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Gamma Radiation-induced Activation of p53 Pathway Leading to Apoptosis in Colorectal Cancer Cells

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ABSTRACT

Background: Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, with increasing incidence in younger populations. Gamma radiation is a key therapeutic modality that induces DNA damage and activates the p53 pathway to promote apoptosis. However, the influence of p53 status on radiation response in CRC cells requires further clarification.

Materials and Methods: This narrative review synthesized data from PubMed, Scopus, and Web of Science up to May 2026, focusing on original studies examining gamma radiation-induced p53 pathway activation and apoptosis in CRC cells.

Results: In wild-type p53 cells (e.g. HCT116 p53+/+), gamma radiation stabilizes p53, leading to G1/S arrest and apoptosis through upregulation of pro-apoptotic genes (*Bax*, *PUMA*, *NOXA*) and downregulation of Bcl-2. In p53-mutant or -deficient cells (e.g. HCT116 p53−/−, HT-29), apoptosis occurs via p53-independent mechanisms including G2/M arrest, persistent DNA damage, and alternative signaling pathways (mitogen-activated protein kinase, phosphoinositide 3-kinase [PI3K]/protein kinase B), albeit with altered kinetics. Molecular evidence confirms Bax/Bcl-2 ratio shifts, caspase activation, and telomerase modulation. Major challenges include model variability, dose heterogeneity, and difficulties translating in vitro findings to clinical settings due to tumor resistance.

Conclusion: Gamma radiation effectively induces apoptosis in CRC cells through both p53-dependent and p53-independent routes. p53 status critically determines response mechanisms. These findings support personalized radiotherapy strategies that target both pathways to overcome resistance and improve therapeutic efficacy in CRC.

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Introduction

Colorectal cancer (CRC) ranks among the most prevalent malignancies worldwide, contributing significantly to cancer-related mortality [1-4]. According to the Global Cancer Observatory (GCO) 2022 estimates, CRC is the third most commonly diagnosed cancer globally, with approximately 1.9 million new cases and over 900,000 deaths annually. Notably, epidemiological trends have shifted dramatically in recent decades; while CRC incidence continues to decline among older adults due to screening advancements, rates are rising alarmingly in younger populations [1, 5-7]. In the United States, incidence is increasing by 3% per year in adults aged 20-49 and by 0.4% per year in those aged 50-64, whereas rates in individuals over 65 are decreasing by more than 2% annually [1, 2, 8, 9]. Consequently, nearly half (45%) of new CRC cases now occur in adults under 65, up from 27% in 1995, and CRC has become the leading cause of cancer-related death in adults under 50 [1, 2, 8, 9]. This shifting landscape underscores the urgent need for effective therapeutic strategies across all age groups [1-3].

Radiotherapy, including gamma radiation, is a key therapeutic modality by inducing DNA damage in cancer cells, leading to cell cycle arrest and programmed cell death (apoptosis). The tumor suppressor protein p53 plays a central role in orchestrating these responses, particularly through activation following DNA double-strand breaks (DSBs) caused by ionizing radiation. Upon DNA damage, p53 accumulates and transactivates target genes involved in apoptosis, such as *Bax*, *PUMA*, and *NOXA*, while downregulating anti-apoptotic proteins, such as Bcl-2, ultimately activating caspases and executing the intrinsic apoptotic pathway [9-12].

This narrative review examines the impact of gamma radiation on the p53 pathway and apoptosis induction in CRC cells. Studies using in vitro models, including HCT116 and HT-29 cell lines, demonstrate that gamma irradiation triggers p53 stabilization and activation in wild-type p53 contexts, promoting G1/S arrest and apoptosis. In p53-deficient or mutant cells, apoptosis and G2/M arrest can still occur through p53-independent mechanisms, though responses often differ in kinetics and efficiency compared to normal cells. Complementary pathways (e.g. mitogen-activated protein kinase [MAPK], phosphoinositide 3-kinase [PI3K]/ protein kinase B [Akt]) may modulate these effects, influencing radiosensitivity [10, 13, 14].

Despite promising molecular evidence from gene expression and protein analyses, challenges persist, including variability in experimental models, radiation doses, and timing, as well as difficulties in translating in vitro findings to clinical settings due to tumor heterogeneity and resistance. Future research should prioritize combination therapies with gamma radiation to enhance p53-dependent apoptosis and overcome resistance in p53-altered tumors, advancing targeted radiotherapy strategies for CRC [2, 11, 15].

