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Original Article

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The Diverse Analysis Identifies Mutated *KRAS* Associated With Radioresistance in Non-Small Cell Lung Cancer

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Abstract

Background: To analyze the relationship between V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) status and radioresistance in non-small cell lung cancer (NSCLC), we identified potential genotypic differences and pathways involved.

Methods: We retrospectively analyzed epidermal growth factor receptor (*EGFR*) and *KRAS* status in patients undergoing definitive radiotherapy for NSCLC between 2004 and 2018. Cox proportional hazard models were used to evaluate local progression-free survival (LPFS). Using clonogenic survival and measurement of γH2AX foci, we analyzed the difference in radiosensitivity between NSCLC cell lines with different *KRAS* status. The Cancer Genome Atlas (TCGA) analysis was used to explore the potential pathways involved.

Results: The results showed that of the 286 patients identified, 68 (24%) had local tumor progression (mean ± standard deviation (SD), 27 ± 17.4 months); of these patients, *KRAS* mutations were found in 14 (23%), and *KRAS* status was associated with LPFS. After adjusting for concurrent chemotherapy, gross tumor volume, and mutation status in multivariate analysis, *KRAS* mutation was associated with shorter LPFS (hazard ratio: 1.961; 95% confidence interval: 1.03 - 2.17; P = 0.032). *KRAS* mutation showed higher radioresistance *in vitro*. TCGA data showed that the ERK1/2 pathway, phosphatidylinositol I3 kinase (PI3K)/mTOR, p38 MAPK pathway, cell cycle checkpoint signaling, DNA damage, repair pathways, and EGFR/PKC/AKT pathway were differentially expressed in patients with *KRAS* mutations or cell lines compared with their expression in the wild-type group.

Conclusions: Diverse analyses identified that *KRAS* mutation was associated with radioresistance in NSCLC. *KRAS* mutation status may be helpful as a biomarker of radioresistance and a potential target to increase radiosensitivity.

Keywords: *KRAS*; Non-small cell lung cancer; Radioresistance; TCGA; Biomarker

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Introduction

Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases [1]. Radiation therapy, alone or combined with chemotherapy, is the standard approach for the definitive treatment of locally advanced NSCLC or early-stage disease in patients who are not candidates for surgery [2, 3]. Even when concurrent chemotherapy is used with standard radiation therapy, local-regional relapse rates are unacceptably high, ranging from 20% to 50% [2-5].

A better understanding of radiation resistance and strategies to overcome it are crucial for improving treatment outcomes in NSCLC [6]. Molecular mechanisms underlying tumor radioresistance are complex and include tumor microenvironment, DNA damage and repair, and DNA checkpoint pathways [7, 8]. In NSCLC, overexpression, or mutation of the genes for epidermal growth factor receptor (*EGFR*) and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) has been linked with lung cancer prognosis [9, 10]. However, their role in radiosensitivity remains unclear.

To date, most clinical studies involving molecular biomarkers have focused on the ability of such markers to predict prognosis or be used as the basis for targeted inhibitors rather than as predictors of radiosensitivity. Few clinical studies on radiotherapy and *KRAS* have reported conflicting results [11-14]. Despite the limited results from clinical studies, numerous laboratory investigations have indicated that *KRAS* genotypes have specific properties that are expected to affect radioresistance [15-18]. Thus, we hypothesized that *KRAS* mutation status could predict radioresistance of a particular tumor. To test this hypothesis, we retrospectively analyzed patients with NSCLC who had received definitive radiation therapy and whose *KRAS* mutation status was known. We investigated potential relationships between local tumor progression and mutation status to identify *KRAS* as a molecular marker of radi-

oresistance using an integrative strategy, combining the results of *in vitro* experiments and the Cancer Genome Atlas (TCGA) data to analyze the role of *KRAS* in radioresistance and the potential gene pathway.

Materials and Methods

Patient selection, local tumor progression, and follow-up

Ethics committee approval was obtained from the Medical Ethics Committee of the NanFang Hospital of Southern Medical University (number: NFEC-2017-031). The study was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration. Patients were selected from a clinical database of patients with NSCLC who had received definitive radiation therapy at a single institution. Inclusion criteria were as follows: 1) histologically confirmed stage I - III NSCLC; 2) receipt of ≥ 60 Gy as definitive radiotherapy (or 60 Gy (RBE)) for proton therapy); and 3) available histologic reports on tumor *EGFR* and *KRAS* status. Patients treated with stereotactic ablative radiation therapy or those with unconfirmed NCSLC, stage IV NSCLC, or small cell lung cancer were excluded. A total of 286 patients who met these criteria were identified; these patients had received radiation therapy between May 15, 2004, and April 2, 2014.

Local tumor progression was defined as disease that persisted or recurred within either the radiation field or at the margin of the field [19]. Briefly, in-field progression occurred inside the planning target volume (PTV) or within the 95% prescribed isodose volume; marginal progression occurred outside the PTV, but ≤ 1 cm from the PTV boundary, or outside the 95% specified isodose volume, but within 1 cm of the 95% isodose line. At least two experienced radiation oncologists, who reviewed radiology reports and computed tomography (CT) scans, positron emission tomography (PET) scans, or PET/CT images, confirmed progression. Biopsy was not required to confirm local progression if serial imaging revealed persistent or recurrent disease [20].

Follow-up visits were conducted at least once before radiation therapy and weekly during treatment; each visit included interval history and physical examinations. Post-treatment follow-up visits were scheduled during the first 1 - 3 months after completing radiation therapy, every 3 - 4 months thereafter for the first 2 - 3 years, and then twice a year until 5 years after completing radiation therapy. Chest CT and PET were performed every 3 - 6 months after radiation therapy.

