig. 2B, left) and mRNA levels in both A549 and HCC827 cell lines (Fig. 2B, right), together with an increase of HSF-1 activity as measured by HSE transactivation assay (Supplementary Fig. S3). Moreover, silencing HSF-1 using siRNA abrogated erlotinib-induced upregulation of Hsp27 (Fig. 2C). Hsp27 can be
phosphorylated by p90Rsk downstream of pAkt and pErk (18), two pathways downstream of pEGFR, and therefore inhibited by erlotinib (data not shown). Although levels of pAkt and pErk do not increase after erlotinib, increase of pHsp27 reflects increased HSF-1 mediated transcription of total Hsp27. Collectively these data indicate that stress-induced activation of HSF-1 by erlotinib leads to increased total Hsp27 levels and a consequent increase in p-Hsp27. This finding, in addition to the positive staining in TMA samples from a variety of NSCLC, implicates Hsp27 in erlotinib resistance and highlights it is a therapeutic target in combination therapy with EGFR TKI.

Hsp27 overexpression reduces erlotinib-induced cell death

We previously reported that Hsp27 overexpression promotes cell survival and confers resistance to paclitaxel in prostate and bladder cancer cell lines (10, 28). Here, we investigated effects of Hsp27 overexpression on A549 cell growth in the presence of erlotinib by comparing cell viability of a stably transfected A549 cell line (A549Hsp27) to A549Empty controls after increasing doses of erlotinib. Cell viability curves in Fig. 3A confirm that A549Hsp27 cells are more resistant to erlotinib compared with control A549Empty cells. Moreover, Western blot analysis (Fig. 3B) indicates reduced PARP cleavage at 10 and 20 μmol/L erlotinib in the A549Hsp27 versus A549Empty cell lines confirming that overexpression of Hsp27 protects lung cancer cells from erlotinib-induced apoptosis.

To better evaluate the role of Hsp27 in erlotinib resistance, we established an HCC827 resistant cell line (IC25 = 1 μmol/L) by maintaining HCC827 parental cells in 500 nmol/L erlotinib for 3 months (Fig. 3C). Hsp27 levels were measured by Western blot analyses (insert). Cell viability was determined after increasing erlotinib doses 72 hours after treatment. D, cleaved PARP, Hsp27, p-Hsp27, p-EGFR, total EGFR, and vinculin as loading control were detected by Western blot analyses for resistant cells at 500 nmol/L erlotinib and parental cells at 0 and 72 hours after 200 nmol/L erlotinib.

OGX-427 sensitizes both HCC827 and A549 to erlotinib

Because the preceding results link Hsp27 to erlotinib resistance, we hypothesized that cotargeting this chaperone in combination with EGFR TKI will increase erlotinib efficacy. Cell viability was assessed in A549 and HCC 827 cells after erlotinib or OGX-427 mono- and combination-therapy. OGX-427 reduces Hsp27 protein expression (Supplementary Fig. S4), and when used in combination with erlotinib, leads to increased cell death compared with single-agent therapy (Fig. 4A). CI, calculated at a ratio OGX-

Figure 3.
Hsp27 overexpression induces erlotinib resistance. A, A549 cells were stably transfected either with an empty vector (A549Empty) or with a vector containing wild-type Hsp27 (A549Hsp27). Cell viability was determined after increasing erlotinib doses 72 hours after treatment. B, p-EGFR, total EGFR, Hsp27, cleaved PARP, and β-actin as loading control were detected by Western blot analysis in both cell lines after 72 hours erlotinib treatment. C, HCC827 erlotinib-resistant cell lines (HCC827resistant) were established maintaining HCC827 parental cells in 500 nmol/L erlotinib for 3 months. Hsp27 levels were measured by Western blot analyses (insert). Cell viability was determined after increasing erlotinib doses 72 hours after treatment. D, cleaved PARP, Hsp27, p-Hsp27, p-EGFR, total EGFR, and vinculin as loading control were detected by Western blot analyses for resistant cells at 500 nmol/L erlotinib and parental cells at 0 and 72 hours after 200 nmol/L erlotinib.
OGX-427 sensitizes both HCC827 and A549 to erlotinib. A, A549 and HCC827 cells were transfected with 50 nmol/L OGX-427 or ScrB for 2 days and treated with the indicated concentration of erlotinib for 48 hours after the second transfection. Cell viability was determined by crystal violet assay 48 hours after erlotinib treatment. B, combination index analysis for erlotinib and OGX-427 treatment in A549 and HCC827 cell lines was performed according to the Chou and Talalay method (49). C, p-EGFR, total EGFR, total Hsp27, cleaved PARP, cleaved caspase-3, caspase-3 and β-actin, as loading control, were detected by Western blot analysis. Inhibition of erlotinib-induced Hsp27 enhances cell apoptosis in both A549 and HCC827. D, cells were transfected with 50 nmol/L OGX-427 or ScrB for 2 days and treated with erlotinib (A549 at 10 μmol/L and HCC827 at 2.5 nmol/L) for 48 hours after the second transfection. Flow-cytometric analysis of A549 and HCC827 shows an increase in sub-G0 and decrease in G0–G1. Bars, SD. **, differs from control (P < 0.01).