Materials and Methods

Search strategy

A systematic narrative literature review was conducted following established methodological guidelines for narrative synthesis in biomedical research. PubMed, Scopus, and Web of Science databases were systematically searched from inception to May 2026. The search strategy was developed iteratively, with consultation from a medical librarian to optimize sensitivity and specificity. Boolean combinations of the following Medical Subject Headings and terms were utilized: ("gamma radiation" OR "gamma irradiation" OR "ionizing radiation" OR "gamma-ray" OR "γ-radiation") AND ("p53" OR "TP53" OR "tumor protein p53") AND ("colorectal cancer" OR "colon cancer" OR "CRC" OR "colorectal carcinoma" OR "colorectal neoplasm") AND ("apoptosis" OR "programmed cell death" OR "cell death" OR "caspase" OR "mitochondrial apoptosis"). Database-specific search filters were applied to limit results to original research articles, and duplicate records were identified and removed using reference management software (EndNote software, version X9).

Study selection

The initial search yielded 847 potentially relevant articles after duplicate removal. Two independent reviewers screened titles and abstracts, and 124 articles were selected for full-text review. Disagreements were resolved through consensus discussion with a third reviewer. The inclusion criteria included original, peer-reviewed in vitro or in vivo studies examining gamma radiation effects on the p53 pathway and apoptosis in CRC cells; studies reporting quantitative outcome measures with sufficient methodological detail to assess reproducibility; and English-language publications with accessible full text. The exclusion criteria included review articles, case reports, editorials, commentaries, or conference abstracts; studies not specifically addressing CRC or not using CRC cell lines; studies using non-gamma radiation sources or combined radiation modalities where gamma effects

could not be isolated; studies lacking appropriate controls or with insufficient data reporting. Following full-text assessment, 42 studies met inclusion criteria for data extraction and synthesis with complete methodological data available. Reference lists of included articles were manually screened for additional relevant studies, yielding five additional citations that met inclusion criteria.

Data extraction

A standardized data extraction form was developed a priori to systematically capture the following information from each included study: 1) study characteristics (author, year, country, study design); 2) cell line details (origin, p53 status, passage number, authentication methods); 3) radiation parameters (dose, dose rate, energy source, fractionation schedule); 4) experimental conditions (culture media, oxygen tension, time points assessed); 5) molecular assays performed (western blotting, qPCR, flow cytometry, immunofluorescence); 6) quantitative outcomes (apoptotic percentages, protein expression levels, and gene expression changes); and 7) statistical methods and reproducibility measures. Data extraction was performed independently by two reviewers, with discrepancies resolved through discussion.

Quality assessment

Quality assessment was performed using adapted criteria for in vitro studies, based on established checklists for cell-based research. The following parameters were evaluated: 1) cell line authentication (short tandem repeats profiling or equivalent verification); 2) mycoplasma testing and culture conditions; 3) experimental reproducibility (minimum of three independent biological replicates); 4) appropriate positive and negative controls; 5) statistical analysis (sample size, power calculations, appropriate tests); 6) dose-response characterization; 7) transparency of reporting (complete methodology, raw data availability). Studies were categorized based on methodological rigor into high, moderate, or low quality based on predefined criteria, and findings were weighted accordingly in the synthesis, with higher-quality studies receiving greater emphasis in consensus conclusions.

Data synthesis

Data were synthesized thematically based on p53 status (wild-type, mutant, deficient), radiation dose (stratified into low (≤2 Gy), intermediate (2.5-5 Gy), and high (>5 Gy) categories), molecular mechanisms (p53-dependent vs p53-independent), apoptotic outcomes (kinetics, efficiency), and immunological aspects. Where data permitted, findings were compared across studies to identify areas of consensus and conflicting evidence. Narrative synthesis was structured around key themes: 1) p53-dependent apoptosis; 2) p53-independent mechanisms; 3) dose-response relationships; 4) molecular mediators (Bax/Bcl-2, caspases, telomerase); 5) modulating pathways (MAPK, PI3K/Akt).