Cell lines and reagents

Lung cancer cell lines with *KRAS* mutation (H460 and A549) and wild-type *KRAS* (H1299 and H661) were used. The cancer cell lines were cultured in RPMI-1640 medium. All experiments were performed using confluent cultures maintained in 10% serum.

Clonogenic assays

Cell lines with *KRAS* mutation (H460 and A549) and wild-type *KRAS* (H1299 and H661) were grown to 40-60% confluency; 50 cells were plated for the control (no radiation) condition with an increased number of cells plated for samples exposed to higher doses of radiation (150 for 2 Gy, 300 for 4 Gy, and 600 for 6 Gy). After irradiation, the plates were placed back into a 37 °C incubator with 5% CO_2 and allowed to divide for 10 - 14 days until sufficient colonies with more than 50 cells per colony were obtained. The medium was then removed, and the cells were stained with 0.5% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) in methanol, rinsed, and colonies containing more than 50 cells were counted. Survival was calculated relative to that of non-irradiated cells (survival = (plating efficiency of treated cells)/(plating efficiency of control cells), where plating efficiency = (number of colonies formed by treated cells)/(number of colonies formed by untreated cells)).

Immunofluorescence

H460 cell lines with *KRAS* mutation and H1299 cell lines with wild-type *KRAS* were grown on glass coverslips, and irradiated with 4 Gy after 1 h, 8 h, and 24 h; washed with phosphate-buffered saline (PBS); fixed in 2% paraformaldehyde/PBS for 10 min; and processed for immunofluorescence using the relevant γH2AX antibody (1:400, Cell Signaling Technology)). The relevant secondary antibodies were fluorochrome-conjugated Cy3 (1:300, Jackson ImmunoResearch). Images were captured using a digital camera (AxioCam MRm; Carl Zeiss MicroImaging, Inc.) attached to a fluorescent microscope (Axioskop2 Mot Plus; Carl Zeiss MicroImaging, Inc.) (× 100 magnification). AxioVision LE 4.3 software (Carl Zeiss MicroImaging, Inc.) was used to capture the individual images. Fluorescence intensity was quantitated using ImageJ software.

TCGA database analysis

Archived data were from The Cancer Genome Atlas for Lung Adenocarcinoma (TCGA LUAD) database (https://tcga-data.nci.nih.gov). Data were selected based on patient and cell samples subjected to reverse-phase protein array (RPPA) analysis. One hundred sixty patients and 160 cell lines were available in the database. RPPA analysis selected at the false discovery rate (FDR) level of 0.10 was used to create the heatmap for patients. For the cell lines, the top 15 were used to create the heat map. The P value (≤ 0.05) hits from the records were then collectively input into the protein association networks (http://genecodis.cnb.csic.es) to determine the pathway.

Statistical analysis

The relationship between *KRAS* status and other clinicopathological characteristics was analyzed using the Chi-squared test. Means of age, radiation dose, and gross tumor volume

(GTV) were compared using Mann-Whitney U tests. Local progression-free survival (LPFS) was calculated from the date of definitive radiation therapy termination using the Kaplan-Meier actuarial method. The influence of variables on survival was studied using univariate and multivariate analyses (Cox proportional hazards models). Independent sample *t*-tests were used to compare the average number of γH2AX foci in H1299 and H460 cells after radiation at different time points. All analyses were performed using Stata version 10.1. The hazard ratios (HR) and 95% confidence intervals (CIs) were calculated. Differences were considered statistically significant at $P < 0.05$.

Results

Mutated *KRAS* increased local tumor progression after definitive radiation therapy for patients with NSCLC

The characteristics of the 286 identified patients are shown in Table 1; 68 patients (24%) had local progression, the mean (± standard deviation (SD) patient age was 63.9 (± 10.4) years, and most patients (252, 88%) had stage III disease. The progression/no progression groups were relatively well balanced, except for age (patients without progression were slightly older than those with progression, P = 0.034), radiation modality (29% of those treated with photons had progression vs. 16% of those treated with protons, P = 0.02), and receipt of induction chemotherapy (32% of those who had received induction chemotherapy vs. 19% of those who had not received induction chemotherapy, P = 0.012). Mean radiation dose was similar between patients who did and did not experience local progression (68.1 ± 5.1 Gy or Gy (RBE) vs. 68.7 ± 6.5 Gy or Gy (RBE), P = 0.785). Although the SD values were large, the GTV was not different for those who did not experience progression. Most patients (262, 94%) received concurrent chemotherapy, but the progression rate was higher among those who received induction therapy (32%) than among those who did not undergo induction chemotherapy (19%, P = 0.02). The median follow-up time for the 68 patients with local progression was 57.4 months (range 1.27 - 93.5 months), and the mean interval to progression was 27 months (± 17.4 months (SD)). Among the patients who experienced local progression, *EGFR* mutations were detected in five of 67 patients (7%) and *KRAS* mutations in 14 of 60 patients (23%) (Table 1). Only one patient had mutations in both *KRAS* and *EGFR* and was excluded from the LPFS analysis. The PET and CT images of a representative patient with local progression are shown in Figure 1.

Univariate and multivariate analyses to identify the potential predictors of local progression are shown in Tables 2 and 3, respectively. In the univariate analysis, only concurrent chemotherapy was associated with better LPFS (HR: 0.385; 95% CI: 0.182 - 0.815; P = 0.013) and having a larger GTV may have been linked with poorer LPFS (HR: 1.002; 95% CI: 0.999 - 1.004; P = 0.058). Neither *EGFR* nor *KRAS* status was associated with LPFS in the univariate analysis (Table 2, Fig. 2a, b). However, in multivariate analysis, after adjustment for concurrent chemotherapy, GTV, and mutation status, *KRAS* mutation was associated with poorer LPFS (*KRAS* mutation: HR: 1.961; 95% CI: 1.062 - 3.622; P = 0.031) (Table 3).

