Short Communications

Editorial Process: Submission:09/04/2024 Acceptance:06/05/2025

Effects of *PIK3CA* Gene Modifications on Radiosensitivity in Colorectal Cancer Cells

Ghazi Alsbeih*, Khaled Al-Hadyan

Abstract

The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha *(PIK3CA)* plays a critical role in cell growth and survival. *PIK3CA* somatic mutations are linked to the *PIK3CA*-related overgrowth spectrum (PROS) syndrome. Cells from those patients appeared to be associated with a moderate but significant radiosensitivity. Mutations or amplifications in this gene are common in breast, colorectal, and lung cancers. Alterations in the *PIK3CA* gene, including amplification and mutation, are common in cancer, but their influence on radiotherapy is not yet fully understood. This report reveals a potential association between *PIK3CA* gene modifications and radiosensitivity ($p < 0.05$) deducted from 8 established colorectal cancer cell lines (HT-29, DLD-1, HCT-116, SW480, HCT-15, Colo-320, and LoVo). Meanwhile gene amplification (> 2) seems to be linked to increased radiation sensitivity, mutations appear to be associated with increased radioresistance in colorectal cancer cells. Leveraging this relationship, *PIK3CA* amplification and mutations could act as biomarker to pinpoint patients who might benefit from more personalized radiotherapy regimens. However, this association is preliminary and hypothesis-generating in view of the limited number of cases. Further studies are needed to confirm this conclusion. By uncovering the distinct mechanistic effects of these *PIK3CA* alterations on radiosensitivity phenotype in both normal and cancerous cells, researchers can lay the groundwork for tailored radiotherapy strategies in colorectal cancer. This insight could enhance treatment effectiveness while reducing side effects, ultimately leading to improved patient outcomes.

Keywords: colorectal cancer- *PIK3CA*- gene amplification- gene mutation- radiosensitivity

Asian Pac J Cancer Prev, **26 (6)**, 1875-1879

Introduction

The complex relationship between genetic alterations and cancer treatment response is a central focus in research, especially in the context of radiotherapy [1]. Among the numerous genetic aberrations linked to cancer, the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene is a significant player in various malignancies [2]. *PIK3CA*, which encodes the catalytic subunit of phosphoinositide 3-kinase (PI3K), is a crucial part of the PI3K/AKT/mTOR pathway, a signalling cascade involved in cell growth, survival, and metabolism. Changes in *PIK3CA* function, due to gene amplification or mutation, have been identified in a range of cancers, including breast, colorectal, and lung cancers [3]. While several studies have reported an association between *PIK3CA* alterations and poor prognosis in cancer patients [4], conflicting reports exist regarding the impact of *PIK3CA* gene mutations on the response of different cancers to chemo-immunotherapy [5-7]. This raises the contentious question of whether these genetic changes also affect the tumor's response to radiotherapy.

Radiation sensitivity, a complex trait reflecting a tumor's susceptibility to ionizing radiation, is a critical factor in determining treatment outcomes [8, 9]. Understanding the role of *PIK3CA* gene amplification and mutation in shaping radiosensitivity could provide valuable insights for developing more effective and personalized therapeutic strategies. While *PIK3CA* mutations have been studied in the context of drug-based therapies [10], their influence along with gene amplification on the response to radiotherapy remains largely unexplored.

Bachelet and colleagues [11] presented the first radiobiological characterization of cells derived from a patient with somatic post-zygotic mutations in the phosphatidylinositol 3-kinase catalytic subunit (PI3KCA, more commonly known as *PIK3CA*) gene, leading to the so-called "*PIK3CA*-related overgrowth spectrum (PROS) syndrome." They found that the patient's cells displayed a moderate but significant increase in radiosensitivity, as measured by several assays, including clonogenic cell survival, gamma-H2AX, micronuclei (both surrogate markers of DNA double-strand breaks and misrepair, respectively), and delayed radiation-induced nucleoshuttling of the ATM kinase. As PROS patients are frequently exposed to ionizing radiation for

*Radiation Biology Section, Biomedical Physics Department, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. *For Correspondence: galsbeih@kfshrc.edu.sa*

diagnostic imaging, the authors proposed a radioprotective approach using bisphosphonates combined with statins, which can partially revert the radiosensitive phenotype and render cells more radioresistant. These findings help to better characterize this disorder. Although no excess of *PIK3CA*-associated adult cancers has been reported in PROS syndrome, the PI3K pathway is frequently altered in cancer, and both *PIK3CA* gene amplification and mutations are commonly found in various tumors [12, 3].

This report enhances our understanding of the controversial issue of the impact of *PIK3CA* gene amplification and mutation on radiosensitivity, illuminating potential implications for personalized cancer treatment strategies.

