Artigo de Revisão

Revista Brasileira de Física Médica (2025) 19:826

Unraveling the Mechanisms of Radiotherapy: Biological Pathways and Therapeutic Innovations

Desvendando os Mecanismos da Radioterapia: Vias Biológicas e Inovações Terapêuticas

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Abstract

Radiotherapy (RT) plays a pivotal role in oncology as a treatment modality for various malignancies, leveraging physical principles such as ionizing radiation to damage cancer cells and inhibit tumor growth. Recent advancements in RT technologies, such as proton therapy, carbon ion radiotherapy (CIRT), and adaptive radiation therapy (ART) have significantly enhanced the precision and effectiveness of radiation delivery. Despite these technological advancements, RT is still limited by factors such as tumor heterogeneity, normal tissue toxicity, and radioresistance, which can significantly impact treatment outcomes. The biological effects of radiation, including DNA damage, cell cycle arrest, and apoptosis, are influenced by the tumor microenvironment (TME) and mechanisms like hypoxia, which contribute to increased resistance. Nanoparticles, with their multifunctional capabilities, have emerged as a promising tool to overcome these limitations. They enable precise targeting, enhance local radiation deposition, and modulate the TME, potentially improving the efficacy of RT. However, concerns regarding their biocompatibility and potential toxicity, particularly with prolonged exposure, must be addressed. Furthermore, the integration of artificial intelligence (AI) and machine learning (ML) into radiotherapy planning is revolutionizing treatment strategies. These technologies allow for more accurate tumor delineation, personalized treatment planning, and optimization of radiation doses, improving therapeutic outcomes. To overcome radioresistance, innovative combination therapies that target both tumor cells and the TME, along with advancements in nanotechnology and AI, are promising avenues to enhance RT efficacy and improve patient survival.
Keywords: radiotherapy; radioresistance; radiotherapy response; cancer.

Resumo

A radioterapia desempenha um papel fundamental na oncologia como modalidade de tratamento para diversos tipos de câncer, utilizando princípios físicos, como a radiação ionizante, para danificar as células tumorais e inibir o crescimento tumoral. Avanços recentes nas tecnologias de RT, como a terapia com prótons, a radioterapia com íons de carbono e a radioterapia adaptativa, aumentaram significativamente a precisão e a eficácia da entrega da radiação. Apesar desses avanços tecnológicos, a RT ainda enfrenta limitações, como a heterogeneidade tumoral, a toxicidade aos tecidos normais e a radioresistência, que podem impactar negativamente os resultados do tratamento. Os efeitos biológicos da radiação, incluindo danos ao DNA, parada do ciclo celular e apoptose, são influenciados pelo microambiente tumoral e por mecanismos como a hipóxia, que contribuem para uma maior resistência. Nanopartículas, com suas capacidades multifuncionais, surgem como uma ferramenta promissora para superar essas limitações. Elas permitem um direcionamento preciso, aumentam a deposição local de radiação e modulam o TME, potencialmente melhorando a eficácia da RT. No entanto, preocupações quanto à biocompatibilidade e à toxicidade potencial das nanopartículas, especialmente com a exposição prolongada, precisam ser abordadas. Além disso, a integração da inteligência artificial e do aprendizado de máquina no planejamento da radioterapia está revolucionando as estratégias de tratamento. Essas tecnologias permitem uma delimitação mais precisa do tumor, um planejamento personalizado do tratamento e a otimização das doses de radiação, melhorando os resultados terapêuticos. Para superar a radioresistência, terapias combinadas inovadoras que atuem tanto sobre as células tumorais quanto sobre o TME, aliadas aos avanços em nanotecnologia e IA, representam caminhos promissores para aumentar a eficácia da RT e melhorar a sobrevida dos pacientes.
Palavras-chave: *radioterapia; radioresistência; resposta à radioterapia; câncer.*

