Cancer Management and Research

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Inhibition of lncRNA PART1 Chemosensitizes Wild Type but Not KRAS Mutant NSCLC Cells

This article was published in the following Dove Press journal:
Cancer Management and Research

Shu-Chen Chen[1]
Yu-Zhu Diao[1]
Zi-Han Zhao[2]
Xiao-Ling Li[1]

[1]Medical Oncology Department of Thoracic Cancer 1, Cancer Hospital of China Medical University, Liaoning Cancer Hospital, Shenyang, Liaoning 110042, People's Republic of China; [2]The Second Clinical College of Dalian Medical University, Dalian, Liaoning 116044, People's Republic of China

Background: Lung cancer has the highest incidence among solid tumors in men and is the third most common cancer in women. Despite improved understanding of genomic and mutational landscape in non-small cell lung cancer (NSCLC), the five-year survival in these patients has remained stagnant at a dismal 15%. The first line of treatment commonly adapted for NSCLC patients with somatic mutation in *EGFR* is tyrosine kinase inhibitor gefitinib or erlotinib. *EGFR* mutant cells seem to be intrinsically sensitive to tyrosine kinase inhibitors; however, the remaining 20–30% patients are resistant to tyrosine kinase inhibitor.
Materials and Methods: Here we show, using in vitro normal and NSCLS cell lines, that the lncRNA Prostate androgen-regulated transcript 1 (*PART1*) is expressed at higher levels in NSCLC cells compared to normal lung epithelial cell line, corroborating two earlier studies.
Results: We additionally show that these cells are resistant to erlotinib which is reversed in some, but not all, cell lines following suppression of *PART1* expression. The differential response to erlotinib following siRNA-mediated knockdown of *PART1* was found to be related to the mutational status of *KRAS*. Only in cells with wild-type *KRAS* suppression of *PART1* sensitized them to erlotinib. Knockdown of mutant *KRAS* did not sensitize those cell lines to erlotinib. But knockdown of mutant *KRAS* along with suppression of *PART1* sensitized the cells to treatment with erlotinib. The results from the study reveal a yet undefined and important role of lncRNA *PART1* in defining sensitivity to erlotinib. This action is mediated by mutation status of *KRAS*.
Conclusion: Even though preliminary, our results indicate *PART1* might be a potential candidate for targeted therapy or used as a predictor of chemosensitivity in patients with NSCLC.

Keywords: lncRNA, PART1, KRAS, non-small cell lung cancer, chemosensitivity, erlotinib

Introduction

China has the sixteenth highest incidence of lung cancer in the world with an age-standardized rate of 35.1 per 100,000.[1] Worldwide, lung cancer is also the most common solid cancer in men and third most common solid cancer in women.[1] Despite all the advances made in diagnostic and therapeutic regimens, the 5-year survival at 15% has largely remained static in lung cancer patients.[2] This highlights the urgent need to define pathogenic mechanisms in lung cancer in order to identify and test more effective therapeutic targets.[3,4] Non-small cell lung cancer (NSCLC) is the major form of lung cancer contributing to approximately 85% cases.

Deregulated tyrosine kinase activity of the transmembrane growth factor receptor epidermal growth factor receptor (EGFR) has been implicated in pathogenesis of lung cancer.[5–8] Indeed, sensitivity to tyrosine kinase inhibitors gefitinib and

Correspondence: Xiao-Ling Li
Medical Oncology Department of Thoracic Cancer 1,Cancer Hospital of China Medical University, Liaoning Cancer Hospital, No. 44, Xiaoheyan Road, Dadong District, Shenyang, Liaoning 110042, People's Republic of China
Tel/Fax +86-24-31916361
Email lixiaoling_med@163.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/CMAR.S245257

erlotinib in lung cancer patients have shown association with somatic mutations mapping to the tyrosine kinase domain of the *EGFR* gene.[9–12] Patients harboring mutations have increased EGFR signaling and are better responders to the gefitinib and/or erlotinib. Thus, gefitinib and erlotinib are routinely the therapy of choice in *EGFR*-mutation positive NSCLC patients, with greater than 70% patients showing durable and positive outcome.[13–15] However, the remaining patients have intrinsic resistance to tyrosine kinase inhibitors.[16] The mechanism of this intrinsic resistance is not completely understood.

There have been an increasing number of studies elucidating that microRNAs and long non-coding RNAs are important regulators of chemosensitivity of cancer cells.[17,18] Even in the context of lung cancer, lncRNAs, including lncRNA *RP11-838N2.4*, have shown association with resistance to tyrosine kinase inhibitors.[19,20] It will not be surprising if additional lncRNAs are also involved in a context-dependent or context-independent fashion in rendering resistance to tyrosine kinase inhibitors in NSCLC patients. Prostate androgen-regulated transcript 1 (*PART1*) was identified as an androgen-regulated and prostate-specific lncRNA.[21] *PART1* is also expressed in lungs of NSCLC patients and was found to be correlated to survival[22] as well as disease progression via JAK-STAT signaling pathway.[23] Given its apparent importance in pathogenesis of NSCLC,[20,23] our objective was to study if *PART1* expression indirectly or directly regulates chemosensitivity of NSCLC cells. Interestingly, our results show that inhibition of *PART1* sensitized NSCLC cell lines to erlotinib treatment whereas its overexpression in normal lung epithelial cells rendered them resistant to erlotinib treatment. But this effect on erlotinib chemosensitivity was restricted to cells with wild-type *KRAS*.

