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Reduced expression of *EI24* confers resistance to gefitinib through IGF-1R signaling in PC9 NSCLC cells

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ABSTRACT

Objectives: Lung cancer is the commonly diagnosed cancer and is the leading cause of cancer-related mortality worldwide. The most prevalent form of lung cancer is NSCLC, comprising 80% of all lung cancer cases, and epidermal growth factor receptor (*EGFR*) is frequently mutated in NSCLC. *EI24* is a p53-responsive gene and plays an important role in tumor suppression. In the current study, we found that reduced expression of *EI24* conferred resistance to EGFR-tyrosine-kinase inhibitor (TKI) in NSCLC cells.
Materials and methods: The correlation between *EI24* expression and EGFR-TKI drug resistance in EGFR-driven tumors were determined from microarray datasets. The phospho-protein expression profiles of receptor tyrosine kinases and protein kinases were examined using antibody arrays method in PC9 cells expressing shRNAs targeting *EI24* and gefitinib-resistant PC9-GR cells expressing exogenous *EI24*.
Results and conclusions: The EGFR-TKI resistant clones had reduced expression of *EI24* mRNA compared to the sensitive clones, and *EI24* knockdown rendered sensitive cells resistant to EGFR-TKI. Receptor tyrosine kinase screening revealed the involvement of a kinase switch in *EI24*-mediated regulation of drug sensitivity. We found that *EI24* modulates the insulin growth factor-1 receptor (IGF-1R) pathway through the induction of IGF-1. Combination treatment with EGFR and IGF-1R inhibitors significantly reduced the viability of *EI24* knockdown-induced resistant cell lines compared to single-agent treatments. We also showed that low *EI24* and high *IGF-1R* expressions in lung cancer patients were correlated with reduced overall survival. Taken together, these results suggest a potential role for *EI24* as a biomarker of drug resistance, and indicate that combination therapy with EGFR and IGF-1R inhibitors would be effective in NSCLC patients with low *EI24* expression.

1. Introduction

Lung cancer is the most commonly diagnosed cancer (1.82 million cases, 12.9% of total cancer diagnoses) and is the leading cause of cancer-related mortality worldwide, accounting for nearly 1.6 million (19.4%) deaths per year [1]. The most prevalent form of lung cancer is NSCLC, which comprises 80% of all lung cancer cases. The development of targeted therapy gave the marked advances to patients who harbor specific genetic alterations in this cancer. The most common genetic alterations in NSCLC are *KRAS* mutations, *EGFR* mutations, and the *ALK* rearrangement. *EGFR* mutations are found in approximately 10–28% of NSCLC cases and are most common in women and non-smokers in East Asian populations [2].

The majority of *EGFR* mutations occur within the kinase domain, leading to ligand-independent activation of EGFR signaling. Although these mutant proteins are clinically sensitive to EGFR-TKIs, this therapy is limited by acquired resistance after varying periods of time [3]. Recently, several groups have identified mechanisms of this resistance, including amplification of MET, activation of AXL, activation of EMT, and upregulation of IGF-1R signaling. The IGF-1R signaling pathway plays a central role in the

Abbreviations: NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; IGF-1R, insulin growth factor-1 receptor; RTK, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; AXL, anaplastic lymphoma kinase; EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition.

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regulation of cell proliferation and metabolism [4] and polymorphism and copy number amplification of IGF network components are associated with an increased risk for developing several cancers [5]. IGF-1R is expressed in most organs, and a higher level of circulating IGF-1 is associated with a higher risk for developing lung cancer [4]. Recently, antibodies against IGF-1R and small molecules that target IGF-1R kinase activity have been evaluated in clinical trials [6].

EI24, a p53 responsive pro-apoptotic factor, plays an important role in the suppression of cell growth and the activation of autophagy [7,8]. Furthermore, reduced expression of *EI24* is associated with the induction of EMT and tumor progression [9]. Loss of *EI24* contributes to etoposide resistance, suggesting that *EI24* status could be used as a prognostic marker for chemotherapy responsiveness [10]. In the current study, we examined the functional role of EI24 on EGFR-TKI-mediated cell viability. EGFR-TKI-resistant clones expressed less *EI24* than sensitive clones, and *EI24* knockdown in gefitinib-sensitive cells induced resistance to EGFR-TKI treatment. Moreover, combined inhibition of EGFR and IGF-1R in *EI24* knockdown cell lines reduced cell viability more effectively than single-agent treatments. We also found that low *EI24* and high *IGF-1R* expressions in lung cancer patients were correlated with reduced overall survival. From this study, we provide a rationale for using *EI24* as a biomarker for EGFR-TKI sensitivity and demonstrate that targeting both IGF-1R and EGFR could be an effective therapy for patients expressing low levels of *EI24*.

2. Materials and methods

2.1. Cell culture and generation of cell lines

The PC9 EGFR-mutant NSCLC cell line and its gefitinib-resistant derivative (PC9-GR) have been previously characterized [11] All cells were cultured in RPMI 1640 supplemented with 10% FBS. The human *EI24*-overexpressing and control cell lines were generated by transfection with a plasmid containing FLAG-tagged *EI24* and the corresponding empty vector (pcDNA3.1), respectively. The human *EI24* stable knockdown cell lines were generated as previously described [9]. The shRNA sequences are as follows:

#TRCN0000159876 (CCGGGCCATTTGGTTTCAGGATATACTCGAGTATATCCTGAAACCAAATGGCTTTTTTG) and #TRCN0000160559 (CCGGCAAAGCATATCTCTTCCAGTTCTCGAGAACTGGAAGAGATATGCTTTGTTTTTTG).