1. Introduction

Given the projected 60% increase in cancer cases worldwide over the next 20 years (1) there is a clear need to improve cancer treatment techniques, making them more personalized and aiming to reduce the effects of metastasis (2). Since cancer is a global disease, it requires specific treatments that focus on the molecular mechanisms of tumor resistance to current clinical methods, including drug delivery and radiotherapy, or a combination of both. This review aims to discuss and show how cellular mechanisms respond to ionizing radiation (3). The effectiveness of ionizing radiation in cancer treatments lies in its ability to cause damage by energy deposition in the double strands of DNA molecules. There are two primary pathways through which ionizing radiation induces stress: direct and indirect effects. The first involves the direct absorption of radiation energy by the DNA, while the second occurs when nearby molecules absorb the energy and release free radicals that can interact with the DNA, potentially causing genetic damage (4) (5). Radiotherapy is a widely used therapeutic modality in cancer treatment that employs ionizing radiation to destroy tumor cells or inhibit their growth. This form of treatment can be applied externally, using radiation beams directed at the tumor (external radiotherapy), or internally, through the insertion of radioactive materials near or inside the tumor (brachytherapy)(6). Radiotherapy primarily acts on the DNA of cells, causing damage that leads to their death or prevents their proliferation. In the oncological context, radiotherapy plays a fundamental role, whether as a primary, adjuvant, or palliative treatment. As a primary treatment, radiotherapy may be the main approach for localized tumors that are sensitive to radiation (7). When used as an adjuvant therapy, it complements other treatments, such as surgery or chemotherapy, enhancing their effectiveness by reducing the risk of recurrence (8). Furthermore, palliative radiotherapy is used to relieve symptoms and improve the quality of life in patients with advanced cancer, controlling tumor growth and reducing pain (9). The continuous evolution of radiotherapy, alongside advances in molecular targeting and personalized treatment strategies are essential in addressing the growing global burden of cancer, improving patient outcomes and minimizing the long-term side effects of treatment. In this review, we will examine the basic principles of radiotherapy, exploring its mechanisms of action and interactions with both tumor cells and the tumor microenvironment. Additionally, we will discuss recent innovations in radiotherapy, focusing specifically on strategies to overcome radioresistance.

2. Radiotherapy: Physical principles

Radiotherapy is based on the irradiation of target tissues through the incidence of ionizing radiation. The term ionizing radiation refers to particles with sufficient energy to cause ionizations in a medium. It includes: (i) charged particles, such as electrons, positrons, protons, and alpha particles, which directly ionize the medium; and (ii) neutral particles, such as photons (X-rays and gamma rays) and neutrons, which ionize indirectly by interacting with atoms and molecules. In contrast, non-ionizing radiation, characterized by low-energy waves – such as radio waves, microwaves, infrared, and visible light – can excite molecules but do not have enough energy to remove electrons or cause ionizations (10). In this review, we will focus on ionizing radiation and its various mechanisms of interaction with matter, which are critical for the development of effective theranostic applications. Theranostics combines tumor diagnosis and tracking with therapeutic intervention, utilizing ionizing radiation to enhance both diagnostic precision and therapeutic efficacy (11). Understanding these interactions is fundamental to advancing cancer treatment with this dual-purpose approach.

3. Radiation Effects on Matter

There are two primary mechanisms by which photons interact with matter: the photoelectric effect and the Compton Effect (Figure 1A). Each mechanism has unique characteristics and different implications for medical imaging and radiation therapy, impacting how radiation energy is deposited in tissues. In the photoelectric effect, a photon interacts with a strongly bound electron within one of the atomic shells of the absorbing material. The photon, with energy hv, is fully absorbed by the atom, leading to the ejection of the electron.

The kinetic energy (κ) of this ejected electron is expressed by $\kappa = hv - w0$, where w0 is the work function, or the binding energy, of the electron within the atom. In other words, the photoelectric effect occurs when an incident photon provides sufficient energy to eject an electron from one of the atomic shells (usually from the inner shells), overcoming the binding energy of that electron. The ejected electron is released as a charged particle, and any excess energy, if present, is converted into the kinetic energy of the electron. When radiation photons, such as X-rays and gamma rays, are absorbed by tissues, their energy is transferred to electrons, causing ionization. This process damages the DNA of tumor cells, leading to cell death or inhibiting their growth; however, healthy cells can also be affected by this damage.

The photoelectric effect is crucial in imaging and diagnostic techniques due to its high contrast in materials with varying densities, like bone and soft tissue in X-rays (12). In contrast, the Compton effect is characterized by the partial transfer of energy from the incident photon to a weakly bound electron in an atom, resulting in the ejection of the electron and the deflection of the photon in a new direction (12). The energy transferred to the electron may be sufficient to eject it, but it does not result in the photoelectric effect.

The scattered photon can interact with other electrons in independent events, but this does not form a continuous cascade effect, as the photon's energy is limited. In high-energy radiotherapy, such as electron beam therapy, electrons primarily interact

with matter through collisional processes (ionization and excitation) and bremsstrahlung radiation, resulting in significant tissue damage along their path. While secondary photons produced by electron interactions can undergo Compton scattering, the predominant mechanism of energy deposition in electron therapy is direct ionization of atomic electrons, which enhances therapeutic effectiveness.