Materials and Methods

Cell Culture

The normal human lung epithelial cell line BEAS-2B, and the NSCLC cell lines NCI-H2444, NCI-H647, A549, and NCI-H23 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA. Both cell lines were cultured in DMEM (Thermo Fisher Scientific, Shanghai, China) containing 10% fetal bovine serum (Thermo Fisher Scientific) and 100 units penicillin/streptomycin (Thermo Fisher Scientific). Cells were incubated at 37°C.

Cell Lysate and Western Blot

At the end of experimental time-point, the media was aspirated off and the cells were washed twice with ice-cold 1X phosphate-buffered saline (PBS). Cells were then lysed using RIPA buffer (20x volumes of cell pellet; Thermo Fisher Scientific) and protein concentration in extracted whole cell lysate was determined using BCA assay (Thermo Fisher Scientific). Fifty micrograms of lysates were resolved by SDS-PAGE and processed for Western blot using standard methodologies. Blots were probed with the following antibodies: anti-KRAS (clone 9.13, 1:1000; ThermoFisher Scientific), anti-EGFR (#2085; 1:2000; Cell Signaling), anti-EGFR (#2234; 1:1000; Cell Signaling) and anti-GAPDH (#5174, 1:4000; Cell Signaling). Blots were probed with anti-GAPDH antibody to ensure equivalent protein loading across samples.

Immunofluorescence (IF) Analysis

Cells were grown on coverslips housed in 24-well plates. Once cells reached exponential growth phase, they were washed with ice-cold PBS and fixed using methanol. Cells were blocked with 10% BSA + 0.1% saponin in PBS for 1 hour. The cells were then incubated overnight at room temperature with EGFR antibody (clone D38B1, 1:250; Cell Signaling Technology, Cambridge, USA). The excess antibody was washed off using three 15-minutes wash with PBS and then the cover slips were incubated with secondary antibody (1:500) for 1 hour followed by three more washes with PBS. The coverslips were then mounted using VECTASHIELD Antifade containing 4,6-diamidino-2-phenylindole (DAPI) Vector Laboratories, Burlingame, USA). Slides were imaged via Confocal Spectral Microscope (TCS-SP8 confocal microscope, Leica, Germany).

Gene Construction and Transfection/Transduction

shRNA targeting *KRAS* was obtained from Dharmacon and was transduced in NCI-H2444 and NCI-H647 cells. Cells were selected with puromycin (2 µg/mL) for 2 weeks. siRNA targeting *PART1* (Assay ID: n260057; 5′-GGAACAACACAGAUGAGAUtt – 3′) or a negative control siRNA (#4390843) were obtained from ThermoFisher Scientific. *PART1* overexpression plasmid or negative control vector was obtained from GeneChem (Shanghai, China). For transfection cells were plated in antibiotic-free media. Cells were transfected with control or *PART1* siRNA (10 nM final concentration) using Polyplus

jetPrime transfection reagent. For transduction, BEAS-2B cells were transduced with either lentiviral particles containing control or *PART1* overexpression construct using polybrene. Cells were selected with puromycin (2 μg/mL) for 2 weeks. All indicated treatments were done 48 hours following siRNA transfection. Successful knockdown or overexpression was verified by quantitative real-time polymerase chain reaction (qRT-PCR).

RNA Isolation and qRT-PCR

Media was aspirated off and cells were rinsed with ice-cold PBS. The cell pellet was then used to isolate RNA using Trizol (Thermo Fisher Scientific). qRT-PCR was performed using SYBR Green (Thermo Fisher Scientific) using the following primers: *EGFR* forward primer – 5′ – AACACCCTGGTCTGGAAGTACG – 3′; *EGFR* reverse primer – 5′ – TCGTTGGACAGCCTTCAAGACC – 3′; *PART1* forward primer – 5′-AAGGCCGTGTCAGAACT CAA-3′; *PART1* reverse primer – 5′-GTTTTCCATCTCA GCCTGGA-3′; *KRAS* forward primer – 5′ -CAGTAG ACACAAAACAGGCTCAG - 3′; *KRAS* reverse primer – 5′ – TGTCGGATCTCCCTCACCAATG – 3′; 18s rRNA forward primer – 5′-GGCCCTGTAATTGGAATGAGTC -3′; 18s rRNA reverse primer - 5′-CCAAGATCCAACT ACGAGCTT-3′. Post-normalization to 18s rRNA expression relative expression of *PART1* was calculated by ΔΔCt method. Data were represented as expression relative to that in BEAS-2B cells (Figure 1A and E), or relative to mock overexpression or knockdown (Figure 2A). Data were represented as mean ± standard error of mean (SEM). qRT-PCR experiments were run in triplicate and P-values were calculated by *t*-test. $P < 0.05$ was considered statistically significant.

Cytotoxicity Assays

Cytotoxicity of erlotinib (Selleckchem, Houston, USA) was determined in dose-dependent manner in indicated cell types and conditions. In each case, cells were treated with indicated dose of erlotinib for 3 days before cell viability was measured using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay (Sigma Aldrich, Shanghai, China). The antiproliferative effect of erlotinib was calculated as a percentage of cell growth inhibition with respect to dose and compared to the respective controls. Data are represented as mean ± standard deviation (SD). P-values were calculated by *t*-test. $P < 0.05$ was considered statistically significant.

Statistical Analysis

All data, except for qRT-PCR, were represented as mean ± standard deviation (SD) of at least three independent replicates. qRT-PCR data were presented as mean + SEM. Statistical significance between groups was analyzed using the Student's *t*-test. A P-value <0.05 was considered statistically significant.