KRAS mutation is associated with radioresistance in human NSCLC cell lines

We hypothesized that NSCLC cell lines with *KRAS* mutation would be more resistant to radiation than cell lines with wild-type *KRAS*. To test this hypothesis, we performed clonogenic assays with two cell lines harboring *KRAS* mutations (H460 and A549) and two cell lines with wild-type *KRAS* (H1299 and H661). In support of our previous results in patients, we found that cell lines with *KRAS* mutations were more resistant to radiation than wild-type cells (Fig. 3a). In contrast, H1299 (*KRAS* wild-type (wt)) and H460 (*KRAS* mutation (mut)) were irradiated with ionizing radiation (4 Gy); immunofluorescence analyses were performed at the indicated time points. The number of γH2AX foci was then counted and quantified. Representative images of γH2AX foci in H460 (*KRAS* mut) and H1299 (*KRAS* WT) cells at each time point are shown in Figure 3b, c. The average (± SD) number of γH2AX foci per cell of control was not different between H460 and H1299 cell lines (7.61 ± 1.42 vs. 5.97 ± 1.13, P = 0.118). γH2AX foci were significantly higher after radiation, and significantly different from that at baseline at 1 h (25.32 ± 4.11 vs. 33.54 ± 1.93, P = 0.042) and 8 h (17.47 ± 3.51 vs. 28.3 ± 6.64, P = 0.043), and significantly higher at 24 h (12.33 ± 1.52 vs. 21.67 ± 3.78, P = 0.007) for the H1299 cell line (Fig. 3d).

TCGA data showed multiple pathways involved in *KRAS* mutation patients and cell lines

TCGA LUAD data showed that the ERK1/2 pathway, phosphatidylinositol I3 kinase (PI3K)/mTOR, p38 MAPK pathway, cell cycle checkpoint signaling, DNA damage, repair pathways, and EGFR/PKC/AKT pathway were differentially expressed in *KRAS* mutations patients or cell lines relative to the wild-type group. Differentially expressed genes for patients with *KRAS* mutations are shown in Figure 4a. Raf-pS338, MEK1 pS217-S221, MAPK-pT202-Y204, and YB.1-Ps102 were upregulated, while ERK2 was downregulated in the ERK1/2 pathway. HER3, mTOR-pS2448, and S6-pS235-S236 were upregulated, and 4EBP1 was downregulated in the PI3K/mTOR pathway. p90RSK-pT359-S363 was upregulated, and PI3K.p110.alpha and STAT5.alpha were downregulated in the p38 MAPK pathway. Regarding cell cycle checkpoint signaling, X53BP1, Chk2, CyclinE1, and CyclinB1 were downregulated. PARP was upregulated and ATM, Ab.3, and PCNA were downregulated in DNA damage and repair signaling. For *KRAS* mutation cell, PKC.alpha, PKC.alpha pS657, PKC.delta pS664, IRS1, transglutaminase, MIG.6, Akt, and Y-box binding protein-1 (pS102) (YB-1 pS102) were upregulated, and EGFR pY1068, GAB2, and ShcpY317 were downregulated in the EGFR/PKC/AKT pathway (Fig. 4b). TCGA data from lung adenocarcinoma patients and cell lines showed that YB-1

Table 1. Patient Characteristics

Characteristic	All	Local progression	No local progression	P value
KRAS status				
WT	187	46	141	0.843
Mutation	54	14	40	
EGFR status				
WT	252	62	190	0.376
Mutation	32	5	27	
Age, years, mean ± SD	63.9 ± 10.4	61.9 ± 9.7	64.6 ± 10.6	0.034
Sex				
Male	144	38	106	0.296
Female	142	30	112	
Race				
Other	31	9	22	0.467
White	255	59	196	
Karnofsky performance status				
> 80	120	26	94	0.476
≤ 80	166	42	124	
Disease stage				
I - II	34	9	25	0.694
III	252	59	193	
Tumor histology				
Squamous cell	67	21	46	0.122
Adenocarcinoma	177	35	142	
NSCLC, other	42	12	30	
Smoking status				
No smoking	42	11	31	0.242
Former	178	37	141	
Current	61	19	42	
Dose, mean ± SD, Gy or Gy (RBE)	68.7 ± 6.2	68.1 ± 5.1	68.7 ± 6.5	0.785
Radiation modality				
Photon	173	50	123	0.012
Proton	113	18	95	
Chemotherapy				
No induction chemotherapy	185	36	149	0.02
Induction chemotherapy	101	32	69	
No concurrent chemotherapy	24	8	16	0.251
Concurrent chemo	262	60	202	
Gross tumor volume, cm^3	122.0 ± 128.1	120.2 ± 120.5	122.5 ± 130.7	0.852

EGFR: epidermal growth factor receptor; *KRAS*: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; WT: wild-type; NSCLC: non-small cell lung cancer.

pS102 was upregulated in the *KRAS* mutation group.