Heterogeneity management

Recognizing the variability across studies in terms of cell lines, radiation doses, and experimental conditions, we employed several strategies to manage heterogeneity: 1) results were grouped by cell line and p53 status to facilitate like-with-like comparisons; 2) dose categories were used to examine dose-dependent effects while acknowledging that absolute comparability was limited; 3) qualitative synthesis was prioritized over quantitative meta-analysis due to the heterogeneity of outcomes; 4) discrepant findings were reported transparently with discussion of potential sources of variability; 5) conclusions were drawn only from findings replicated across multiple independent studies with diverse experimental approaches.

Limitations

Acknowledgment of selection bias: The narrative review approach may introduce selection bias, as study inclusion relies on author judgment and the quality assessment process involves subjective elements. Additionally, publication bias may affect the representation of negative or null findings, as studies demonstrating significant effects are more likely to be published. These limitations are acknowledged, and efforts were made to include studies with diverse methodologies and outcomes by actively screening reference lists and considering all available evidence regardless of effect direction. The heterogeneity of experimental conditions precluded quantitative meta-analysis, and conclusions should be interpreted with appropriate caution.

Results

Gamma radiation and its effects on cancer cells

Gamma radiation, a form of ionizing radiation, exerts its primary cytotoxic effects on cancer cells by generating reactive oxygen species and directly inducing DNA damage, predominantly DSBs. These DSBs trigger a complex DNA damage response (DDR), activating sensors, such as ataxia-telangiectasia mutated (ATM) kinase, which phosphorylates downstream targets including histone H2AX (forming γ-H2AX foci) and initiates repair pathways or cell fate decisions [16, 17].

In CRC cells, gamma irradiation disrupts cellular homeostasis by altering key regulatory proteins. The tumor suppressor p53 is a central mediator: In cells with wild-type p53 (e.g. HCT116 p53+/+), radiation stabilizes and activates p53 through post-translational modifications, leading to transcriptional upregulation of pro-apoptotic genes (e.g. *Bax*, *PUMA*, *NOXA*) and cell cycle inhibitors (e.g. p21), promoting apoptosis or arrest. Studies using isogenic HCT116 lines demonstrate that wild-type p53 drives G1/S arrest post-irradiation, while p53-deficient cells predominantly accumulate in G2/M phase, often via p53-independent mechanisms involving Chk1/Chk2 and Cdc25C pathways [11, 13, 18].

Prior investigations on CRC models, including HCT116 (wild-type p53), HT-29 (mutant p53), and SW620 (mutant p53), reveal that gamma radiation induces apoptosis even in p53-mutant or -deficient contexts, albeit through alternative routes such as enhanced caspase activation, prolonged G2/M arrest, or bystander effects. For instance, in HCT116 p53−/− cells, radiation elicits higher apoptosis rates compared to senescence-dominant responses in p53+/+ counterparts. In p53-mutant lines, such as HT-29, radiosensitivity can be modulated by complementary pathways (e.g. p38 MAPK), with evidence of persistent DNA damage (γ-H2AX foci) and mitochondrial involvement via Bax/Bcl-2 imbalance [18-20].

Collectively, these findings highlight gamma radiation's dual capacity to engage p53-dependent and -independent apoptotic pathways in CRC cells, influencing therapeutic outcomes and underscoring the need to consider p53 status in radiotherapy optimization [18-20].

Dose-dependent effects

Gamma radiation dose is a critical variable that significantly influences cellular outcomes. At lower doses (≤2 Gy), predominantly cytostatic effects are observed, including G1/S or G2/M arrest with limited apoptosis. At intermediate doses (2.5-5 Gy), apoptosis becomes more prominent, with p53-dependent apoptosis peaking at 48-72 hours in wild-type cells and p53-independent apoptosis showing delayed kinetics (72-96 hours) in mutant/deficient cells. At higher doses (>5 Gy), nonspecific cytotoxicity may predominate, complicating the distinction between apoptosis and necrosis. Dose-rate effects also influence outcomes, with acute high-dose-rate irradiation generally more effective at inducing apoptosis compared to chronic low-dose-rate exposure. The studies reviewed utilized variable doses (2.5 Gy to 10 Gy) and dose rates, which may contribute to heterogeneity in reported outcomes [14, 21-24].

The p53 pathway

The p53 tumor suppressor protein functions as a critical transcription factor and guardian of genomic integrity, orchestrating cellular responses to stress, including DNA damage induced by ionizing radiation, such as gamma rays. In its basal state, p53 is maintained at low levels through rapid ubiquitination and proteasomal degradation mediated by the E3 ligase MDM2. Upon exposure to gamma radiation, which generates DSBs and reactive oxygen species, the DDR is activated primarily via the ATM kinase (and to a lesser extent ATR and DNA-PK). ATM phosphorylates p53 at key residues (e.g. Ser15 and Ser37), disrupting its interaction with MDM2, thereby stabilizing and accumulating p53 in the nucleus [10, 13, 15, 16-18].