Results

We initially determined mRNA and protein expression of *EGFR* in the normal lung cell line BEAS-2B and the NSCLC cell lines, NCI-H647, NCI-H24444, NCI-H23, and A549. Steady state mRNA expression of *EGFR* was not significantly different among the NSCLC cell lines compared to the normal lung cell line BEAS-2B (Figure 1A). Both Western blot (Figure 1B) and immunofluorescence analysis (Figure 1C) showed equivalent EGFR protein expression in these cell lines. We also tested for phosphorylated (Tyrosine 1068) EGFR in these cell lines. Given that the cells were not treated with EGF we did not observe any phosphorylated EGFR along expected lines (data not shown). We next determined the chemosensitivity of these cells to increasing concentration of erlotinib over 72 hours. Cells were treated with 0–20 μM erlotinib and relative viability was measured after 72 hours. The IC_{50} for BEAS-2B was 3.4185 ± 0.54 μM (Figure 1D). The IC_{50} for each of the NSCLC cell lines was greater than 20 μM (Figure 1D), indicating that these cell lines were resistant to erlotinib.

Our broad question was whether lncRNA *PART1* was involved in the observed erlotinib resistance in these cells. We initially determined the relative expression of lncRNA *PART1* in BEAS-2B and the NCI-H647, NCI-H24444, NCI-H23, and A549 cell lines. Similar levels of high *PART1* expression were observed in NCI-H647, NCI-H24444, NCI-H23, and A549 cells, which was significantly higher than that observed in the normal lung cell line BEAS-2B (Figure 1E; P<0.05 in each case). This indicated that lncRNA *PART1* is overexpressed in NSCLC cell lines, corroborating previous studies.[22,23]

We then investigated if modulating *PART1* expression will alter chemosensitivity of BEAS-2B or the NSCLC cell lines. *PART1* was overexpressed in BEAS-2B cells, whereas the NSCLC cells lines were transfected with *PART1* siRNA. Overexpression of *PART1* in BEAS-2B and knockdown in NCI-H647, NCI-H24444, NCI-H23, and A549 cell lines were confirmed by qRT-PCR (Figure 2A). To determine if

