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 chemotheraphy, gross tumor volume, and mutation status in multivariate analysis, KRAS mutation status was associated with LPFS (hazard ratio: 1.961; 95% confidence interval: 1.03 - 2.17; \( P = 0.032 \)). KRAS mutation showed higher radiosensitivity 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/PIK3/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

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: NFE-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% CO₂ 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-buff ered 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 fluochrome-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 heatmap. 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). Among the patients who experienced local progression, \( \text{H}_{1299} \) and \( \text{H}_{1299} \text{WT} \) 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 \( \text{H}_{1299} \) (\( \text{KRAS} \) mut) and \( \text{H}_{1299} \) (\( \text{KRAS} \) 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 \( \text{H}_{1299} \) and \( \text{H}_{1299} \text{cell lines (7.61 ± 1.42 vs. 5.97 ± 1.13, P = 0.118) \gamma H2AX foci were significantly higher after radiation, and significantly different from that at baseline at 1 h (23.52 ± 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 \( \text{H}_{1299} \) 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 (\( \text{PI3K} / \text{mTOR} \)), p38 MAPK pathway, cell cycle checkpoint signaling, DNA damage, repair pathways, and EGFR/PKC/AKT pathway were differentially expressed in \( \text{KRAS} \) mutations patients or cell lines relative to the wild-type group. Differentially expressed genes for patients with \( \text{KRAS} \) mutations are shown in Figure 4a. \( \text{Raf-pS338, MEK1 pS217-S221, MAPK-pT202-Y204, and YB1-pS102} \) were upregulated, while \( \text{ERK2} \) was downregulated in the \( \text{ERK1/2} \) pathway. \( \text{HER3, mTOR-pS2448, and S6-pS235-S236} \) were downregulated, and \( \text{4EBP1} \) was downregulated in the \( \text{PI3K/mTOR} \) pathway. \( \text{p90RSK-pT359-S363} \) was upregulated, and \( \text{PI3K.p110} \) and \( \text{STAT5} \) were downregulated in the p38 MAPK pathway. Regarding cell cycle checkpoint signaling, \( \text{X53BP1, Chk2, CyclinE1, and CyclinB1} \) were downregulated. \( \text{PARP} \) was upregulated and \( \text{ATM} \) and \( \text{PCNA} \) were downregulated in DNA damage and repair signaling. For \( \text{KRAS} \) mutation cell, \( \text{PKC.alpha, PKC.delta pS664, IRS1, transglutaminase, MIG.6, Akt, and Y-box binding protein-1} \) (\( \text{pS102} \)) (\( \text{YB1-pS102} \)) were upregulated, and \( \text{EGFR} \) \( \text{pY1068, GAB2, and ShpY317} \) were downregulated in the EGFR/PKC/AKT pathway (Fig. 4b). TCGA data from lung adenocarcinoma patients and cell lines showed that \( \text{YB1} \)
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 control.

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                            | 120          | 26               | 94                   | 0.785   |
| Radiation modality                    |              |                  |                      |         |
| Photon                                | 173          | 90               | 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, cm³               | 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
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 $68.7 \pm 6.2$ 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

**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.
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 \textit{KRAS} mutation status [32]. Another cellular radiosensitivity biomarker is γH2AX foci.

### Table 2. Univariate Analysis of Independent Predictors of Local Progression

|                         | HR     | 95% CI        | P value |
|-------------------------|--------|---------------|---------|
| \textit{KRAS} status    |        |               |         |
| WT (reference)          |        |               |         |
| Mutation                | 1.585  | 0.865 - 2.901 | 0.135   |
| \textit{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, cm³ | 1.002  | 0.999 - 1.004 | 0.058   |

\textit{EGFR}: epidermal growth factor receptor; \textit{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.
Mutated KRAS and Radiation Resistance

H2AX is a central component of numerous signaling pathways in response to DNA DSBs. 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 and 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. 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, which is involved in the resistance of NSCLC to radiotherapy.

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. YB-1 is involved in many pathways, including the E2F pathway, PI3K/Akt kinase signaling, MAPK/ERK

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                                | 0.601 (0.237 - 1.52)                 | 0.283   |
| Mutation             |                                      |                                      |                                      |
| EGFR status          |                                      |                                      |                                      |
| WT (reference)       | 0.301 (0.132 - 0.683)                | 0.004                                | 0.352 (0.156 - 0.798)                | 0.012   |
| Mutation             |                                      |                                      |                                      |
| Chemotherapy         |                                      |                                      |                                      |
| No concurrent chemotherapy (reference) | 1.003 (1.000 - 1.004) | 0.013                         | 1.002 (1.000 - 1.005) | 0.017 |
| Concurrent chemotherapy |                                   |                                      |                                      |

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

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.
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.

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Figure 4. TCGA LUAD data show differentially expressed genes in the ERK1/2 pathway, phosphatidylinositol 3 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.

(continued)
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 Hu 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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