Discussion

In this study, we found that the presence of mutated *KRAS* in patients undergoing definitive radiation therapy for NSCLC was associated with inferior local control after adjustment for concurrent chemotherapy and GTV, suggesting that *KRAS* mutations may confer radioresistance in NSCLC. To the best of our knowledge, this is the first clinical study to show an association between *KRAS* mutation status and local tumor

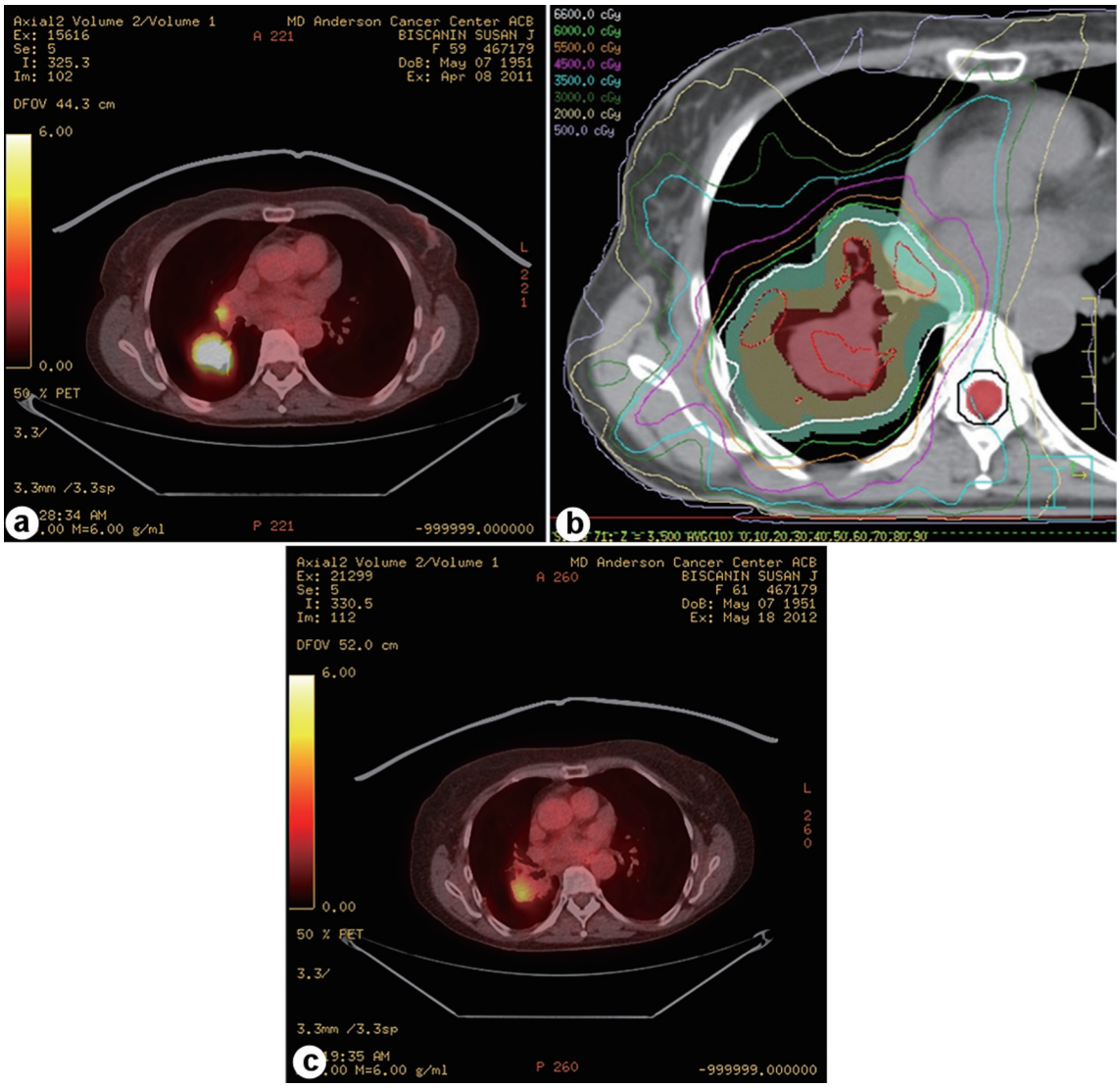

Figure 1. Local tumor progression in a patient with stage III adenocarcinoma lung cancer. (a) Positron emission tomography (PET) image from diagnosis. (b) Intensity-modulated photon radiation therapy plan with isodose lines and planning target volume (PTV) in green colorwash. (c) Post-treatment PET scan shows local tumor progression inside the PTV.

control in patients with NSCLC after definitive radiation therapy.

Local control is strongly linked with improved overall survival in locally advanced NSCLC [19, 21]. Assessment of local control after radiation therapy is governed by both the accuracy of detecting such diseases after treatment and the observation interval between treatment completion and progression or recurrence [22-24]. Hazuka et al [24] suggested that local progression can be diagnosed based on clinical, bronchoscopic, or radiographic evidence of tumor regrowth within the irradiated field. Martel et al [23] indicated that LPFS rates should be calculated at ≥ 30 months after radiation therapy. We also defined local progression according to PET, CT, and biopsy findings, and our median follow-up time for LPFS extended well beyond the recommended 30-month minimum (median, 57.4 months; range, 1.27 - 93.5 months).

Although others have found local control to be associated with performance status, concurrent chemotherapy, and radiotherapy dose [19], we found only one such association between local control and concurrent chemotherapy. Because the prescribed dose in our study was 68.7 ± 6.2 Gy (or Gy (RBE)), dose escalation did not improve local control [25], and the dose to the tumor field in our study met the requirement that 95% of the PTV received 100% of the prescription dose, we conclude that these instances of local tumor progression indicated intrinsic radioresistance.