2.2. Plasmids and reagents

The human *EI24* construct was used as previously described [9]. The shRNAs against human *EI24* were purchased from Sigma–Aldrich. Gefitinib was obtained from Cayman Chemical. PQ-401 and AG-1024 were purchased from Selleck Chemicals. Human IGF-1 and recombinant IGF-1 were purchased from R&D Systems.

2.3. Immunoblotting and antibodies

Protein extraction was performed as previously described [12]. Exposures were acquired using a LAS-3000 Imager (Fujifilm). The following antibodies were used: anti-phospho-EGFR, anti-EGFR, anti-phospho-IRS-1, anti-IRS-1, anti-AKT, anti-phospho-AKT, anti-ERK1/2, and anti-phospho-ERK1/2. All antibodies were purchased from Cell Signaling Technology.

2.4. RNA isolation and real-time qPCR

Total RNA was prepared using Trizol (Invitrogen). One microgram of total RNA was reverse-transcribed to cDNA using the Superscript III First-Strand Synthesis System with oligo-dT primers (Invitrogen). Real-time qPCR was performed as previously described [9]. The primer sequences used were previously described [9,13], or were obtained from OriGene (www.origene.com).

2.5. Cell viability assay

Cells were seeded at a density of 3×10^4 cells per well in a 96-well culture plate and incubated for 24 h. After cells were exposed to drugs for 48 h, 0.4 mg/ml of MTT was added to the medium in the well. After incubating for 4 h at 37 °C, formazan crystals in viable cells were solubilized with 100 μl of dimethyl sulfoxide (DMSO). The optical density of the formazan product was read at 590 nm on a microplate reader (Bio-Rad). All experiments were conducted in triplicate.

2.6. IGF-1 quantification

Supernatants from cultured cells were collected and centrifuged for 5 min to remove dead cells and cellular debris. An enzyme-linked immunosorbent assay (ELISA) for IGF-1 was conducted by using a kit according to the manufacturer's instructions (#DG100, R&D Systems).

2.7. Human RTK and kinase array

Proteome Profiler Human Phospho-RTK Antibody Array and Human Phospho-Kinase Antibody Array kits (R&D Systems) were used according to the manufacturer's instructions. Briefly, fresh proteins were incubated overnight at 4 °C with nitrocellulose membranes dotted with duplicate antibody spots for 49 RTKs (RTK array) or 43 kinases and 2 related total proteins (kinase array), with each containing reference controls. The bound proteins were detected with 50 μl of mouse anti-phospho-tyrosine antibody conjugated to horseradish peroxidase (HRP) and a chemiluminescence assay.

2.8. GEO2R analysis

Microarray datasets containing the gene expression values of EGFR-TKI-sensitive and EGFR-TKI-resistant clones were obtained from Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo). We selected GSE38121 [14], GSE38404 [15], and GSE37700 [16] datasets to evaluate *EI24* expression. The data were analyzed through the GEO2R interface (http://www.ncbi.nlm.nih.gov/geo/geo2r/) as previously described [17].

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism. Unpaired *t*-tests were used, unless otherwise stated. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Reduced EI24 expression in EGFR-TKI-resistant EGFR-driven lung tumors

To identify the possible role of EI24 in mediating EGFR-TKI resistance, publicly available GEO microarray datasets were analyzed to determine the correlation between *EI24* expression and EGFR-TKI drug resistance in EGFR-driven tumors. In the Zhang dataset [14], we found reduced *EI24* expression in erlotinib-resistant HCC827 clones compared to the sensitive HCC827 NSCLC cell line (Fig. 1A). Additionally, in the Cortot [15] and Ercan [16] datasets, we observed a marked decrease in *EI24* expression in both dacomitinib- and WZ4002- resistant clones compared to each