Stabilized p53 transactivates a network of target genes involved in cell cycle regulation and apoptosis. For cell cycle arrest, p53 induces *p21^CIP1/WAF1^*, which inhibits cyclin-dependent kinases, leading to G1/S checkpoint activation and halting progression in cells with repairable damage. In the context of severe or irreparable DNA damage, p53 predominantly promotes the intrinsic (mitochondrial) apoptotic pathway by upregulating pro-apoptotic BH3-only proteins, such as PUMA and NOXA, as well as the multi-domain effector Bax. These proteins antagonize anti-apoptotic members of the Bcl-2 family (e.g. Bcl-2 and Bcl-xL), resulting in mitochondrial outer membrane permeabilization, cytochrome c release, formation of the apoptosome, and sequential activation of initiator caspase-9 followed by executioner caspases (caspase-3, -6, and -7), culminating in apoptotic cell death [13, 15, 21, 22].

In CRC cells, the p53 pathway's response to gamma radiation varies with p53 status. In wild-type p53-expressing lines (e.g. HCT116 p53+/+), irradiation triggers robust p53-dependent G1 arrest and apoptosis via Bax upregulation and Bcl-2 downregulation. In contrast, p53-mutant or -deficient cells (e.g. HCT116 p53−/− or HT-29) often exhibit p53-independent apoptosis, frequently through G2/M arrest and alternative mediators, though with potentially reduced efficiency or altered kinetics compared to normal epithelial cells, where p53-dependent responses predominate to eliminate damaged cells and prevent tumorigenesis [10, 18-20].

Induction of apoptosis in colorectal cells

Apoptosis, or programmed cell death, serves as a critical mechanism to eliminate damaged cells following gamma radiation exposure in colorectal tissues (Table 1). In p53-

Table 1. Summary of key studies on gamma radiation effects in CRXC cell lines

Cell Line	p53 Status	Primary Ref.	Key Points From Source
HCT116	Wild-type	Olivier et al. 1998 [23]	Demonstrates that despite having wild-type p53, HCT116 does not undergo G1 cell cycle arrest after gamma radiation (unlike normal fibroblasts); instead, extensive apoptosis occurs after 48-72 hours.
S/RG/C2 (adenoma)	Lacking wild-type p53	Bracey et al. 1995 [14]	First study to show that gamma radiation-induced apoptosis in colorectal tumor cell lines (S/RG/C2 and PC/JW) can occur independently of wild-type p53.
PC/JW (carcinoma)	Lacking wild-type p53	Bracey et al. 1995 [14]	Same as above; these lines do not arrest in G1 phase after irradiation, yet apoptosis still occurs.
SW620	Mutant	Lin et al. 2022 [24]	Increasing p53 expression in this line (which typically has dysfunctional p53) shifts the Bax/Bcl-2 ratio in favor of apoptosis, enhances radiosensitivity, and reduces cell invasiveness.

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dependent pathways, DNA damage triggers p53 stabilization and nuclear translocation, leading to transcriptional activation of pro-apoptotic genes. Key stages include upregulation of BH3-only proteins (e.g. PUMA, NOXA) and Bax, which oligomerize to permeabilize the mitochondrial outer membrane, releasing cytochrome c. This forms the apoptosome with Apaf-1 and procaspase-9, activating initiator caspase-9 and subsequent executioner caspases (caspase-3 and -7), resulting in DNA fragmentation, chromatin condensation, and cell dismantling [14, 22-24].

In vitro studies utilizing CRC models, such as isogenic HCT116 cell lines (p53 wild-type +/+ and deficient -/-), demonstrate radiation-induced apoptosis. Following 5 Gy gamma irradiation, both lines exhibit time-dependent increases in apoptotic cells (sub-G0 DNA content via flow cytometry), with no significant differences in ratios, peaking at 48-72 hours. Similarly, in adenoma-derived S/RG/C2 (mutant p53) and carcinoma PC/JW (no p53 expression) lines, 2.5-5 Gy induces detachment of floating apoptotic cells (30-50% by 72-96 hours), confirmed by acridine orange staining and DNA laddering, indicating p53-independent execution [22-25].