Tumor radioresistance, whether inherent or acquired, is a significant obstacle in the effective treatment of NSCLC. The mechanisms influencing intrinsic radiosensitivity have suggested that up to 80% of this variability could have a genetic basis [26-28]. DNA double-strand breaks (DSBs) are thought to be the most severe molecular consequences of radiation

Table 2. Univariate Analysis of Independent Predictors of Local Progression

	HR	95% CI	P value
KRAS status			
WT (reference)			
Mutation	1.585	0.865 - 2.901	0.135
EGFR status			
WT (reference)			
Mutation	0.475	0.190 - 1.119	0.113
Age, years	0.986	0.961 - 1.012	0.301
Sex			
Male (reference)			
Female	0.754	0.462 - 1.229	0.258
Race			
Non-white (reference)			
White	0.97	0.479 - 1.96	0.933
Karnofsky performance status			
> 80 (reference)			
≤ 80	1.149	0.698 - 1.891	0.585
Disease stage			
I - II (reference)			
III	0.966	0.476 - 1.957	0.923
Tumor histology			
Squamous cell (reference)			
Adenocarcinoma	0.783	0.452 - 1.355	0.382
NSCLC, other	0.78	0.383 - 1.588	0.494
Smoking status			
No smoking (reference)			
Former	0.886	0.449 - 1.748	0.727
Current	1.217	0.578 - 2.564	0.605
Radiation dose, Gy or Gy (RBE)	1.012	0.969 - 1.057	0.574
Radiation modality			
Photon (reference)			
Proton	0.772	0.449 - 1.326	0.348
Chemotherapy			
No induction chemotherapy (reference)			
Induction chemotherapy	1.091	0.673 - 1.768	0.724
No concurrent chemotherapy (reference)			
Concurrent chemotherapy	0.385	0.182 - 0.815	0.013
Gross tumor volume, cm^3	1.002	0.999 - 1.004	0.058

EGFR: epidermal growth factor receptor; *KRAS*: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; WT: wild-type; NSCLC: non-small cell lung cancer; HR: hazard ratio; CI: confidence interval.

therapy [29], and DSB repair is a determinant of cellular radiosensitivity [30]. Although several promising biomarkers of cellular radiosensitivity have been tested, there is insufficient evidence of their utility in clinical practice [31]. Therefore, we used the gold standard colony-survival assay to evaluate radiation sensitivity and the efficiency of DSB repair in NSCLC cell lines harboring different *KRAS* mutation status [32]. Another cellular radiosensitivity biomarker is γH2AX foci

Table 3. Multivariate Analysis of Independent Predictors of Local Progression

	KRAS status		*EGFR* status	
	HR (95% CI)	P value	HR (95% CI)	P value
KRAS status				
WT (reference)	1.961 (1.062 - 3.622)	0.031		
Mutation				
EGFR status				
WT (reference)			0.601 (0.237 - 1.52)	0.283
Mutation				
Chemotherapy				
No concurrent chemotherapy (reference)	0.301 (0.132 - 0.683)	0.004	0.352 (0.156 - 0.798)	0.012
Concurrent chemotherapy				
Gross tumor volume, cm^3	1.003 (1.000 - 1.004)	0.013	1.002 (1.000 - 1.005)	0.017

EGFR: epidermal growth factor receptor; *KRAS*: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; WT: wild-type; HR: hazard ratio; CI: confidence interval.

quantification [33]; H2AX is a central component of numerous signaling pathways in response to DSBs [34]. It is rapidly phosphorylated in response to DNA DSBs and contributes to repair protein recruitment to these damaged sites. H460 cells (*KRAS* mutation) showed lower induction of γH2AX a lower rate of foci disappearance after irradiation compared to H1299 cells (*KRAS* WT), which were considered to be correlated with radioresistance.

Our findings provide *in vitro* evidence that NSCLC cell lines transfected with a *KRAS* mutant are more resistant to radiation, consistent with the presence of a *KRAS* mutation associated with local control after definitive radiation therapy for NSCLC.

How does the mutation status of *KRAS* contribute to radio response? We compared the gene expression of different *KRAS* statuses in the TCGA LUAD database, which includes data on multiple pathways in *KRAS* signaling. Some studies have shown consistency with TCGA analysis [35-37]. After radiation, ligand-independent phosphorylation of EGFR can activate the RAS/RAF/MEK/MAPK, PI3K/AKT, and STAT3/STAT5 pathways; *KRAS*-mutated human tumor cell lines might activate EGFR via upregulated autocrine/paracrine production and secretion of EGFR ligands, resulting in an upregulation of the EGFR-PI3K-AKT-survival pathway [35], which is involved in the resistance of NSCLC to radiotherapy. It is considered responsible for the accelerated repopulation of tumor clonogenic cells during radiotherapy [38].

Interestingly, data from both the patient and cell lines showed that YB-1 pS102 was upregulated in the *KRAS* mutation group. YB-1 belongs to a family of DNA-binding proteins [39]. YB-1 is involved in many pathways, including the E2F pathway [40], PI3K/Akt kinase signaling [41], MAPK/ERK