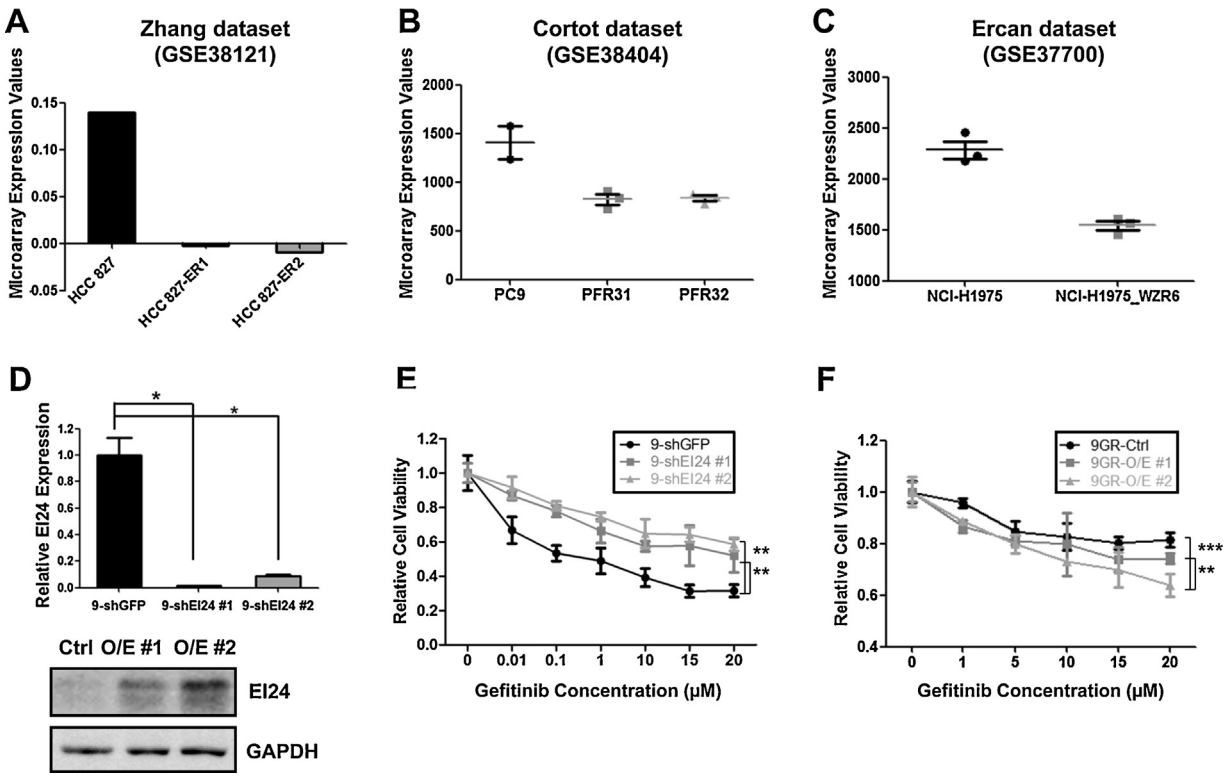

Fig. 1. Reduced expression of *EI24* in EGFR-TKI-resistant EGFR-driven lung tumors. (A) Bar graph showing *EI24* gene expression values from a microarray dataset in erlotinib-sensitive HCC827 cells and erlotinib-resistance clones HCC827-ER1 and HCC827-ER2. (B and C) Scatter plots showing *EI24* gene expression values from the indicated microarray dataset in drug-sensitive clones (PC9 and NCI-H1975) and drug-resistant clones (PFR31, PFR32, and NCI-H1975-WZR6). All *p*-values were <0.05 (D) Real-time qPCR analysis of *EI24* expression in PC9 cells infected with shRNA containing *GFP*-targeting sequence (9-sh*GFP*) or an *EI24*-targeting sequences (9-sh*EI24* #1 and #2). Data shown are the mean ± S.D., $^{*}p < 0.0005$. Immunoblot analysis of *EI24* expression in PC9-GR cells expressing either control vector (9GR-Ctrl) or exogenous *EI24* (9GR-O/E #1 and #2). (E and F) Cells were treated with the indicated concentrations of gefitinib for 48 h before cell viability was measured by the MTT assay. Error bars, S.D.; p-values were calculated using paired 2-tailed *t*-test. $^{**}p < 0.005$, $^{***}p < 0.05$.

parental clones, respectively (Fig. 1B and C). These data indicate that reduced expression of *EI24* in EGFR-TKI-sensitive cells might render them resistant to EGFR-TKIs, demonstrating that *EI24* may inhibit EGFR-TKI resistance.

3.2. *Reduced* EI24 *expression confers resistance to gefitinib in NSCLC cell lines*

To validate the role of *EI24* in EGFR-TKI resistance, we established PC9 cells expressing shRNAs targeting *EI24* (*EI24*-knockdown cells) and PC9-GR (gefitinib-resistant clone of PC9) cells expressing exogenous *EI24* (Fig. 1D). The *EI24*-knockdown PC9 cells displayed decreased sensitivity to gefitinib with a significant increase in cell viability at all gefitinib concentrations tested (Fig. 1E). Next, we tested whether the overexpression of *EI24* would alter gefitinib sensitivity in the gefitinib-resistant PC9-GR cell line. Interestingly, ectopic expression of *EI24* reduced cell viability up to 18% upon exposure to 20 μM gefitinib (Fig. 1F). These data demonstrate that reduced *EI24* expression renders NSCLC cells resistant to the EGFR-TKI gefitinib.

3.3. EI24 *knockdown drives gefitinib resistance through the activation of IGF-1R signaling*

To explore the molecular mechanism of *EI24*-mediated EGFR-TKI sensitivity, we examined the protein phosphorylation of EGFR, AKT, and ERK1/2 after gefitinib treatment in *EI24* knockdown (9-sh*EI24*) and control (9-sh*GFP*) cells. 9-sh*EI24* cells exhibited increased p-EGFR, p-AKT, and p-ERK1/2 expression compared to the control cells (Fig. 2A). Gefitinib significantly reduced the phosphorylation of EGFR in both cells, indicating that EI24-mediated alteration of drug sensitivity was not due to retention of activated EGFR signaling (Fig. 2A). Although gefitinib reduced AKT phosphorylation in the control cells, this was attenuated in 9-sh*EI24* cells. We also observed that the AKT phosphorylation was diminished by ectopic expression of *EI24* upon treatment with gefitinib in PC9-GR cells (Fig. 2B). These results indicate that signaling upstream of AKT might regulate *EI24* loss-mediated acquired drug resistance.

Recent studies have shown that other receptor tyrosine kinases (RTKs) participate in driving EGFR-independence in NSCLC cell lines that become resistant to EGFR-TKIs [18]. In this context, we investigated whether aberrant activation of other RTKs might be involved in *EI24* knockdown-mediated EGFR-TKI resistance. Phospho-RTK profiling of lysates from 9-sh*GFP* and 9-sh*EI24* cells revealed increased activation of the insulin-like growth factor-1 receptor (IGF-1R) and decreased activation of ERBB2 and ERBB3 by *EI24* knockdown (Fig. 2C). We also examined the changes of downstream targets by analyzing the phosphorylation profiles of kinases and their protein substrates upon *EI24* knockdown. The Phospho-kinase array showed significant increases in phosphorylation of proteins downstream of the IGF-1R pathway such as p-p38, p-AKT, and p-ERK1/2 with *EI24* knockdown (Fig. 2D). Moreover, we observed an inhibitory effect of *EI24* on the activation of p-IGF-1R, p-p38, p-AKT, and p-ERK1/2 in gefitinib-resistant PC9-GR cells (Fig. 2E and F). These data suggest that EI24 regulates survival signaling in NSCLC cells through the IGF-1R pathway.