However, it also presents challenges, particularly in terms of damage to healthy tissues and its effectiveness in treating tumors located in low-density areas. The Compton Effect contributes to image degradation in radiography and is a primary mechanism for radiation scatter in radiotherapy. These interactions are essential for understanding how radiation interacts with different tissues and are key to designing effective diagnostic and therapeutic techniques in radiology and oncology.

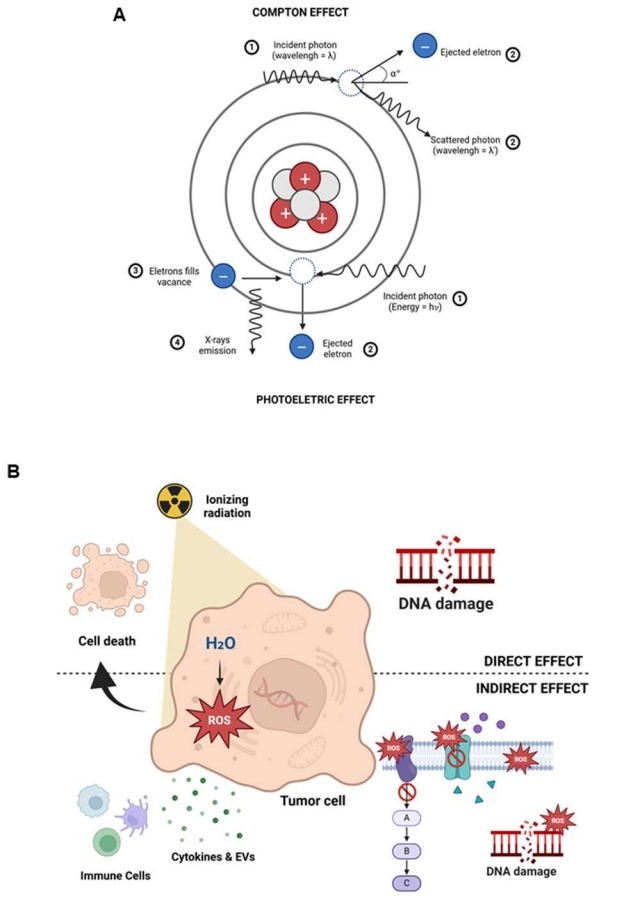

Figure 1. Interaction of photons with matter and biological effects of radiotherapy. (A) Schematic representation of the main mechanisms by which photons interact with matter: the photoelectric effect and the Compton effect. (B) Biological impacts of radiotherapy on cancer cells, emphasizing direct DNA damage (e.g., double-strand breaks caused by directly photon energy deposition in DNA molecules) and indirect effects mediated through the generation of reactive oxygen species (ROS). These ROS lead to further damage to cellular components such as DNA, proteins, and membranes, alongside modulating the immune response. Source: The authors (2025) by Biorender.

4. Dosimetry and Units

Dosimetry is the field of physics dedicated to measuring and quantifying the radiation dose absorbed by a substance or biological tissue. It plays a crucial role in medical applications, particularly in radiotherapy, where precise radiation doses are essential for effective tumor treatment while minimizing damage to healthy tissues. In addition, dosimetry is fundamental in radiation protection, where it is used to monitor radiation exposure and ensure safety in environments where ionizing radiation is used or handled (13).

Radiation dose refers to the amount of energy deposited by ionizing radiation in a material, especially biological tissues. There are various ways to measure radiation dose, each tailored to specific contexts, such as radiotherapy or radiation protection. The two main quantities used to describe radiation dose are absorbed dose and equivalent/effective dose. Absorbed dose is the amount of energy deposited per unit mass of tissue, directly measuring the energy transferred from the radiation to the material. The unit for absorbed dose in the International System (SI) is the Gray (Gy), where 1 Gy corresponds to the absorption of 1 joule of energy per kilogram of tissue. In radiotherapy, this unit is critical for determining the precise amount of radiation that a tumor or specific tissue receives (14). To assess the biological impact of radiation, two additional concepts are used: equivalent dose and effective dose, both of which are measured in Sieverts (Sv). The equivalent dose accounts for the type of radiation (e.g., X-rays, alpha particles, neutrons), recognizing that different types of radiation have varying biological effects even if they deposit the same amount of energy. For example, alpha particles are more damaging to biological tissues than X-rays for an equivalent absorbed dose. The effective dose goes a step further, considering the biological risks relative to the sensitivity of different organs and tissues exposed to radiation, allowing for an overall assessment of biological risk based on the specific tissues exposed.