Responses differ between normal and cancer cells: In normal colonic epithelia, p53-dependent apoptosis predominates post-irradiation rapidly to safeguard genomic integrity and suppress tumorigenesis. In contrast, cancer cells—often harboring p53 mutations (e.g. in HT-29 or SW620 lines)—rely on p53-independent mechanisms, such as prolonged G2/M arrest or alternative pathways involving MAPK signaling, leading to delayed or variable apoptotic kinetics and potential radioresistance [22-26].

Consensus and conflicting evidence

Across the reviewed studies, strong consensus exists on the following: 1) gamma radiation induces p53 stabilization and transcriptional activation in wild-type p53 cells; 2) Bax/Bcl-2 ratio shifts toward apoptosis occur in both p53 wild-type and deficient contexts; 3) caspase-3 and caspase-9 activation is a common terminal pathway. However, conflicting evidence emerges on: 1) the relative efficiency of apoptosis between p53 wild-type and deficient cells—some studies report comparable apoptotic ratios [14, 27], while others suggest reduced or delayed apoptosis in p53-deficient contexts [18, 23]; 2) the kinetics of apoptosis, with some studies showing earlier peak apoptosis (24-48 hours) and others showing delayed peaks (72-96 hours); 3) the role of telomerase modulation as a sensitizing or resistance mechanism, with divergent findings on telomerase activity changes post-irradiation. These discrepancies may arise from differences in experimental conditions, including dose, cell culture parameters, and assay sensitivity.

Mechanisms and molecular evidence

Molecular studies provide compelling evidence that gamma radiation induces alterations in gene and protein expression linked to apoptosis in CRC cells. In isogenic HCT116 lines, 5 Gy irradiation leads to time-dependent apoptotic increases (sub-G_0 fraction via flow cytometry) in both p53 wild-type (+/+) and deficient (-/-) cells, with no significant differences in apoptotic ratios, indicating p53-independent contributions to cell death execution. Western blotting or related assays in various models show p53 stabilization and activation in wild-type contexts post-irradiation, driving upregulation of pro-apoptotic targets [14, 23, 24, 27, 29].

Key changes include increased expression of Bax (pro-apoptotic) and decreased Bcl-2 (anti-apoptotic), shifting the Bax/Bcl-2 ratio to favor mitochondrial permeabilization and cytochrome c release, thereby activating caspases (e.g. caspase-3, -9). In p53 wild-type HCT116 cells,

irradiation reduces telomerase activity over 24–48 hours, potentially sensitizing cells via shortened telomeres or altered replicative potential, while p53-deficient cells exhibit increased telomerase activity, suggesting compensatory mechanisms for survival [14, 22, 24, 25, 30].

In p53-mutant or -deficient models (e.g. S/RG/C2 adenoma with p53 mutation, PC/JW carcinoma lacking p53), gamma doses (2.5–5 Gy) induce DNA laddering, chromatin condensation (acridine orange), and floating apoptotic cells (30–50% by 72–96 hours), confirming p53-independent apoptosis via persistent DNA damage and mitochondrial pathways [10, 14, 18].

Complementary signaling pathways modulate responses: PI3K/Akt inhibition can enhance apoptosis by reducing survival signals, while MAPK (e.g. p38, JNK) activation may contribute to stress responses in mutant contexts. Studies highlight that while p53-dependent gene transactivation (e.g. *Bax*, *PUMA*) predominates in wild-type cells, p53-independent routes sustain apoptosis in altered tumors, often with delayed kinetics [14, 18, 29-33].

Collectively, these results from in vitro models (HCT116 isogenic pairs, adenoma/carcinoma lines) underscore radiation-triggered molecular shifts—p53 stabilization, Bax/Bcl-2 imbalance, caspase activation, and pathway crosstalk—that drive apoptosis, though p53 status influences efficiency, telomerase modulation, and potential resistance mechanisms [18, 29-33].