Combining OGX-427 with erlotinib enhances tumor growth inhibition in vivo

The combination effect of OGX-427 and erlotinib was next evaluated in an in vivo model using A549 xenografts. Tumor-bearing mice were randomly assigned to groups treated with ScrB-diluent, ScrB+erlotinib, OGX-427+diluents, or OGX-427+erlotinib when A549 tumors reached 100 mm³. Mean tumor volume were similar in all groups at baseline and all treatments were performed for 7 weeks. Consistent with in vitro findings, OGX-427 was able to reduce Hsp27 protein level, and the combination therapy of OGX-427 plus erlotinib significantly reduced tumor growth rates (Fig. 5A–C) compared with all other groups (P = 0.0002, 0.03, and 0.01 against ScrB+diluent, ScrB+erlotinib, and OGX-427+diluent, respectively). Moreover, OGX-427 plus erlotinib-treated tumors had higher apoptotic rates as shown by increased TUNEL staining compared with the other groups (Fig. 5D). These studies indicated that this combination treatment significantly delays growth and increases apoptotic rates of A549 xenografts.

Combination therapy of OGX-427 plus cytotoxic agents increases tumor cell death

Hsp27 is known to be induced by cytotoxic agents in many cancers, and can confer resistance to cytotoxics (29, 30). We next evaluated effects of OGX-427 in combination with pemetrexed, a first-line therapy for the treatment of NSCLC. Pemetrexed increased total and phospho-Hsp27 levels in a time- and dose-dependent manner in A549 cells (Fig. 6A). Combined OGX-427 plus pemetrexed reduced A549 cell viability compared to cells...
treated with either drug alone and the CI analysis demonstrated synergistic effects with combination therapy (Fig. 6B). Similar results were observed in A549 cells after OGX-427 treatment in combination with cisplatin, paclitaxel, and gemcitabine, all cytotoxic agents used clinically in patients with NSCLC (Fig. 6C). Western blot analysis in A549 cells indicated marked increase in apoptosis as measured by cleaved PARP with OGX-427 combination regimens compared with single agents (Fig. 6D), supporting inhibition of Hsp27 as a cotargeting strategy with cytotoxic agents for the treatment of NSCLC.

Discussion

Anticancer treatments induce stress responses that inhibit apoptosis and promote emergence of an acquired treatment-resistant phenotype. Molecular chaperones play key roles in these stress responses by regulating many prosurvival signaling and transcriptional networks. Two strategies have recently emerged that are revolutionizing the treatment landscape in cancer: the use of molecular-targeted agents and the selective inhibition of adaptive responses. In this work, we focus on the role of treatment-induced Hsp27 expression and treatment response in NSCLC, the most common type of lung cancer and the largest cause of cancer-related death worldwide, where chemotherapy or EGFR-TKIs are first-line treatment. EGFR belongs to a family of membrane-bound receptors with a tyrosine kinase domain that, when phosphorylated, activates a number of downstream prosurvival pathways, including the PI3K/Akt and RAS/MAPK pathways (4). Some patients, for whom EGFR-TKI is recommended, have a mutated EGFR that is constitutively activated. For these individuals, the use of EGFR-TKI is recommended over chemotherapy as first line of treatment (31, 32). Although EGFR TKIs have been shown to prolong life, most patients acquire resistance with a median progression-free survival of 10 months (33) and eventually die of metastatic disease.

Hsp27 is a stress-induced molecular chaperone that regulates many prosurvival cellular functions. Many solid tumors, including bladder, prostate, breast and colorectal cancers, express high...
Hsp27 levels that correlate with poor prognosis. Hsp27 maintains protein homeostasis by stabilizing protein conformation or facilitating proapoptotic degradation (34, 35), inhibits apoptosis by interacting with proapoptotic molecules such as cytochrome c (36) or pro-caspase 3 (37), and enhances oncogenic signaling pathways implicated in cancer progression and resistance to chemo- and hormonal therapy (29, 39).