The Gray is primarily used to measure absorbed dose in clinical settings like radiotherapy, particularly tumors, where precise control over dose delivery is essential. On the other hand, Sievert is widely used in radiation protection and occupational safety, helping to guide safety limits and minimize health risks. A summary is illustrated by Table 1.

5. Emergent technologies in radiotherapy

5.1 Photon versus proton therapy

Currently, radiation treatments are mainly mostly based on photon therapy (Xray or Gamma-ray), which is the most common and standardized form of radiotherapy to treat tumors, as it allows for the treatment of a broad range of tumors, including deep seated ones. There are also other techniques, in the case of electrons beams, which are employed in specific cases, primarily for the treatment of superficial tumors. In recent years, another form of radiation that has garnered attention in the oncology therapy field is proton radiation. Although this therapy was first used in 1954 it has not been widely used due to cost and the lack of comparative clinical studies between photon and proton therapy (15, 16). However, with the interest in improving the

therapeutic ratio of radiotherapy, new studies have emerged to improve treatment and patient care.

Photons are high-energy rays that can be targeted to a region of the body depositing energy and generating interactions through the tissues to induce cell damage. However, the radiation dose from photons can extend beyond the tumor, potentially affecting surrounding healthy tissues and depositing the maximum dose close to the skin surface. While photon therapy has advanced significantly, high doses in normal tissues limit its use in certain tumors. In contrast, proton therapy employs beams of protons that release their energy precisely at the tumor in a specific depth causing less damage to adjacent healthy tissues (17). This method uses positively charged particles, unlike photons that have no charge and no mass.

Table 1. Key units of radiation dose and their applications.

Quantity	Unit (SI)	Description	Application
Absorbed Dose	Gray (Gy)	Amount of energy deposited per unit mass of tissue	Radiotherapy, controls dose to specific tissues (tumors)
Equivalent Dose	Sievert (Sv)	Accounts for type of radiation, assessing the biological impact	Radiation protection, measures biological risk
Effective Dose	Sievert (Sv)	Considers the sensitivity of various organs and tissues to radiation exposure	Radiation protection, evaluates systemic biological risk

Source: The authors (2025).

5.2 Proton therapy

The precise targeting of tumors in this type of radiotherapy relies on its dose distribution and the Bragg peak" (Figure 2) (18). The Bragg Peak illustrates how charged particles deposit energy in a medium like water, resulting in a unique depth-dose distribution. This distribution concentrates high energy in a limited area, thereby minimizing additional irradiation and sparing surrounding normal organs and tissues (19). Another important parameter that differentiates radiation types and is essential for selecting the appropriate therapy is the Linear Energy Transfer (LET). LET describes the spatial pattern of energy deposition. High-LET radiation, such as alpha particles or heavy ions, deposits concentrated energy in a short path, causing significant biological damage (20). High Linear Energy Transfer radiation is effective in overcoming tumor hypoxia, which often limits the effectiveness of conventional low-LET radiation. High-LET radiation induces complex DNA damage, such as clusters of double-strand breaks, which are challenging for cells to repair. In contrast, low-LET rays distribute energy more sparsely than High-LET, resulting in less overall damage, mostly inducing single-strand DNA breaks (SSBs) and reactive oxygen species (ROS). Additionally, low-LET radiation, such as gamma and X-rays, is oxygen dependent, meaning that is effectiveness can be increased in the presence of oxygen (21). While protons, like photons, are classified as low-LET radiation, they differ significantly due to their clinical application of the Spread-Out Bragg Peak (SOBP). This characteristic enables a more precise and uniform dose distribution within the tumor, allowing for targeted energy deposition with minimal impact on surrounding healthy tissue. Protons exhibit a higher relative biological effectiveness (RBE) compared to X-rays, meaning that they can cause more biological damage for the same dose. RBE is a measure of the amount of radiation required to produce a specific biological effect relative to a reference dose.

The most commonly used form of proton therapy in clinical practice is Intensity-Modulated Proton Therapy (IMPT). This technique employs a pencil beam of protons controlled by magnets to deliver targeted doses. IMPT allows for highly accurate dose delivery, effectively reducing toxicity while increasing precision and effectiveness in treating complex tumor, such as head and neck tumors (22). Studies to date have shown that proton therapy may offer significant benefits in several specific cases, including pediatric cancer, specific cancer, tumors located near critical organs, cases requiring repeated radiation, and cancer with radioresistance (23, 24).