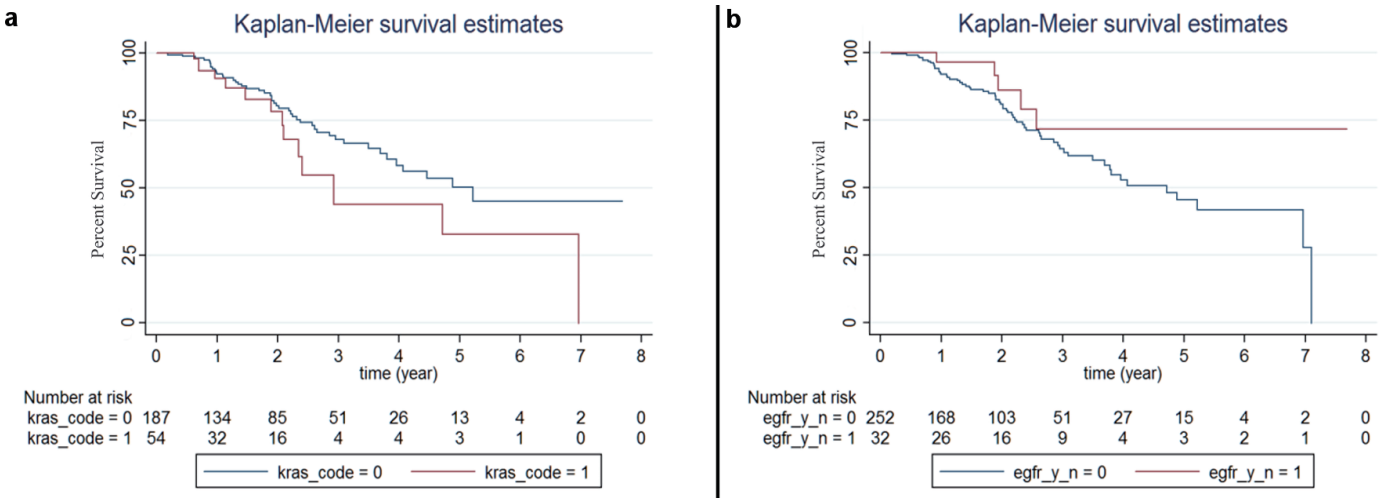

Figure 2. Kaplan-Meier plots of local progression-free survival (LPFS) according to *EGFR* and *KRAS* mutation status. (a) LPFS curves for patients with *KRAS* mutation (red line; krasm = 1), *KRAS* wild-type (WT; blue line (krasm = 0), P = 0.129). (b) LPFS curves for patients with *EGFR* mutation (red line; egfr = 1), *EGFR* WT (blue line; egfr = 0) P = 0.099. *EGFR*: epidermal growth factor receptor; *KRAS*: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog.

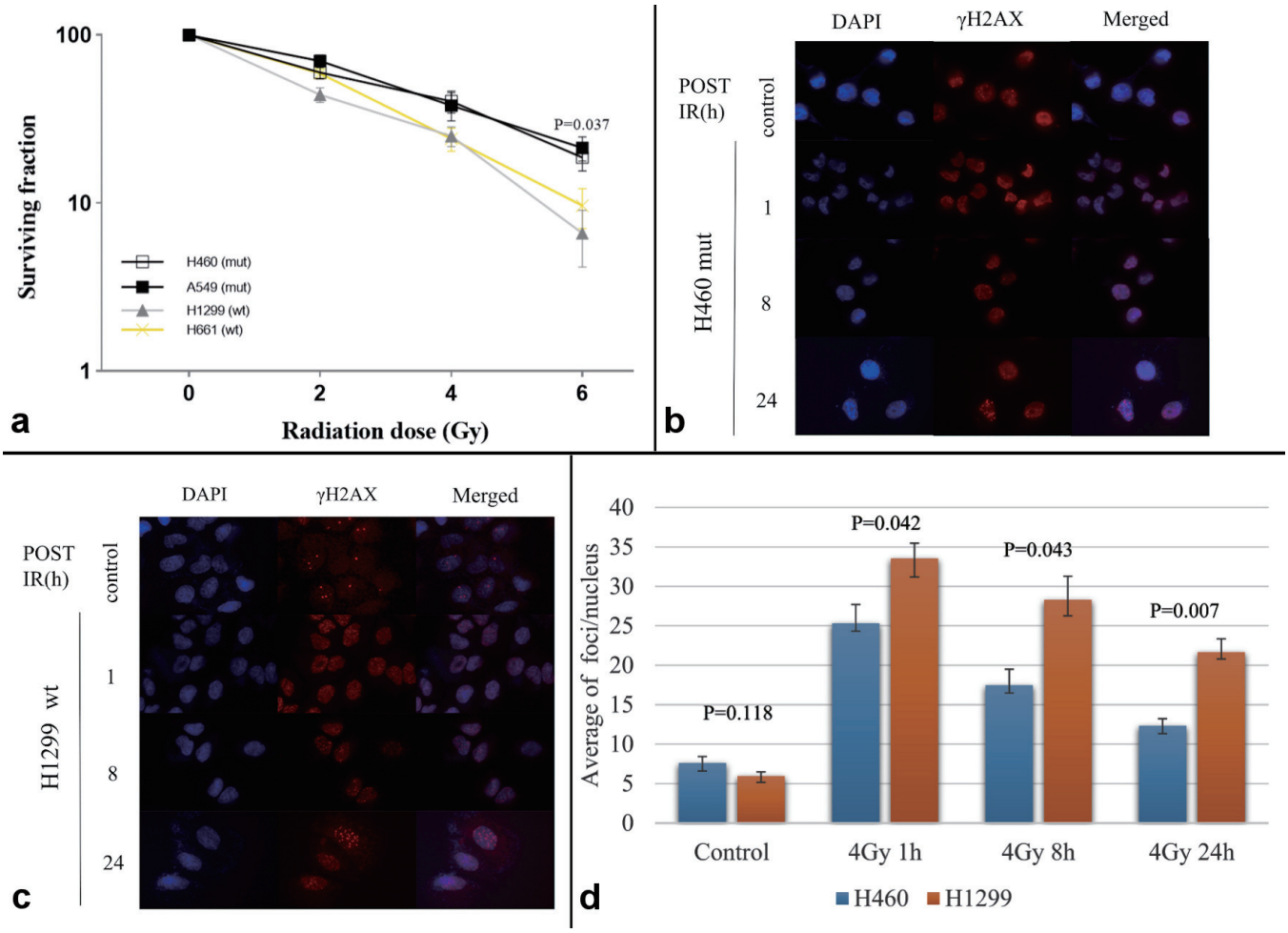

Figure 3. (a) Clonogenic assays showed that cell lines with *KRAS* mutation (H460 and A549) are more resistant than wild-type cells (H1299 and H661) to radiation. (b) H460 (*KRAS* mutation) and (c) H1299 (*KRAS* wild-type) treated with 4 Gy X-ray for 1, 8, and 24 h. Cells were then fixed and immune stained for γH2AX foci (red). Nuclei were counterstained with DAPI (blue). (d) For each time point, five to 10 images were captured and used for quantification of γH2AX foci number. The graph represents an average of three independent experiments ± SD. *KRAS*: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; DAPI: 4',6-diamidino-2-phenylindole; SD: standard deviation.