PIK3CA gene amplification and radiosensitivity

Gene amplification (an increase in number to >2 copies) is a primary mechanism for cancer cells to augment the expression of gene products that confer growth or survival advantages [13]. A previous collaborative study used fluorescence in situ hybridization to evaluate *PIK3CA* amplification in tissue microarray and DNA chip (Affymetrix HG 250K Sty) analysis of 448 colorectal (CR) carcinoma samples. It found that survival was significantly longer in patients with *PIK3CA*-amplified cancers [14]. This association was independent of stage, grade, histology subtype, gender, and age categories. However, this finding is not unanimous, and aggressive cancers with poor prognosis have also been associated with *PIK3CA* gene amplification [4]. Thus, the prognostic value of *PIK3CA* marker remains unclear, particularly if the amplified gene is mutated.

The influence of *PIK3CA* gene amplification on radiosensitivity was examined in 8 established colorectal cancer cell lines (HT-29, DLD-1, HCT-116, SW480, CaCo-2, HCT-15, Colo-320, and LoVo) available in our laboratory. These cell lines were previously obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA) and were routinely cultured in the recommended cell culture medium. These cell lines are widely used in colorectal cancer research. They have various genetic makeup, origin, and phenotypic characteristics. Together, they represent key aspects of colorectal cancer heterogeneity, including common mutations (such as in *APC, KRAS, TP53* and *PIK3CA* genes), and differentiation states. However, they are simplified in vitro models and do not capture the full complexity of patient tumors, particularly regarding the tumor microenvironment and intra-tumor heterogeneity. The method for assessing *PIK3CA* gene amplification was detailed previously [14]. The technique primarily relies on the DNA chip "Affymetrix HG 250K Sty," which enables the detection of DNA copy number alterations, for instance in the *PIK3CA* genomic locus. In brief, copy numbers were derived from the Chromosome Copy Number Tool (CNAT, version 4.0) and displayed as log2 ratios. Amplification is defined as an estimated absolute copy number > 2 for the region encompassing the *PIK3CA* gene. Results were validated using fluorescence in situ hybridization (FISH) confirming *PIK3CA*/centromere 3 ratio > 2, and quantitative real-time (qPCR) PCR demonstrating a significant fold-increase over diploid controls (LINE1), supporting the accuracy of the assessment. The findings revealed that SW480, HCT-15, Colo-320, and LoVo exhibited *PIK3CA* gene amplification ranging from 3 to 10 copies (Table 1).

Radiosensitivity was assessed using the gold-standard clonogenic survival assay, as detailed in previous work [15]. In brief, cells in exponential growth were trypsinized, counted, diluted, and seeded in sufficient quantities to produce at least 50 colonies in each of three flasks. The cells were incubated for 3–4 h to allow attachment before being exposed to a single radiation dose ranging from 0 to 10 Gy. This irradiation was conducted at room temperature using a 6-MV photon linear accelerator at a dose rate of 2 Gy/min. Following a 2-week incubation period, the cells were fixed and stained with crystal violet. Colonies consisting of at least 50 cells were classified as survivors. This process was repeated in three independent experiments for each cell line. For data analysis, surviving fractions from replicate experiments were combined and fitted to the linear quadratic model of cell killing: $SF = \exp(-\alpha D - \beta D^2)$. The surviving fraction at 2 Gy (SF2) was calculated from the entire survival curve and utilized as a singular measure of cellular radiosensitivity [16].

The results of the clonogenic survival assay for the 8 cell lines are presented in Figure 1. The survival curves demonstrated a broad spectrum of radiosensitivity. The SF2 varied between 0.30 and 0.68 (Table 1), with an average of 0.48 (standard deviation "SD" = 0.13). The HT-29, DLD-1, HCT-116, and CaCo-2 cell lines, which maintain the normal copy number of the *PIK3CA* gene

Table 1. Studied Colorectal Cancer Cell Lines, *PIK3CA* Copy Number, Mutation, and Surviving Fraction at 2 Gy Radiation Dose (SF2).

Cell strain	PIK3CA copy number	PIK3CA gene mutation	SF2 (CI 95%)*
HT-29	2	Yes	0.68 (0.62–0.75)
DLD-1	2	Yes	0.61 (0.58–0.65)
HCT-116	2	Yes	0.54 (0.50–0.58)
CaCo-2	2	No	0.45 (0.40–0.50)
SW480	3	No	0.47 (0.44–0.53)
HCT-15	6	Yes	0.45 (0.42–0.48)
Colo-320	8	No	0.34 (0.31–0.37)
LoVo	10	No	0.30 (0.27–0.33)

* CI 95%: 95% confidence interval.