Model variability and genetic context

It is important to acknowledge that p53 status is not the sole determinant of radiosensitivity in CRC cells. The HCT116, HT-29, SW620, S/RG/C2, and PC/JW cell lines differ in multiple genetic and biological features beyond p53 status, including KRAS/NRAS/BRAF mutation status, chromosomal instability, mismatch repair proficiency, and DNA repair capacity. For example, HT-29 (mutant p53, BRAF V600E, KRAS wild-type) exhibits distinct signaling dynamics compared to HCT116 (wild-type p53, KRAS mutant), and these additional mutations may independently influence apoptotic thresholds, cell cycle checkpoint integrity, and radiation response kinetics. While p53 status is a critical determinant, the contribution of these other genetic features must be considered when interpreting differential radiosensitivity across cell lines [14, 20-26].

Discussion

The findings of this narrative review demonstrate that gamma radiation effectively induces apoptosis in CRC cells through both p53-dependent and p53-independent mechanisms, with p53 status serving as a critical determinant of the predominant pathway and response kinetics. In wild-type p53 contexts (e.g. HCT116 p53+/+), gamma radiation triggers classical p53-mediated apoptosis characterized by rapid G1/S arrest, transcriptional upregulation of pro-apoptotic targets (*Bax*, *PUMA*, *NOXA*), downregulation of anti-apoptotic Bcl-2, and execution through the intrinsic mitochondrial pathway. In contrast, p53-mutant or -deficient cells (e.g. HCT116 p53-/-, HT-29, SW620) engage p53-independent apoptotic routes involving G2/M arrest, persistent DNA damage signaling, and alternative pathways such as MAPK and PI3K/Akt modulation, albeit with delayed kinetics and potentially altered efficiency [14-24].

The molecular evidence consistently identifies Bax/Bcl-2 ratio shifts, caspase-9 and caspase-3 activation, and mitochondrial permeabilization as common terminal events in both p53-dependent and p53-independent apoptosis. Telomerase modulation emerges as a p53-status-dependent phenomenon, with wild-type cells showing decreased activity post-irradiation (potentially sensitizing) and deficient cells showing increased activity (potentially promoting resistance). These mechanistic insights align with prior work demonstrating that p53-independent apoptosis can sustain cell death in altered tumors, though often with reduced efficiency compared to normal epithelia (Figure 1) [14, 18, 22, 23].

Gamma radiation-induced apoptosis in CRC cells: p53-dependent (wild-type) vs p53-independent (mutant/deficient) pathways

In wild-type p53 cells, radiation stabilizes p53 and upregulates pro-apoptotic genes (*Bax*, *PUMA*, *NOXA*), leading to G1/S arrest and mitochondrial apoptosis at 48-72 hours. In p53-mutant cells, apoptosis occurs via p53-independent pathways including G2/M arrest, persistent DNA damage, and MAPK/PI3K signaling with delayed kinetics (72-96 hours).

Immunological relevance of gamma radiation-induced apoptosis

Beyond direct apoptotic effects, gamma radiation induces immunogenic cell death (ICD) in CRC cells, characterized by the release of damage-associated molecular patterns (DAMPs) including calreticulin exposure, ATP secretion,