To determine how *EI24* regulates the IGF-1R signaling pathway, we evaluated the expression of the IGF-1R activating ligand (IGF-1) in *EI24* knockdown PC9 and *EI24*-overexpressing PC9-GR cells.

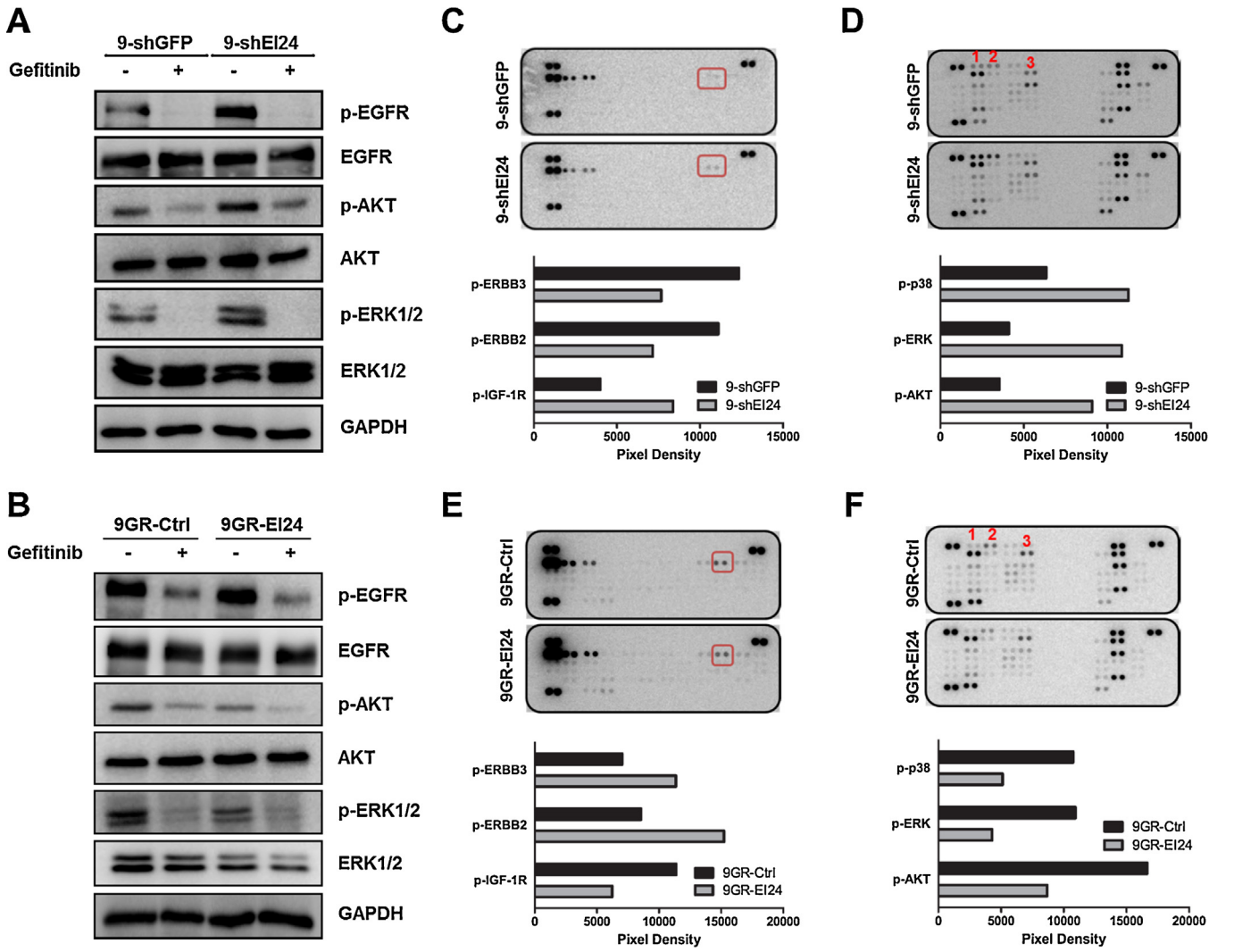

Fig. 2. Involvement of EI24 in IGF-1R signaling. (A and B) 9-sh*GFP*, 9-sh*EI24*, 9GR-Ctrl, and 9GR-*EI24* cells were left untreated or treated with gefitinib for 3–6 h before lysis for immunoblotting against the indicated proteins. (C–F) A phospho-RTK (C and E) and a phospho-kinase array (D and F) were performed with the lysates from *EI24* knockdown or *EI24*-overexpressing cells compared to each control cells. Representative blots are shown (1: p-p38, 2: p-ERK1/2, 3: p-AKT). Quantification of each protein was determined by measuring pixel density.

9-sh*EI24* cells contained approximately 2-fold higher IGF-1 mRNA compared to the control cells, while *EI24*-overexpressing PC9-GR cells exhibited decreased expression of IGF-1 mRNA (Fig. 3A and B). To determine the effect of EI24 on IGF-1 secretion, we measured IGF-1 protein in the culture medium using an ELISA. The amount of secreted IGF-1 protein was significantly increased in *EI24*-knockdown cells, and was reduced by exogenous *EI24* expression (Fig. 3C and D). Moreover, stimulation of PC9-GR control cells with IGF-1 resulted in increased expression of p-IRS, p-AKT, and p-ERK1/2, but this was diminished in *EI24*-overexpressing PC9-GR cells (Fig. 3E). These data demonstrate that EI24 modulates survival signaling in NSCLC cells through the IGF-1R pathway by regulating the expression of *IGF-1*.