Because Hsp27 functions as a regulatory ‘hub’ in multiple regulatory pathways, its inhibition will simultaneously suppress many pathways implicated in cancer progression and resistance to hormone- and chemotherapies. Hsp27 acts through an ATP-independent mechanism and is therefore not amenable to inhibition by 17-AAG–derived small molecules. Strategies to inhibit Hsp27 at the mRNA level are an alternative approach to inhibiting “non-druggable” targets; indeed, siRNA or antisense oligonucleotides targeting Hsp27 suppresses proliferation and EMT in prostate (10, 39–41) and breast cancer cells (42). Hsp27 silencing also decreases clonogenic survival and induces cell senescence in HCT116 human colon cancer (43). Hsp27 silencing chemosensitizes SK-BR-3 HR breast cancer cells to Herceptin (44), A549 lung cancer cells to 17-AAG (45), MUC-3 bladder cancer cells and PC-3 prostate cancer cells to paclitaxel (28, 40).

In this study, we show that Hsp27 and phospho-Hsp27 are commonly expressed in a TMA of 440 SCCs, adenocarcinomas, and LCCs of the lung. We also show that treatment stress with erlotinib or chemotheraphy increases Hsp27 expression via the transcription factor HSF-1 and that high levels of Hsp27 induce resistance to erlotinib. These findings implicate a role for Hsp27 in cell survival of NSCLC under EGFR inhibition, and support testing cotargeting Hsp27 with erlotinib or other chemotherapeutics for the treatment of NSCLC. Inhibition of treatment-induced Hsp27 using OGX-427 synergistically enhanced inhibitory effects of erlotinib on cell proliferation and cell viability in NSCLC cell lines in vitro. Similarly, combination therapy with erlotinib plus OGX-427 significantly inhibited A549 tumor growth in vivo compared with monotherapy. Consistent with these results, Hsp27 is upregulated by HSF-1 in c-MET addicted gastric cell lines when c-MET is inhibited, and functions to limit the activity of c-MET-targeted therapies (46). In this gastric cell line, the activation of K-RAS by transfection of G12V K-RAS downstream of c-MET reverses the effect of c-MET inhibitors on Hsp27 upregulation (46). We observed an increase of Hsp27 even in A549 lung cancer cells that have a constitutively activated MAPK pathway due to a K-RAS activating mutation (G12S), demonstrating that in A549 lung cancer cells, Hsp27 upregulation via HSF-1 is a response to EGFR inhibition even when the MAPK pathway is constitutively active.

Because platinum-based doublets with gemcitabine or pemetrexed are the first line of treatment for NSCLC in patients that do not carry an EGFR mutation, we also evaluated effects of...
Hsp27 inhibition as cotreatment with these cytotoxics. OGX-427 synergistically enhanced growth-inhibitory effects of pemetrexed, gemcitabine, cisplatin, and paclitaxel in A549 cells by enhancing treatment-induced apoptosis. These results provide a framework for building new drug combinations based on mechanism-based interventions to overcome drug resistance, and preclinical proof-of-principle supporting clinical trials of OGX-427 combination regimens for lung and other cancers. OGX-427 has completed single-agent and docetaxel-combination dose-escalation phase I trials in prostate, bladder, breast, and lung cancer (47). OGX-427 is well tolerated, with the majority of the adverse events reported being grade 1 or grade 2, although a symptom complex of rigors, pruritus, and erythema during or shortly after infusion of drug has required steroid prophylaxis and/or treatment in some patients at higher doses. Preliminary data from a randomized phase II trial of OGX-427 plus prednisone in castration-resistant prostate cancer reported a PSA decline $\geq$ 30% in 55% of patients and an acceptable safety profile (48). Several randomized phase II trials of OGX-427 in combination with chemotherapy are actively accruing, including untreated stage IV NSCLC comparing carboplatin plus pemetrexed with or without OGX-427 (SPRUCE, clinicaltrials.gov NCT01829113).

Disclosure of Potential Conflicts of Interest
M.E. Gleave reports receiving commercial research support from, has ownership interest (including patents) in, and is a consultant/advisory board member for OncoGenex. No potential conflicts of interest were disclosed by the other authors.

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References

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