signaling [42], and EGFR pathways. It was also shown that radiation or mutated *KRAS* overexpression in breast cancer cell lines enhanced basal YB-1 phosphorylation and increased DNA DSB repair and post-irradiation survival [42]. Our future work will explore the role of YB-1 pS102 in *KRAS* mutation NSCLC.

Radiation therapy is the definitive treatment for NSCLC but is associated with high rates of local failure. *KRAS* is an essential predictor of the prognosis of NSCLC. However, its role in tumor response to radiation is not entirely clear. This study determined *KRAS* mutation status in conjunction with local tumor progression after definitive radiation therapy for NSCLC, indicating intrinsic radioresistance. Verified using a clonogenic assay and *in vitro* immunofluorescence, TCGA analysis was used to explore differential gene expression and potential pathways. Our findings could be helpful for the baseline prediction of outcomes according to *KRAS* genotype and may provide a potential target for radiosensitization in future studies.

Our conclusion from the current study was that *KRAS* mutations are associated with NSCLC. *KRAS* mutation status may be helpful as a biomarker of radioresistance and a potential target for increasing radiosensitivity.

Acknowledgments

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Financial Disclosure

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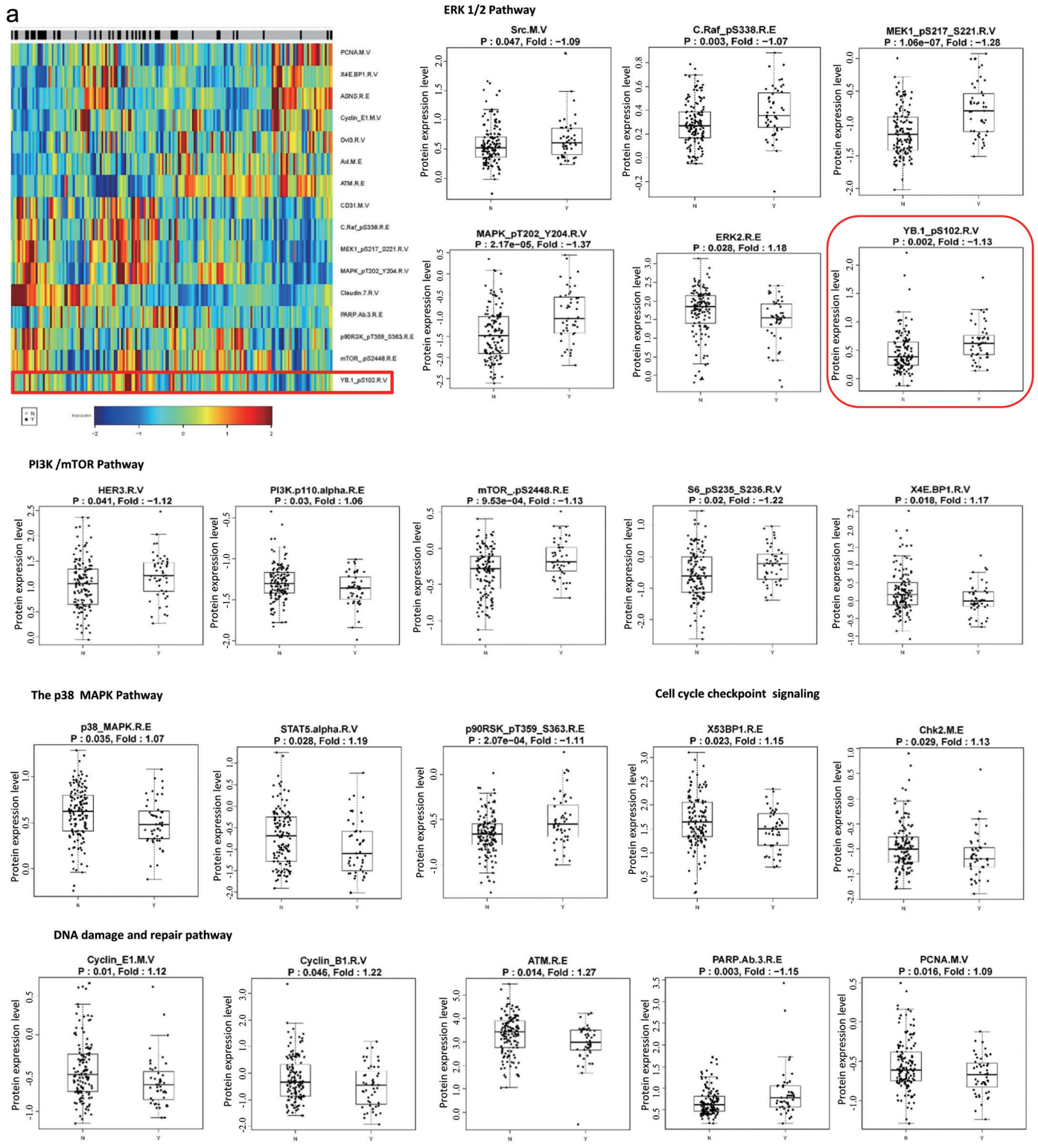

Figure 4. TCGA LUAD data show differentially expressed genes in the ERK1/2 pathway, phosphatidylinositol l3 kinase (PI3K)/mTOR, p38 MAPK pathway, cell cycle checkpoint signaling, and DNA damage. The repair and EGFR/PKC/AKT pathways presented with differential expression in patients (a) and cell lines (b) with *KRAS* mutations compared with the wild-type group. Y: patients or cell line with mutation; N: patients or cell line without mutation. TCGA LUAD: The Cancer Genome Atlas for Lung Adenocarcinoma.