3.4. *The EGFR and IGF-1R pathways exhibit combinatorial effects on* EI24*-mediated drug resistance*

Given the likely importance of IGF-1R activation in gefitinib resistance, we reasoned that EGFR-mutant cells with activated IGF-1R signaling following *EI24* knockdown could be inhibited by a combination of drugs against both EGFR and IGF-1R. Upon treatment with both EGFR and IGF-1R inhibitors, we found that combination treatments were more effective than single-agent treatment at reducing cell viability and clonogenic potential in PC9 *EI24*-knockdown cells (Fig. 4A–D). These results indicate that the efficacy of EGFR-TKI therapy can be improved by co-inhibition of the IGF-1R pathway in instances of *EI24*-mediated drug resistance.

Based on these data, we analyzed the correlation between the *EI24* expression level and the clinical outcomes for lung cancer patients by using the Kaplan–Meier Plotter, which can perform a meta-analysis of many microarray datasets from primary tumors of lung cancer patients [19]. We found that reduced expression of *EI24* was significantly correlated with reduced overall survival of lung cancer patients (Fig. 4E). We used the same approach to evaluate *IGF-1R* expression, and found a significant correlation between high IGF-1R expression and reduced overall survival (Fig. 4F). Taken together, these data suggest that low *EI24* and high IGF-1R expressions are relevant to the survival of lung cancer patients.

4. Discussion

Here, we show that *EI24* loss contributes to the resistance of EGFR-mutant NSCLC cells to gefitinib treatment through the upregulation of IGF-1R signaling. IGF-1R signaling involves several

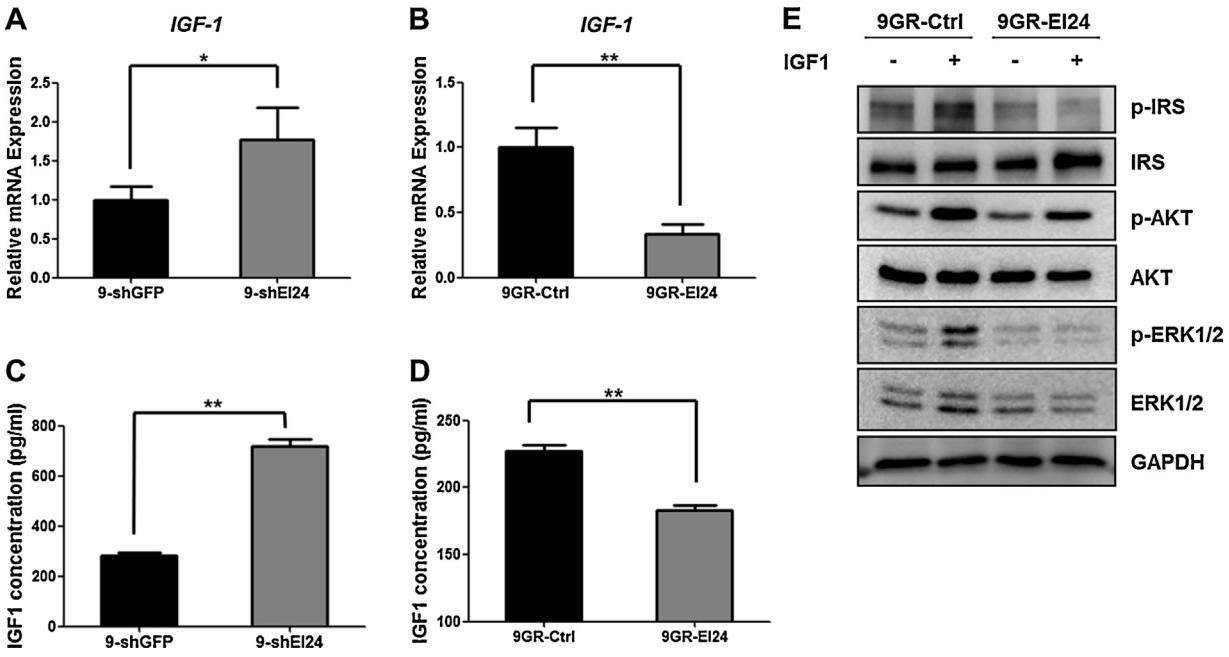

Fig. 3. EI24 modulates IGF-1R signaling through the regulation of IGF-1. (A and B) Relative mRNA expression levels of *IGF-1* in indicated cells were analyzed by real-time qPCR. Data shown are the mean ± S.D. of two independent experiments with each sample assayed in triplicate. *p*-Values were calculated by unpaired *t*-test. $^{*}p < 0.05$, $^{**}p < 0.005$. (C and D) The levels of secreted IGF-1 protein were measured by ELISA using supernatants from the indicated cell types. *p*-Values were calculated by unpaired *t*-test. $^{**}p < 0.005$. (E) 9GR-Ctrl and 9GR-*EI24* cells were left untreated or treated with IGF-1 (50 ng/ml) for 30 min before lysis for immunoblotting against the indicated proteins.