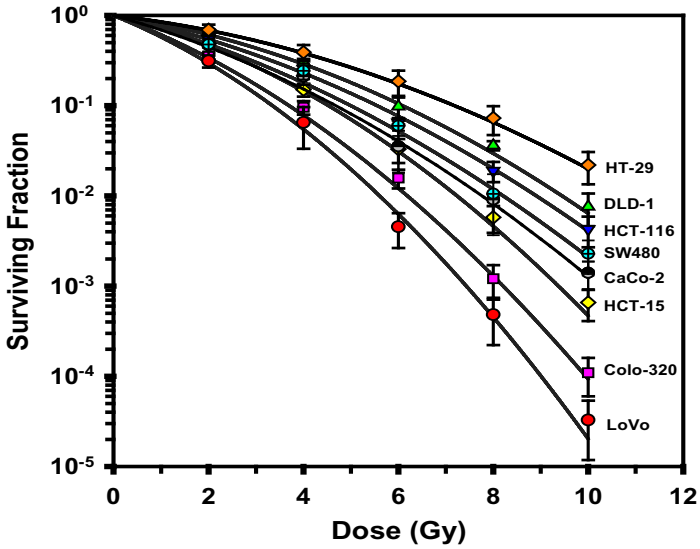

Figure 1. Clonogenic Survival Data for the Eight Colorectal Cancer Cell Lines. The survival curves were fitted to the linear quadratic model of cell killing by ionizing radiation. The data points represent the mean surviving fraction from three independent experiments. The error bars represent the 95% confidence interval.

(2-copies), exhibited higher SF2 (mean = 0.57, SD = 0.10) than the SW480, HCT-15, Colo-320, and LoVo cell lines, which have an increased *PIK3CA* gene copy number (mean SF2 = 0.39, SD = 0.08) (Figure 2-A). The T-test revealed a statistically significant difference in the mean SF2 between *PIK3CA*-amplified and non-amplified cell lines (two-tailed p-value = 0.033), suggesting that the presence of *PIK3CA* gene amplification is linked to increased sensitivity to radiation therapy.

PIK3CA gene mutation and radiosensitivity

Conversely, Abubaker and colleagues [17] reported that of the 13 established colorectal cell lines tested, 4 cell lines (DLD-1, HCT-116, HCT15, and SW948) harbored *PIK3CA* mutations using PCR amplification and direct sequencing. The analysis focused on exons 9 and 20 of the *PIK3CA* gene, as these regions contain the most frequent mutation hotspots. The amplified PCR products were subjected to direct sequencing using an automated DNA sequencer (ABI PRISM 3100 Genetic Analyzer). The mutations were missense resulting in amino acid substitutions rather than truncations or frameshifts, consistent with their role in activating *PI3K* signaling. The identified mutations (e.g., E542K, E545K, and H1047R) are known to increase PI3K activity, supporting their oncogenic role in colorectal cancer. Furthermore, HT-29 is known to harbor a *PIK3CA P449T* mutation [18]. Thus, the 4 lines (HT-29, DLD-1, HCT-116 and HCT15) included in this report contain *PIK3CA* mutations, unlike CaCo-2, SW480, Colo-320, and LoVo, which have the wild type *PIK3CA* gene. The SF2 of these cell lines were distinguishable from each other (Table 1). The average