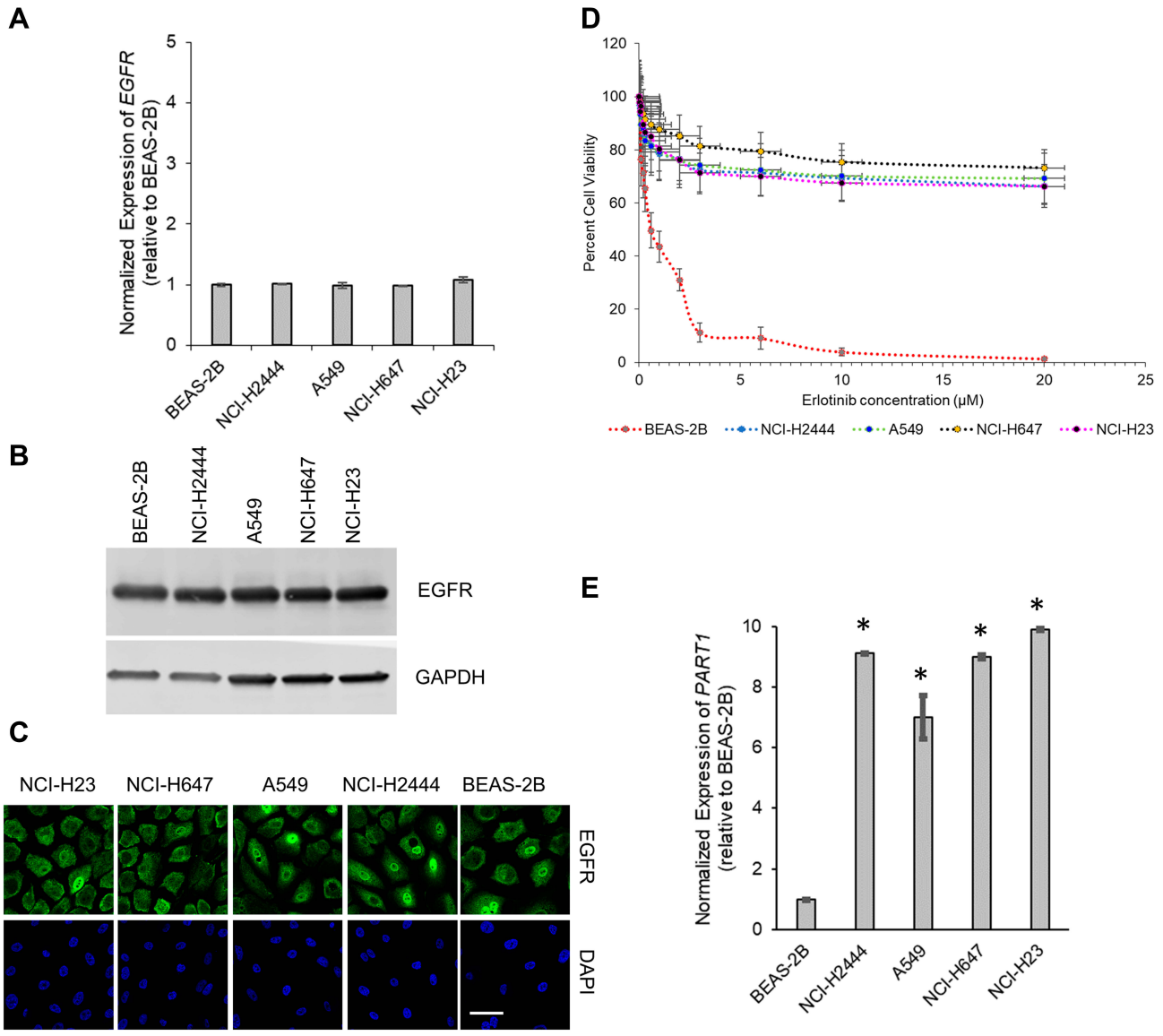

Figure 1 Differential *PART1* expression and resistance to erlotinib in NSCLC cells. (**A**) Relative expression of *EGFR* determined by qRT-PCR. Post-normalization to 18s rRNA expression, expression relative to BEAS-2B is shown. Error bars, SEM. Error bars, SD. *P<0.05. (**B, C**) Western blot (**B**) and IF (**C**) analysis of EGFR expression in the normal lung epithelial cell line BEAS-2B or the NSCLC cell line, H-2444, A549, NCI-H647, and NCI-H23. Scale bar, 35 μm. (**D**) The normal lung epithelial cell line BEAS-2B or the NSCLC cell line, H-2444, A549, NCI-H647, and NCI-H23 cells were treated with indicated doses of erlotinib for 3 days and cell viability was measured. Data is representative of three independent experiments, each done in triplicate. Error bars, SD. (**E**) Relative expression of lncRNA *PART1* determined by qRT-PCR. Post-normalization to 18s rRNA expression, expression relative to BEAS-2B is shown. Error bars, SEM. Error bars, SD. *P<0.05.

overexpression of *PART1* in the BEAS-2B cells alter their sensitivity to erlotinib, we treated the mock or *PART1* transduced BEAS-2B cells to different doses of erlotinib for 72 hours and quantified their relative viability by MTT assay. Overexpression of *PART1* increased the IC_{50} from 2.86 ± 0.31 μM to 13.48 ± 0.03 μM (Figure 2B; P<0.05). This indicated that *PART1* expression level is potentially regulated to sensitivity to erlotinib in lung cancer cells.

We next investigated if decreasing *PART1* expression in the NSCLC cell lines will increase their sensitivity to erlotinib. Mock transfected or *PART1* siRNA transfected NSCLC cells were treated with different doses of erlotinib for 3 days and relative viability were determined. The IC_{50} in NCI-H2444 (26.55 ± 2.34 μM in *PART1* siRNA vs 26.69 ± 1.29 μM control siRNA; P>0.05) and NCI-H647 (31.27 ± 1.09 μM in *PART1* siRNA vs 32.27 ± 3.28 μM control siRNA; P>0.05) did not change even after *PART1* knockdown (Figure 3A and B). However, the IC_{50} of A549 (6.97 ± 1.21 μM in *PART1* siRNA vs 27.02 ± 1.34 μM control siRNA; P<0.05) and NCI-H23 (9.12 ± 3.1 μM