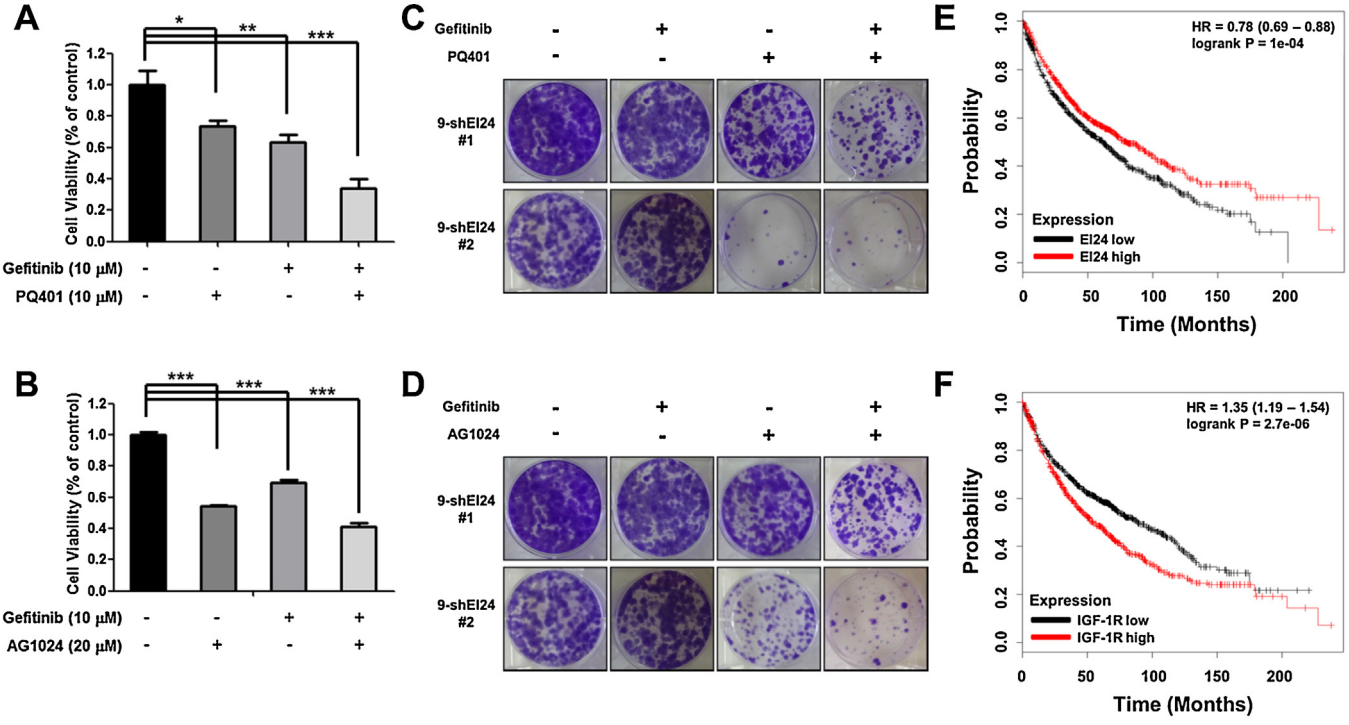

Fig. 4. IGF-1R is key determinant of the EI24-mediated gefitinib sensitivity. (A and B) *EI24*-knockdown cells were treated with the indicated concentrations of inhibitors for 24 h before cell viability was measured by the MTT assay. Error bars, S.D., *p*-values were calculated using unpaired *t*-test. $^{*}p < 0.01$, $^{**}p = 0.0003$, $^{***}p < 0.0001$. (C and D) *EI24*-knockdown cells were fixed and stained with crystal violet after 14 days treatment with concentrations of gefitinib (0.1 μM), PQ401 (5 μM), and AG1024 (20 μM). (E and F) Kaplan–Meier survival plots were obtained using Kaplan–Meier Plotter and display the probability of overall survival of lung cancer patients grouped according to *EI24* (208289_s_at, E) and *IGF-1R* (203627_at, F) expression. *p*-Values were calculated using the log-rank test.

feedback loops and is implicated in promoting oncogenic transformation, growth, and the survival of cancer cells [4,20]. Others have found that IGF-1R mediates resistance to anti-EGFR therapy through the continued activation of the PI3K-AKT pathway, and ablation of EGFR-dependent activation of the anti-apoptotic protein AKT is significantly associated with gefitinib sensitivity [21,22]. Additionally, a recent study utilizing combined treatment of gefitinib and IGF-1R inhibitors in NSCLC cell lines showed induction of apoptosis, inhibition of cell proliferation, and decreased expression of p-AKT [23]. In our study, AKT activation that persisted in

the presence of gefitinib in *EI24*-knockdown cells suggested the involvement of IGF-1R pathway. Moreover, *IGF-1R* expression and its activation have been linked to disease progression, increased resistance to radiotherapy, and poor prognosis [4,24,25]. We found that lung cancer patients with reduced expression of *EI24* and increased expression of IGF-1R have a poor prognosis, reinforcing the relevance of *EI24* in NSCLC. Further study will be necessary to determine which of these signaling pathways is most critical for EI24-mediated resistance to EGFR TKIs in EGFR-mutant NSCLC. Such studies may identify additional evidences for therapeutic intervention in the context of the acquisition of resistance to EGFR TKIs driven by EI24. On the basis of our data, we propose that inhibition of IGF-1R signaling may enhance responses to treatment with EGFR TKI-resistant *EGFR*-mutated NSCLC.

Recent studies have shown that EMT plays a role in acquired resistance to EGFR-TKIs, indicating that mesenchymal status is correlated to the resistance to these drugs in NSCLC cell lines and clinical samples [26–28]. Furthermore, it has been reported that phosphorylation of EGFR, ERBB2, and ERBB3 is attenuated in mesenchymal-like erlotinib-resistant cells, suggesting that EMT confers resistance by generating a switch in kinase pathway activation [29]. We found decreased ERBB2 and ERBB3 phosphorylation and increased IGF-1R phosphorylation in *EI24* knockdown NSCLC cells, suggesting the existence of an EMT-mediated kinase switch. We previously found that loss of *EI24* induces EMT [9], and PC9 cells acquire mesenchymal-like morphology upon *EI24* knockdown (data not shown). These results support previous data demonstrating the crosstalk between EMT and IGF-1R signaling in EGFR-TKI-resistant cell lines [30], and implicate EI24 as candidate molecule for studying the relationship of EMT and the IGF-1R pathway in drug sensitivity.