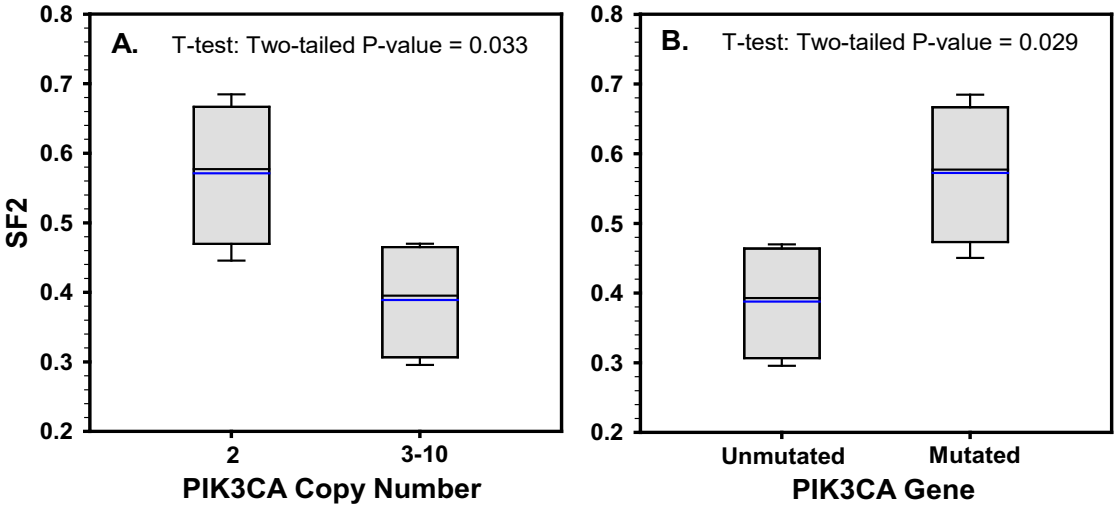

Figure 2. Box Plot Analysis of the Relationship between *PIK3CA* Copy Number (A), gene mutation (B), and radiosensitivity at 2 Gy radiation dose (SF2) in eight colorectal cancer cell lines. Lines within the boxes indicate the median (black) and the mean (blue) SF2. The upper and lower boundaries of the boxes represent the 75th and 25th percentiles, respectively. The bars above and below the boxes represent the 90th and 10th percentiles, respectively.

SF2 of the 4 cell lines harboring *PIK3CA* mutations was 0.57 (range: 0.45–0.68, SD = 0.10), which is higher than that of cell lines without *PIK3CA* mutations (average 0.39, range: 0.30–0.45, SD = 0.08). The T-test revealed a statistically significant difference in the mean SF2 between *PIK3CA*-mutated and unmutated cell lines (two-tailed p-value = 0.029), suggesting that the presence of *PIK3CA* gene amplification is linked to increased radiation sensitivity (Figure 2-B).

While the mutational analysis may not be definitive, the existing data suggest that *PIK3CA* mutations could confer radioresistance. In line with this finding, a study involving lung cancer patients discovered that those with *PIK3CA* mutations are at a significantly higher risk of local failure following radiation therapy for brain metastases, implying in vivo radiation resistance [19]. Nevertheless, these results may seem inconsistent with the finding of the *PIK3CA* mutation associated with PROS syndrome, which was characterized as moderately radiosensitive [11]. However, carcinogenic transformation involves numerous mutations in other genes, in addition to gene amplification and loss, which can also influence radiosensitivity. This mutagenic phenotype may underlie differences in individual radiosensitivity and, consequently, tumor response and normal tissue reactions to radiotherapy by affecting the fidelity of DNA double-strand break (DSB) repair [20].

It is well understood that the observation of potential association between radiosensitivity in samples with *PIK3CA* gene mutation and amplification emerged from the analysis of small number (n = 8) of tumors, which indeed represents a relatively small cohort. While the statistical association reached significance ($p < 0.05$), the limited number of cases restricts the generalizability of this finding and increases the risk of overfitting or chance observations. Therefore, this association is preliminary and hypothesis-generating rather than definitive. In addition, other confounding factors such as potential biological mechanisms involved in PI3K3CA pathway activation, and mutations in other genes could underlie this observation, which remain speculative without additional functional studies. Therefore, future studies with larger sample sizes are needed to confirm the radiosensitivity phenotype and elucidate its mechanistic basis.

Conclusion

This report uncovers a potential significant link between *PIK3CA* gene mutation, amplification and radiosensitivity in colorectal cancer cells. While increased *PIK3CA* copy number (> 2) is associated with increased tumor radiosensitivity, the *PIK3CA* gene mutation appear to be associated with decreased radiosensitivity (increased tumor resistance). This may imply that *PIK3CA* amplification and mutation might act as potential independent biomarkers for pinpointing patients who could gain from personalized radiotherapy protocols to improve treatment outcome. However, this finding should be interpreted with caution and requires validation in larger, independent cohorts to clarify the impact of *PIK3CA* modifications on radiation response in both normal and cancerous cells. Deciphering these mechanisms is vital for the creation of personalized radiotherapy approaches that utilize *PIK3CA* status to enhance treatment outcome for colorectal cancer patients.

Author Contribution Statement

Ghazi Alsbeih: conceived the work and prepared original draft. Khaled Alhadyan: review and editing.

Acknowledgements

We thank Dr. Zeenath Jehan, Dr. Shahab Uddin for *PIK3CA* gene amplification and Ms. Najla Alharbi and Sara Bin Judia for their assistance.

Funding

The work was supported by King Faisal Specialist Hospital and Research Centre (KFSHRC), grant RAC # 2000 031.

Ethics Declaration

The work was approved by the Research Ethic Committee at KFSHRC.

Availability of data

This work incorporates data previously published, all findings are included in the respective section and figures.

Conflict of Interest

No conflict of interest to declare.

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