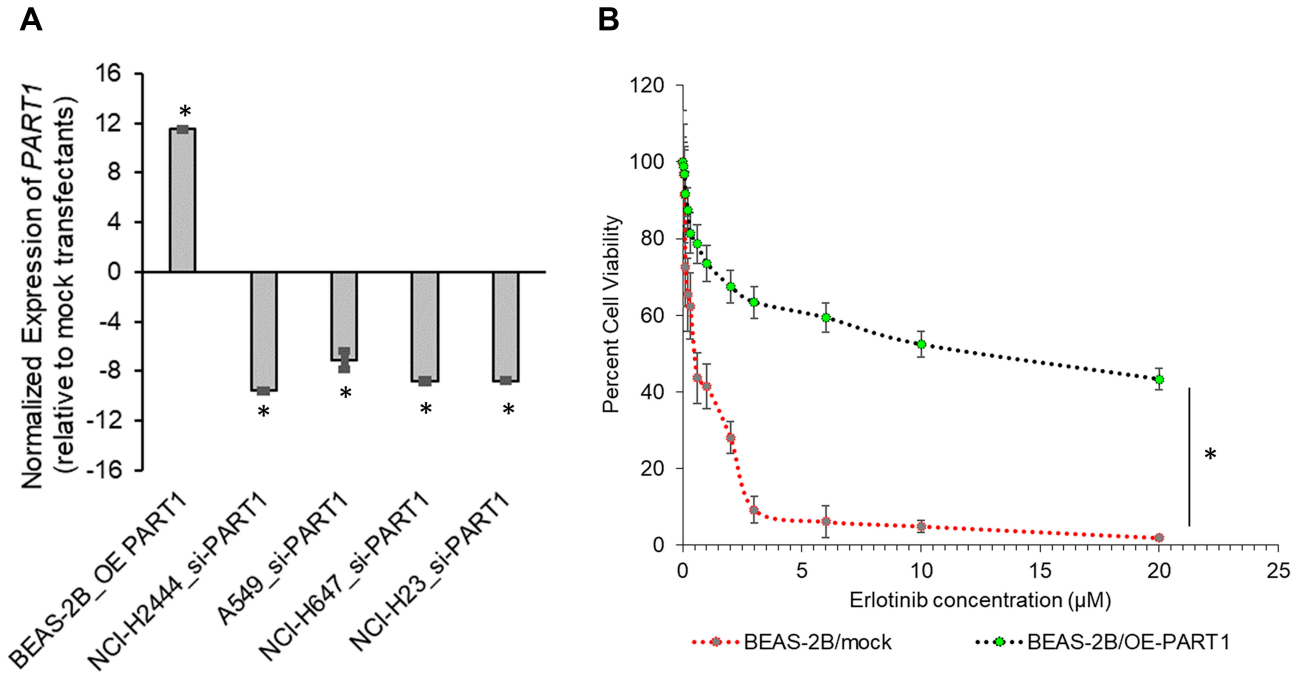

Figure 2 Overexpression of *PART1* makes BEAS-2B cells resistant to erlotinib. (**A**) Relative expression of lncRNA *PART1* determined by qRT-PCR. Post-normalization to 18s rRNA expression, expression relative to mock transduction (BEAS-2B) or transfection (other cell lines). Error bars, SEM. Error bars, SD. *P<0.05. (**B**) Mock or *PART1* transduced BEAS-2B cells were treated with indicated doses of erlotinib for 3 days and cell viability was measured. Data is representative of three independent experiments, each done in triplicate. Error bars, SD. *P<0.05.

in *PART1* siRNA vs 25.32 ± 1.12 μM control siRNA; P<0.05) significantly decreased following knockdown of *PART1* (Figure 3C and D). The differential response observed was not due to different efficiency of *PART1* knockdown (Figure 2A). This indicated that there are secondary factors whose expression is different in the NSCLC cell lines and that mediate the effect of *PART1* on sensitivity to erlotinib.

We evaluated the mutation status of the NSCLC cell lines from published literature.[24] This search revealed that both NCI-H23 and A549 cell lines harbor *PTEN* mutation whereas the NCI-H2444 and NCI-H647 cell lines harbored *KRAS* mutation. NCI-H2444 harbors *KRAS* G12V mutation whereas NCI-H647 harbors *KRAS* G13D mutation. Whereas the *PTEN* mutation results in constitutive PI3K/AKT signaling, the *KRAS* mutations result in hyperactivation of the MAPK pathway. Given that both cell lines with *KRAS* mutation were resistant to erlotinib irrespective of *PART1* expression we next determined if altering *KRAS* expression in these cell lines would sensitize them to erlotinib treatment. Hence, we transduced NCI-H2444 and NCI-H647 cells with shRNA targeting *KRAS* alone or in combination with siRNA targeting *PART1* and confirmed knockdown by qRT-PCR (Figure 4A) immunoblot analysis (Figure 4B).

The *KRAS* knockdown cells were then transfected with *PART1* or negative control siRNA and knockdown by qRT-PCR (Figure 4A). The sensitivity to erlotinib was subsequently analyzed by MTT assay. Stable knockdown of *KRAS* did not make either the NCI-H2444 (IC_{50} 26.84 ± 0.09 μM) or NCI-H647 cells (IC_{50} 27.02 ± 2.91 μM) (Figure 4C and D). However, knockdown of *PART1* in the *KRAS* knockdown NCI-H2444 and NCI-H647 cells sensitized them to erlotinib treatment decreasing the IC_{50} to 5.89 ± 0.39 μM and 3.14 ± 0.14 μM, respectively (Figure 4C and D; P<0.05 in each case). Taken together, these results confirmed that decreasing *PART1* expression can sensitize NSCLC cells with wild-type *KRAS*, but such a strategy will not work in cells with mutant *KRAS*. Our results also indicate that *PART1* mediates its chemoresistant activity to erlotinib via KRAS.

Discussion

The *PART1* lncRNA was originally characterized in the prostate gland and was shown to be regulated by androgens.[21,25,26] *PART1* has been indicated previously in glioblastoma,[27] esophageal squamous cell carcinoma (ESCC),[28] oral cancer,[29] colorectal cancer,[30] as well as in survival and disease progression of NSCLC.[22,23] However, there has been no previous study elucidating a role of *PART1* in chemoresistance to erlotinib in NSCLC.