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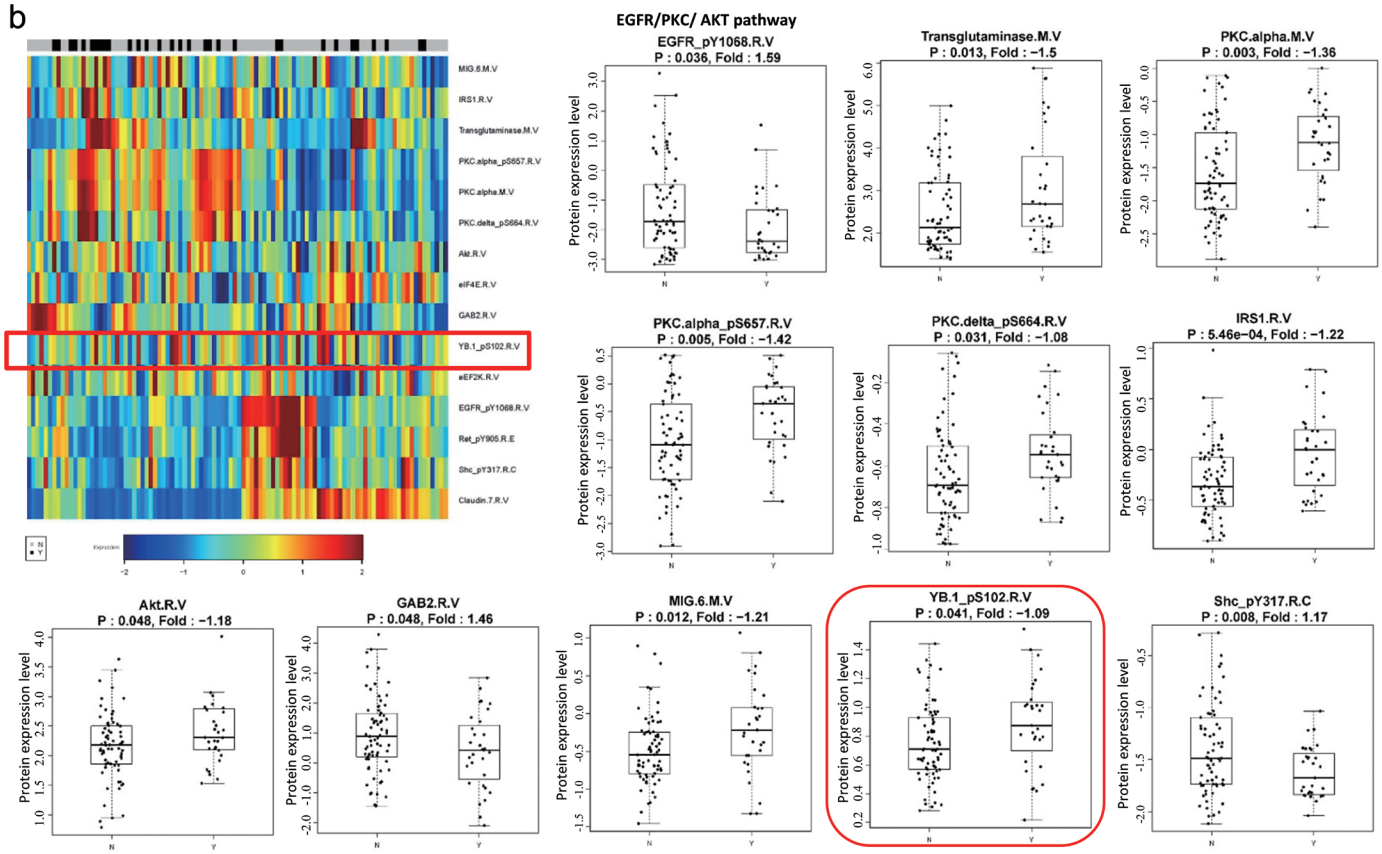

Figure 4. *(continued)* TCGA LUAD data show differentially expressed genes in the ERK1/2 pathway, phosphatidylinositol l3 kinase (PI3K)/mTOR, p38 MAPK pathway, cell cycle checkpoint signaling, and DNA damage. The repair and EGFR/PKC/AKT pathways presented with differential expression in patients (a) and cell lines (b) with *KRAS* mutations compared with the wild-type group. Y: patients or cell line with mutation; N: patients or cell line without mutation. TCGA LUAD: The Cancer Genome Atlas for Lung Adenocarcinoma.

Conflict of Interest

All authors have no conflict of interest to declare.

Informed Consent

Informed consents were obtained from all patients.

Author Contributions

Wei Xiao and Qin Fan designed this study. Dao Qi Zhu, Qin Fan, and Ai Wu Li coordinated the study and finalized the manuscript. Zhi Jian Yu, Ru Hua Zhang, and Feng Ying Gong performed the experiments. Dao Qi Zhu, Ying Liu, and Wei Wang analyzed the data. Ying Liu and Dao Qi Zhu wrote the paper.

Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations

WT: wild-type; HR: hazard ratio; CI: confidence interval; FDR: false discovery rate; GTV: gross tumor volume

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