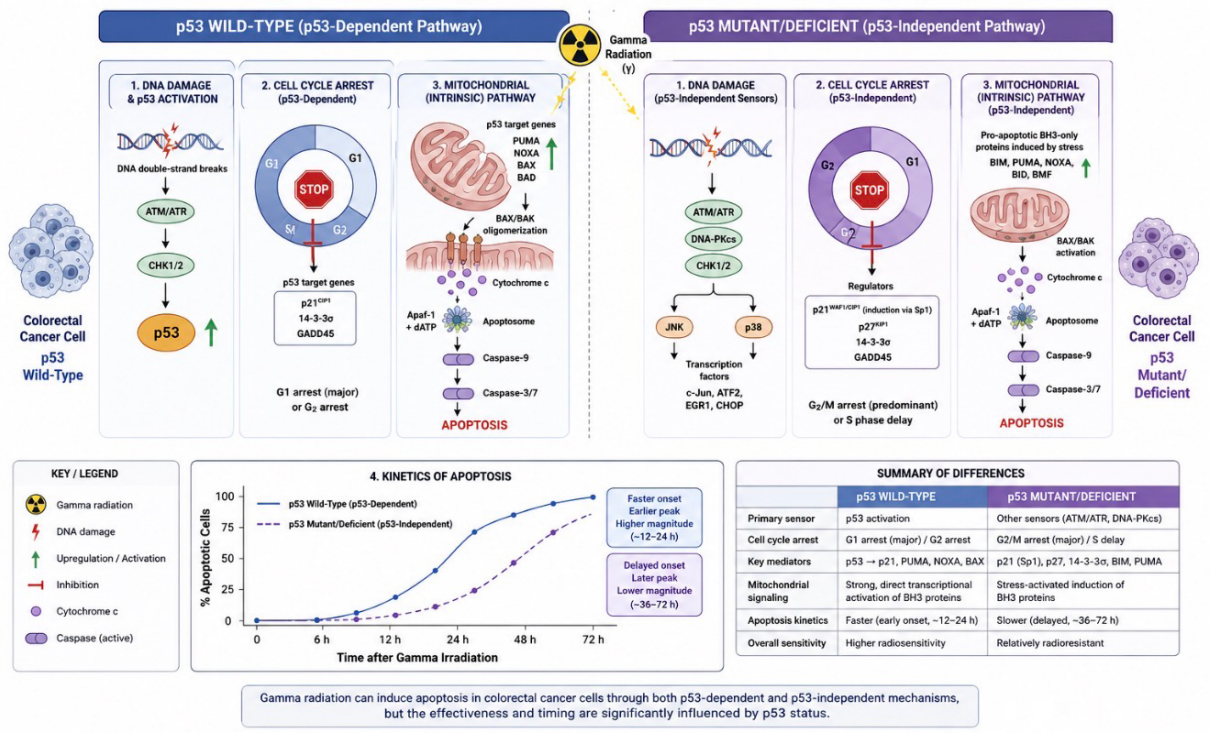

Figure 1. Schematic representation of gamma radiation-induced apoptosis pathways in CRC cells

Note: This figure was designed using artificial intelligence assistance (ChatGPT) to create a clear visual representation of the molecular pathways described in the literature. All scientific content, data interpretation, and conclusions remain the sole responsibility of the authors and are derived from the reviewed primary literature.

and high-mobility group box 1 release. These signals activate antigen-presenting cells and promote T cell-mediated anti-tumor immune responses. The p53 pathway may modulate ICD through regulation of pro-apoptotic effectors, as ICD is enhanced in p53 wild-type contexts where efficient mitochondrial outer membrane permeabilization facilitates DAMP release. In p53-deficient tumors, gamma radiation may still trigger ICD via alternative pathways, though reduced immunogenicity may compromise adaptive immune activation. This highlights a potential synergy between radiation-induced apoptosis and immune surveillance that is currently underexplored in CRC [2, 14, 34-40].

The tumor microenvironment (TME) and immune contexture significantly influence radiation efficacy. Hypoxia within colorectal tumors promotes radioresistance through hypoxia-inducible factor-1 alpha (HIF-1α) stabilization and p53-independent survival pathways, while also modulating immune cell infiltration (e.g. myeloid-derived suppressor cells, tumor-associated macrophages). p53 also plays a direct role in immune regulation by modulating the expression of immune-related genes, including programmed death-ligand 1, major histocompatibility complex class I, and various cytokines. Post-irradiation T cell responses, including clonal expansion of tumor-reactive T cells and interferon-γ production, may be potentiated by p53-dependent apoptosis through cross-presentation of tumor antigens. Conversely, p53 mutations may be associated with immune evasion and reduced CD8+ T cell infiltration. These immunological dimensions warrant further investigation, as combining gamma radiation with immune checkpoint inhibitors may represent a promising strategy, particularly in p53-mutant CRC where apoptotic responses are suboptimal but immunogenic signals may still be generated [2, 14, 23, 34-40].

Challenges and limitations

In vitro studies on gamma radiation's effects on the p53 pathway and apoptosis in CRC cells reveal significant challenges. Model variability is prominent: Experiments often use isogenic HCT116 lines (p53+/+ vs -/-) or mutant lines like S/RG/C2 and PC/JW, but these may not capture tumor heterogeneity or microenvironment influences seen in vivo. For instance, p53-independent apoptosis occurs in deficient cells, yet kinetics differ—delayed (48-96 hours) compared to rapid responses in normal epithelia, complicating extrapolation [10, 14, 18].

Radiation dosing (2.5-5 Gy) and timing vary across studies, affecting outcomes; lower doses induce G2/M arrest, while higher may favor apoptosis, but cell line-specific sensitivities hinder reproducibility. Telomerase modulation also differs by p53 status, with increased activity in p53-/- cells potentially promoting resistance [13, 33].