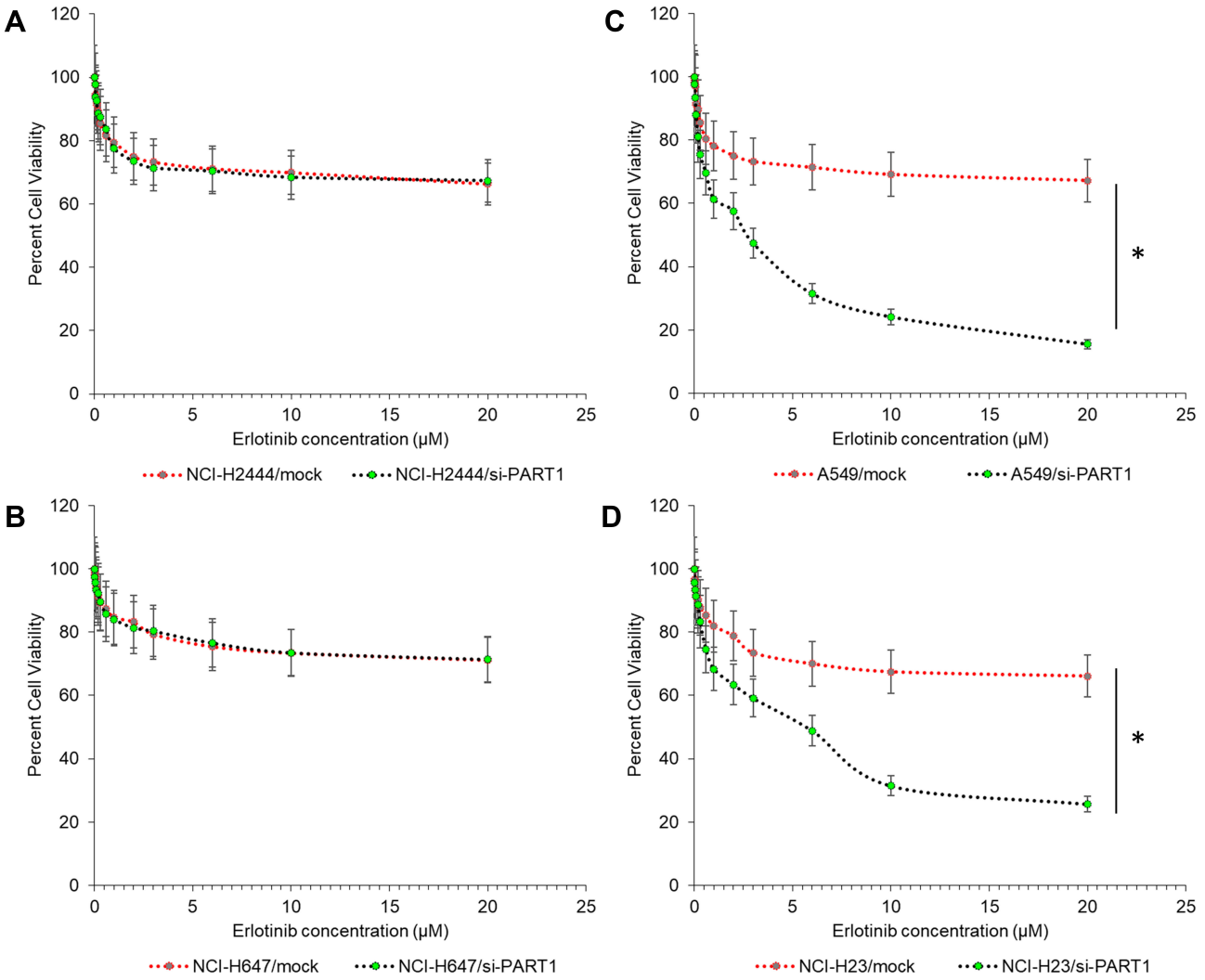

Figure 3 Differential sensitivity of NSCLC cell lines to erlotinib following knockdown of *PART1*. Indicated cells were transfected with *PART1* or negative control siRNA for 48 hours. Post-48 hours of transfection, cells (NCI-H2444 (**A**), NCI-H647 (**B**), A549 (**C**), and NCI-H23 (**D**)) were treated with indicated doses of erlotinib for 3 days and cell viability was measured. Data is representative of three independent experiments, each done in triplicate. Error bars, SD. *P<0.05.

In ESCC, it has been shown that exosome-mediated transfer of *PART1* can induce resistance to the tyrosine kinase inhibitor gefitinib. In this context, *PART1* has also been shown to function as a competing endogenous RNA (ceRNA). *PART1* was found to sponge miR-129 and activate Bcl2 in ESCC.[28] Whether *PART1* functions also mediate resistance to the tyrosine kinase inhibitor erlotinib in NSCLC by functioning as a ceRNA has to be determined in future studies.

Despite the best of efforts in understanding molecular mechanism dictating chemoresistance in cancer cells it still continues to be an enigma.[31] Recent evidences have suggested that lncRNAs and miRNAs can both be critical regulators of this process and our current study corroborates those findings in that *PART1* expression was higher in chemoresistant cells and downregulating its expression in cells with wild type *KRAS* sensitized the cells to erlotinib.

It is not entirely without precedence that *KRAS* mutation status is dictating chemosensitivity of cancer cells. It has been shown that the let-7b, which is a tumor suppressor, replenishment sensitizes *KRAS* mutant cancer cells to paclitaxel and gemcitabine treatment.[32] In the current study, we found that *PART1* requires wild-type *KRAS* activity to render resistance to erlotinib. Furthermore, knockdown of mutant *KRAS* alone did not sensitize the cells to erlotinib treatment. Only when both mutant *KRAS* and *PART1* expression were downregulated the cells became sensitive to erlotinib highlighting *PART1* mediates the resistance but requires *KRAS* activity.