As a strong mediator of cell proliferation, differentiation, and a potent inhibitor of apoptosis, we focused on the induction of IGF-1 by EI24 knockdown. Since the action of IGF-1 is predominantly mediated through the IGF-1R [31], the regulation of IGF-1R activation by EI24 is critical for the EI24-mediated regulation of drug sensitivity in NSCLC cells. Recent study has identified possible interconnection between IGF-1 and NF-κB signaling in patients with chemotherapy resistance [32] and we found NF-κB regulation through autophagy by EI24 [9] and many NF-κB binding motifs at IGF-1 promoter region (data not shown). Along these lines, it is likely that EI24/NF-κB/IGF-1 axis would be an important signaling pathway to confer drug resistance though the more detailed investigations are needed on the molecular mechanism.

To confirm the clinical relevance of *EI24* expression, we evaluated the impact of *EI24* expression on overall survival in lung cancer patients. We found that low *EI24* and high *IGF-1R* expressions were correlated with poor survival in lung cancer patients. Our results suggest that combination treatment with EGFR and IGF-1R inhibitors could be more effective than single-agent treatments in EGFR-TKI resistant EGFR-mutant NSCLC patients who have low *EI24* expression level, highlighting as a new therapeutic strategy for effectively treating these patients.

Acknowledgments

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References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer (2014).
[2] W. Pao, J. Chmielecki, Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer, Nat. Rev. Cancer 10 (2010) 760–774.
[3] D. Jackman, W. Pao, G.J. Riely, J.A. Engelman, M.G. Kris, P.A. Janne, T. Lynch, B.E. Johnson, V.A. Miller, Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer, J. Clin. Oncol. 28 (2010) 357–360.
[4] M. Pollak, Insulin and insulin-like growth factor signalling in neoplasia, Nat. Rev. Cancer 8 (2008) 915–928.
[5] P. Massoner, M. Ladurner-Rennau, I.E. Eder, H. Klocker, Insulin-like growth factors and insulin control a multifunctional signalling network of significant importance in cancer, Br. J. Cancer 103 (2010) 1479–1484.
[6] C. Garcia-Echeverria, Medicinal chemistry approaches to target the kinase activity of IGF-1R, IDrugs 9 (2006) 415–419.
[7] Y.G. Zhao, H. Zhao, L. Miao, L. Wang, F. Sun, H. Zhang, The p53-induced gene Ei24 is an essential component of the basal autophagy pathway, J. Biol. Chem. 287 (2012) 42053–42063.
[8] Z. Gu, C. Flemington, T. Chittenden, G.P. Zambetti, ei24, a p53 response gene involved in growth suppression and apoptosis, Mol. Cell. Biol. 20 (2000) 233–241.
[9] J.M. Choi, S. Devkota, Y.H. Sung, H.W. Lee, EI24 regulates epithelial-to-mesenchymal transition and tumor progression by suppressing TRAF2-mediated NF-kappaB activity, Oncotarget 4 (2013) 2383–2396.
[10] C.N. Mork, D.V. Faller, R.A. Spanjaard, Loss of putative tumor suppressor EI24/PIG8 confers resistance to etoposide, FEBS Lett. 581 (2007) 5440–5444.
[11] S.M. Kim, O.J. Kwon, Y.K. Hong, J.H. Kim, F. Solca, S.J. Ha, R.A. Soo, J.G. Christensen, J.H. Lee, B.C. Cho, Activation of IL-6R/JAK1/STAT3 signaling induces de novo resistance to irreversible EGFR inhibitors in non-small cell lung cancer with T790M resistance mutation, Mol. Cancer Ther. 11 (2012) 2254–2264.
[12] S. Devkota, Y.H. Sung, J.M. Choi, J. Lee, N.Y. Ha, H. Kim, B.C. Cho, J. Song, H.W. Lee, Ei24-deficiency attenuates protein kinase Calpha signaling and skin carcinogenesis in mice, Int. J. Biochem. Cell Biol. 44 (2012) 1887–1896.
[13] H. Sasaki, J. Hayakawa, Y. Terai, M. Kanemura, A. Tanabe-Kimura, H. Kamegai, H. Seino-Noda, S. Ezoe, I. Matsumura, Y. Kanakura, M. Sakata, K. Tasaka, M. Ohmichi, Difference between genomic actions of estrogen versus raloxifene in human ovarian cancer cell lines, Oncogene 27 (2008) 2737–2745.
[14] Z. Zhang, J.C. Lee, L. Lin, V. Olivas, V. Au, T. LaFramboise, M. Abdel-Rahman, X. Wang, A.D. Levine, J.K. Rho, Y.J. Choi, C.M. Choi, S.W. Kim, S.J. Jang, Y.S. Park, W.S. Kim, D.H. Lee, J.S. Lee, V.A. Miller, M. Arcila, M. Ladanyi, P. Moonsamy, C. Sawyers, T.J. Boggon, P.C. Ma, C. Costa, M. Taron, R. Rosell, B. Halmos, T.G. Bivona, Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer, Nat. Genet. 44 (2012) 852–860.
[15] A.B. Cortot, C.E. Repellin, T. Shimamura, M. Capelletti, K. Zejnullahu, D. Ercan, J.G. Christensen, K.K. Wong, N.S. Gray, P.A. Janne, Resistance to irreversible EGF receptor tyrosine kinase inhibitors through a multistep mechanism involving the IGF1R pathway, Cancer Res. 73 (2013) 834–843.
[16] D. Ercan, C. Xu, M. Yanagita, C.S. Monast, C.A. Pratilas, J. Montero, M. Butaney, T. Shimamura, L. Sholl, E.V. Ivanova, M. Tadi, A. Rogers, C. Repellin, M. Capelletti, O. Maertens, E.M. Goetz, A. Letai, L.A. Garraway, M.J. Lazzara, N. Rosen, N.S. Gray, K.K. Wong, P.A. Janne, Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors, Cancer Discov. 2 (2012) 934–947.
[17] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevsky, K.A. Marshall, K.H. Phillippy, P.M. Sherman, M. Holko, A. Yefanov, H. Lee, N. Zhang, C.L. Robertson, N. Serova, S. Davis, A. Soboleva, NCBI GEO archive for functional genomics data sets—update, Nucleic Acids Res. 41 (2013) D991–D995.
[18] H.A. Yu, G.J. Riely, C.M. Lovly, Therapeutic strategies utilized in the setting of acquired resistance to egfr tyrosine kinase inhibitors, Clin. Cancer Res. 20 (2014) 5898–5907.
[19] B. Gyorffy, P. Surowiak, J. Budczies, A. Lanczky, Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer, PLoS One 8 (2013) e82241.
[20] H.M. Khandwala, I.E. McCutcheon, A. Flyvbjerg, K.E. Friend, The effects of insulin-like growth factors on tumorigenesis and neoplastic growth, Endocr. Rev. 21 (2000) 215–244.
[21] A. Chakravarti, J.S. Loeffler, N.J. Dyson, Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling, Cancer Res. 62 (2002) 200–207.
[22] B. Dufourny, J. Alblas, T. van, H.A. eeffelen, S. van, F.M. chaik, van, B. der, B. urg, P.H. Steenbergh, J.S. Sussenbach, Mitogenic signaling of insulin-like growth factor I in MCF-7 human breast cancer cells requires phosphatidylinositol 3-kinase and is independent of mitogen-activated protein kinase, J. Biol. Chem. 272 (1997) 31163–31171.
[23] H.W. Qi, Z. Shen, L.H. Fan, Combined inhibition of insulin-like growth factor-1 receptor enhances the effects of gefitinib in a human non-small cell lung cancer resistant cell line, Exp. Ther. Med. 2 (2011) 1091–1095.
[24] V.A. Blakesley, B.S. Stannard, T. Kalebic, L.J. Helman, D. LeRoith, Role of the IGF-I receptor in mutagenesis and tumor promotion, J. Endocrinol. 152 (1997) 339–344.
[25] S.I. Choi, S.Y. Kim, J. Lee, E.W. Cho, I.G. Kim, TM4SF4 overexpression in radiation-resistant lung carcinoma cells activates IGF1R via elevation of IGF1, Oncotarget 5 (2014) 9823–9837.
[26] J.K. Rho, Y.J. Choi, J.K. Lee, B.Y. Ryoo, I.I. Na, S.H. Yang, C.H. Kim, J.C. Lee, Epithelial to mesenchymal transition derived from repeated exposure to gefitinib determines the sensitivity to EGFR inhibitors in A549, a non-small cell lung cancer cell line, Lung Cancer 63 (2009) 219–226.