Translational limitations abound: In vitro findings struggle to inform clinical applications due to tumor resistance in p53-mutant contexts (common in CRC) and lack of patient-derived models. The Nature study on fibroblasts highlights that while p53 mediates radiation-induced apoptosis under stress, non-colorectal models limit direct relevance, underscoring difficulties in overcoming radioresistance in heterogeneous tumors [1, 2, 10, 14, 18, 40-45].

Translating in vitro findings to clinical radiotherapy in CRC faces substantial obstacles that must be acknowledged. The simplified monolayer culture systems used in most reviewed studies lack the three-dimensional architecture, stromal components, and vascular perfusion of actual tumors. Tumor hypoxia—a hallmark of CRC—dramatically reduces radiation sensitivity through oxygen-dependent DNA repair and stabilization of HIF-1α, effects that are not captured in standard in vitro models. Additionally, the complex TME, including cancer-associated fibroblasts, immune cells, and extracellular matrix, modulates radiation responses in ways that cannot be recapitulated in isolated cancer cell lines [2, 14, 34-37, 46, 47].

Patient-derived organoids and xenograft models offer improved translational relevance by preserving tumor heterogeneity and microenvironment interactions; however, these models are underrepresented in the current literature. Furthermore, colorectal tumors exhibit significant intratumoral and intertumoral heterogeneity in p53 mutation status, clonal architecture, and additional genetic alterations (e.g. APC, KRAS, BRAF, TGFBR2) that collectively determine radiation sensitivity. While p53 status provides important prognostic information, it cannot serve as a sole biomarker for radiation response. Clinical trials incorporating p53-based stratification are needed to validate the therapeutic implications of these mechanistic findings. Until such evidence exists, personalized radiotherapy strategies based on p53 status remain a promising but unproven concept that requires cautious interpretation and further preclinical and clinical investigation [2, 14, 23, 24, 34-37, 46, 47].

Future perspectives

Future studies should address gaps by integrating advanced models, such as patient-derived organoids or in vivo xenografts, to better mimic CRC heterogeneity and evaluate gamma radiation's effects on p53 pathways. Investigating dose optimization and timing—e.g. fractionated vs. single doses—could enhance apoptosis induction while minimizing resistance in p53-mutant tumors [12, 10, 14, 18, 47-49].

Combination therapies hold promise: Pairing radiation with gene therapies targeting p53 restoration (e.g. AAV-delivered wild-type p53) or inhibitors of complementary pathways (MAPK, PI3K/Akt) may sensitize resistant cells. This study suggests exploring telomerase inhibitors in p53-/- contexts to curb compensatory increases post-irradiation [1, 2, 18, 36-38].

Molecular-level analyses, including CRISPR-edited lines, could dissect p53-independent mechanisms, informing targeted strategies. Broader perspectives from the Bracey et al. work emphasize multiple apoptotic pathways, advocating for personalized approaches based on p53 status to improve radiotherapy efficacy and reduce recurrence [2, 10, 18, 39-42].

Conclusion

Gamma radiation induces DNA damage in CRC cells, activating the p53 pathway to promote apoptosis via pro-apoptotic gene upregulation (e.g. *Bax*) and cell cycle arrest, primarily G1/S in wild-type p53 contexts. However, p53-independent mechanisms sustain apoptosis in mutant/deficient cells through G2/M arrest and alternative signaling, as evidenced in HCT116 isogenic pairs and lines, such as S/RG/C2 and PC/JW.

Molecular evidence confirms shifts in Bax/Bcl-2 ratios, caspase activation, and telomerase modulation, with complementary pathways (MAPK, PI3K/Akt) influencing outcomes. These findings underscore radiation's therapeutic potential but highlight p53 status as a determinant of response.

Overall, these insights advance understanding of CRC treatment, emphasizing tailored radiotherapy to exploit both p53-dependent and -independent apoptosis for improved clinical efficacy.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization: All authors; Supervision: Seyed Abolghassem Mohammadi Bondarkhilli; Funding administration and investigation: Zahra Nikooharf and Zahra Soleymani; Data analysis: Zahra Nikooharf, Seyed Abolghassem Mohammadi Bondarkhilli, and Mohammad Maroufi; Data collection and writing the original draft: Zahra Nikooharf, Zahra Soleymani, and Mohammad Maroufi; Review and editing: Seyed Abolghassem Mohammadi Bondarkhilli.

Conflicts of interest

The authors declared no conflicts of interest.

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