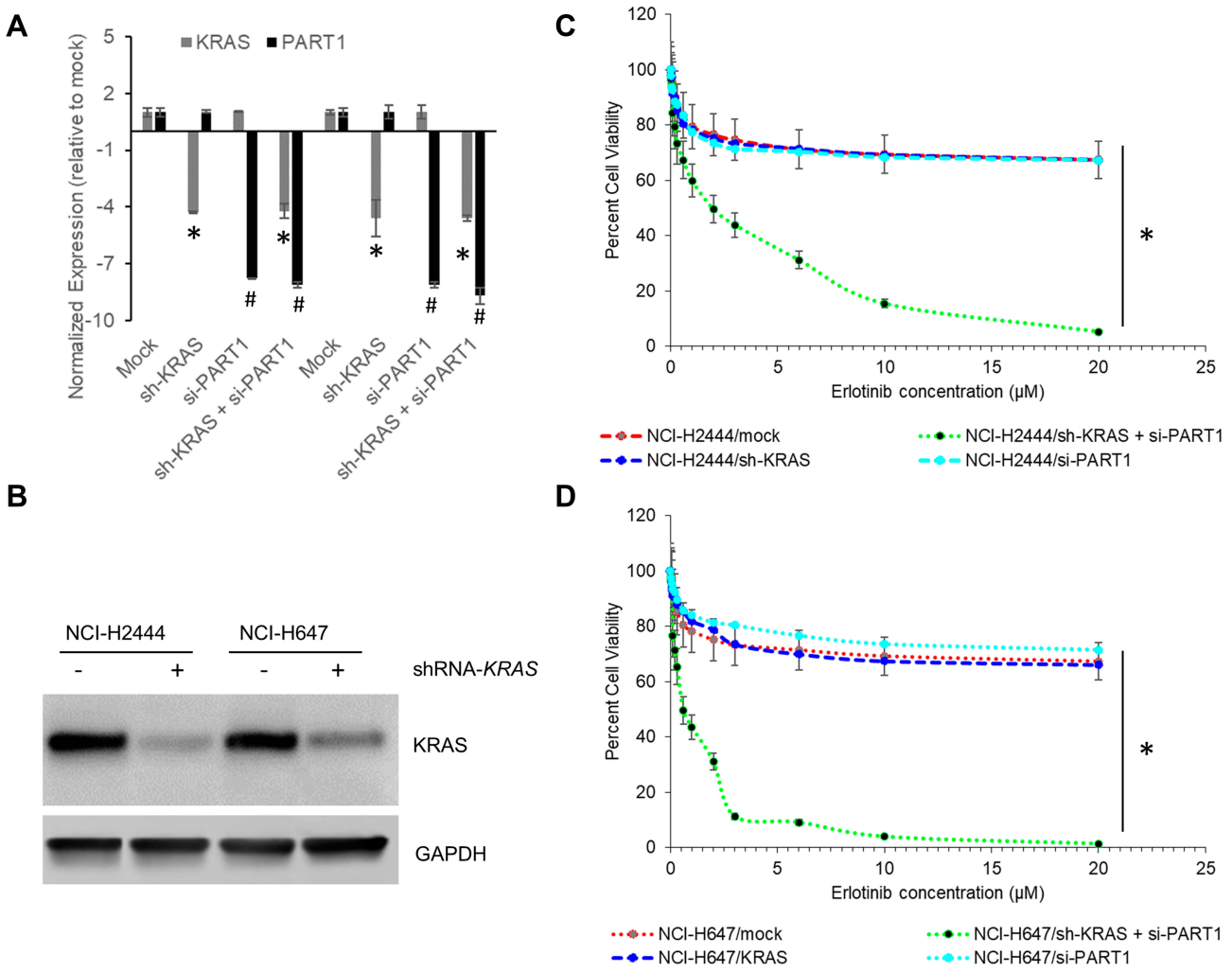

Figure 4 NSCLC cells with mutant *KRAS* become sensitive to erlotinib following combined knockdown of *PART1* and *KRAS*. (**A**) Relative expression of *KRAS* determined by qRT-PCR. Post-normalization to 18s rRNA expression, expression relative to respective controls are shown. Error bars, SEM. Error bars, SD. *P<0.05 versus mock-KRAS group; #P<0.05 versus mock-PART1 group. (**B**) Immunoblot analysis of KRAS in NCI-H2444 and NCI-H647 cells transduced with negative control or *KRAS* shRNA. Blots were probed with GAPDH to confirm loading across lanes. Shown are representative blots from three independent experiments. (**C, D**) *KRAS* knockdown NCI-H2444 (**C**) and NCI-H647 (**D**) cells were transiently transfected with negative control (mock) or *PART1* siRNA. Post-48 hours of transfection, cells (NCI-H2444 (**C**), and NCI-H647 (**D**)) were treated with indicated doses of erlotinib for 3 days and cell viability was measured. Data is representative of three independent experiments, each done in triplicate. Error bars, SD. *P<0.05, #P<0.05.

The *KRAS* mutations are known to cause constitutive activation of MAPK pathway so there might be a possibility of crosstalk between MAPK signaling and EGFR signaling. Hyperactive MAPK pathway perhaps overrides the effect of *PART1* downregulation and maintains chemoresistance in the NSCLC cells. To completely define the specific molecules mediating this effect, gene expression analysis in the different cell types needs to be performed.

Conclusion

One potential limitation of our study is that it was done using in vitro cells. The results need to be expanded and verified to other in vivo models of NSCLS as well as tested in clinical samples. In summary, our results show that *PART1* mediates resistance to erlotinib in NSCLC cells with wild-type *KRAS* and modulating its expression can potentially be tailored to targeted therapies or *PART1* expression can be used as a predictor of resistance in NSCLSC patients without somatic mutations in *EGFR*.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492