[27] K.S. Park, M. Raffeld, Y.W. Moon, L. Xi, C. Bianco, T. Pham, L.C. Lee, T. Mitsudomi, Y. Yatabe, I. Okamoto, D. Subramaniam, T. Mok, R. Rosell, J. Luo, D.S. Salomon, Y. Wang, G. Giaccone, CRIPTO1 expression in EGFR-mutant NSCLC elicits intrinsic EGFR-inhibitor resistance, J. Clin. Invest. 124 (2014) 3003–3015.
[28] J.H. Chung, J.K. Rho, X. Xu, J.S. Lee, H.I. Yoon, C.T. Lee, Y.J. Choi, H.R. Kim, C.H. Kim, J.C. Lee, Clinical and molecular evidences of epithelial to mesenchymal transition in acquired resistance to EGFR-TKIs, Lung Cancer 73 (2011) 176–182.
[29] S. Thomson, F. Petti, I. Sujka-Kwok, D. Epstein, J.D. Haley, Kinase switching in mesenchymal-like non-small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy, Clin. Exp. Metastasis 25 (2008) 843–854.
[30] A. Vazquez-Martin, S. Cufi, C. Oliveras-Ferraros, V.Z. Torres-Garcia, B. Corominas-Faja, E. Cuyas, R. Bonavia, J. Visa, B. Martin-Castillo, E. Barrajon-Catalan, V. Micol, J. Bosch-Barrera, J.A. Menendez, IGF-1R/epithelial-to-mesenchymal transition (EMT) crosstalk suppresses the erlotinib-sensitizing effect of EGFR exon 19 deletion mutations, Sci. Rep. 3 (2013) 2560.
[31] G. Furstenberger, H.J. Senn, Insulin-like growth factors and cancer, Lancet Oncol. 3 (2002) 298–302.
[32] M. Koti, R.J. Gooding, P. Nuin, A. Haslehurst, C. Crane, J. Weberpals, T. Childs, P. Bryson, M. Dharsee, K. Evans, H.E. Feilotter, P.C. Park, J.A. Squire, Identification of the IGF1/PI3K/NF kappaB/ERK gene signalling networks associated with chemotherapy resistance and treatment response in high-grade serous epithelial ovarian cancer, BMC Cancer 13 (2013) 549.