2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. *N Engl J Med*. 2008;359(13):1367–1380. doi:10.1056/NEJMra0802714
3. Yan Y, Xu Z, Qian L, et al. Identification of CAV1 and DCN as potential predictive biomarkers for lung adenocarcinoma. *Am J Physiol Lung Cell Mol Physiol*. 2019;316(4):L630–L643.
4. Wei J, Yan Y, Chen X, et al. The roles of plant-derived triptolide on non-small cell lung cancer. *Oncol Res*. 2019;27(7):849–858. doi:10.3727/096504018X15447833065047
5. Gurtner K, Deuse Y, Butof R, et al. Diverse effects of combined radiotherapy and EGFR inhibition with antibodies or TK inhibitors on local tumour control and correlation with EGFR gene expression. *Radiother Oncol*. 2011;99(3):323–330. doi:10.1016/j.radonc.2011.05.035
6. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res*. 2001;7(10):2958–2970.
7. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene*. 2000;19(56):6550–6565. doi:10.1038/sj.onc.1204082
8. Woodburn JR. The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol Ther*. 1999;82(2–3):241–250. doi:10.1016/S0163-7258(98)00045-X
9. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350(21):2129–2139. doi:10.1056/NEJMoa040938
10. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304(5676):1497–1500. doi:10.1126/science.1099314
11. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A*. 2004;101(36):13306–13311. doi:10.1073/pnas.0405220101
12. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non–small-cell lung cancer to gefitinib. *N Engl J Med*. 2005;352(8):786–792. doi:10.1056/NEJMoa044238
13. Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a Phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol*. 2011;29(21):2866–2874. doi:10.1200/JCO.2010.33.4235
14. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2018;29(Suppl 4):iv192–iv237.
15. Tamura K, Okamoto I, Kashii T, et al. Multicentre prospective Phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403). *Br J Cancer*. 2008;98(5):907–914. doi:10.1038/sj.bjc.6604249
16. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010;362(25):2380–2388. doi:10.1056/NEJMoa0909530
17. Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. *Cancer Res*. 2017;77(15):3965–3981. doi:10.1158/0008-5472.CAN-16-2634
18. Yan Y, Xu Z, Chen X, et al. Novel function of lncRNA ADAMTS9-AS2 in promoting temozolomide resistance in glioblastoma via upregulating the FUS/MDM2 ubiquitination axis. *Front Cell Dev Biol*. 2019;7:217. doi:10.3389/fcell.2019.00217
19. Zhang W, Cai X, Yu J, Lu X, Qian Q, Qian W. Exosome-mediated transfer of lncRNA RP11838N2.4 promotes erlotinib resistance in non-small cell lung cancer. *Int J Oncol*. 2018;53(2):527–538. doi:10.3892/ijo.2018.4412
20. Ma P, Zhang M, Nie F, et al. Transcriptome analysis of EGFR tyrosine kinase inhibitors resistance associated long noncoding RNA in non-small cell lung cancer. *Biomed Pharmacother*. 2017;87:20–26. doi:10.1016/j.biopha.2016.12.079
21. Lin B, White JT, Ferguson C, et al. PART-1: a novel human prostate-specific, androgen-regulated gene that maps to chromosome 5q12. *Cancer Res*. 2000;60(4):858–863.
22. Li M, Zhang W, Zhang S, Wang C, Lin Y. PART1 expression is associated with poor prognosis and tumor recurrence in stage I-III non-small cell lung cancer. *J Cancer*. 2017;8(10):1795–1800. doi:10.7150/jca.18848
23. Zhu D, Yu Y, Wang W, et al. Long noncoding RNA PART1 promotes progression of non-small cell lung cancer cells via JAK-STAT signaling pathway. *Cancer Med*. 2019;8(13):6064–6081. doi:10.1002/cam4.2494
24. Blanco R, Iwakawa R, Tang M, et al. A gene-alteration profile of human lung cancer cell lines. *Hum Mutat*. 2009;30(8):1199–1206. doi:10.1002/humu.21028
25. Sidiropoulos M, Chang A, Jung K, Diamandis EP. Expression and regulation of prostate androgen regulated transcript-1 (PART-1) and identification of differential expression in prostatic cancer. *Br J Cancer*. 2001;85(3):393–397. doi:10.1054/bjoc.2001.1883
26. Yu L, Blackburn GL, Zhou JR. Genistein and daidzein downregulate prostate androgen-regulated transcript-1 (PART-1) gene expression induced by dihydrotestosterone in human prostate LNCaP cancer cells. *J Nutr*. 2003;133(2):389–392. doi:10.1093/jn/133.2.389
27. Zhang XQ, Sun S, Lam KF, et al. A long non-coding RNA signature in glioblastoma multiforme predicts survival. *Neurobiol Dis*. 2013;58:123–131. doi:10.1016/j.nbd.2013.05.011
28. Kang M, Ren M, Li Y, Fu Y, Deng M, Li C. Exosome-mediated transfer of lncRNA PART1 induces gefitinib resistance in esophageal squamous cell carcinoma via functioning as a competing endogenous RNA. *J Exp Clin Cancer Res*. 2018;37(1):171. doi:10.1186/s13046-018-0845-9
29. Li S, Chen X, Liu X, et al. Complex integrated analysis of lncRNAs-miRNAs-mRNAs in oral squamous cell carcinoma. *Oral Oncol*. 2017;73:1–9. doi:10.1016/j.oraloncology.2017.07.026
30. Hu Y, Ma Z, He Y, Liu W, Su Y, Tang Z. PART-1 functions as a competitive endogenous RNA for promoting tumor progression by sponging miR-143 in colorectal cancer. *Biochem Biophys Res Commun*. 2017;490(2):317–323. doi:10.1016/j.bbrc.2017.06.042
31. Jensen NF, Stenvang J, Beck MK, et al. Establishment and characterization of models of chemotherapy resistance in colorectal cancer: towards a predictive signature of chemoresistance. *Mol Oncol*. 2015;9(6):1169–1185. doi:10.1016/j.molonc.2015.02.008
32. Dai X, Jiang Y, Tan C, Batra SK. Let-7 sensitizes KRAS mutant tumor cells to chemotherapy. *PLoS One*. 2015;10(5):e0126653. doi:10.1371/journal.pone.0126653