5.3 Carbon ion therapy (CIRT)

CIRT is the second most commonly used charged particle radiotherapy after proton therapy and offers features similar to proton radiation with unique physical and biological advantages. Like protons, CIRT releases the majority of its energy at the Bragg peak, effectively spearing healthy tissues and organs. However, because carbon ions are heavier and have larger mass, they can deliver their energy more concentrated, allowing for more precise targeting of tumors. CIRT is classified as high-LET radiation, presenting a higher RBE than photons and protons. This makes CIRT particularly successful in treating challenging tumors, increasing the potential for improved therapeutic outcomes. While the RBE for carbon ions is accepted in a range between 2-3 or higher, RBE for photons is considered 1 and 1.1 for protons (25, 26).

The use of CIRT in clinical trials is emerging, with studies demonstrating its efficacy against more common cancers such as prostate, glioblastoma, sarcomas, and head and neck cancers (27, 28). Additionally, CIRT has shown promising results in treating hypoxic, radioresistant tumors (29). As research progresses, CIRT has the potential to become a valuable treatment option for combating challenging and resistant cancers.

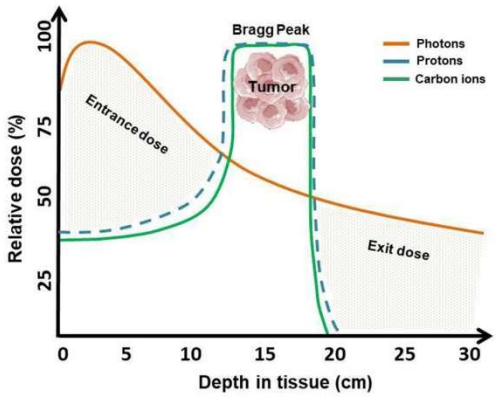

Figure 2. Dose-depth curves for different types of radiation: photons, protons, and carbon ions. The curves illustrate how the radiation dose is distributed as a function of depth in tissue. Photons exhibit an exponential decrease in dose with depth, characteristic of their high penetration and lack of a defined peak. Protons demonstrate a Bragg peak, where the maximum dose is delivered at a specific depth before a rapid fall-off, minimizing damage to surrounding tissues. Carbon ions also show a pronounced Bragg peak, but with a sharper rise and fall, providing greater precision and effectiveness in targeting tumors while sparing healthy tissue. Source: The authors (2025).

5.4 Adaptive radiation therapy (ART)

ART emerged over 20 years ago but is only now being implemented in clinical practice. The primary goal of ART is to improve accuracy and outcomes by adjusting the radiation therapy plan during a course of treatment. To achieve this, ART combines several tools such as image guidance, deformable image registration (DIR), and artificial intelligence (AI). This technology allows for real-time adaptation in response to anatomic alterations such as weight loss, variation in respiratory patterns, and tumor shrinkage. Moreover, ART can consider factors like toxicity responses and tumor biology, including metabolic activity and hypoxia (30). Thus, ART aims to provide more personalized and effective treatments for patients with cancer.

Additionally, ART supports the implementation of different workflows: online, offline, or real-time (31). Offline ART is used between treatment sessions to focus on changes that occur during therapy. In some cases, the offline model cannot address certain variations, so the online model is used before each session to adjust and deliver the correct treatment. When changes are observed during treatment delivery, like variation in breathing, a real-time technique is used to automatically adjust the parameters without the need for operator intervention (32). All these aspects show how advanced and sophisticated this therapy is. Besides, there are different ART techniques designed to enhance tumor radiotherapy, and the use of IA provides benefits. IA improves algorithms that assist the image reconstruction, anatomic modeling, contour segmentation, and quality assurance. The baseline treatment plan usually is established after evaluating a patient's tumor using computed tomography (CT) or the cone beam CT (CBCT), which captures 3D volumetric images to detect changes in patient geometry. To determine the optimal treatment plan, imaging data, treatment volume, radiation beam angles, and other parameters are considered. At this step, the use of Image guided radiation therapy (IGRT) has become essential to preserve surrounding healthy tissues and reduce side effects. IGRT precisely indicates the tumor location and patient's position before the treatment. Given potential variations in these parameters, frequent monitoring of the patient during treatment is necessary. One technique that contributes to monitoring and the adjustment of treatment plan is the Deformable Image Registration (DIR), which aligns several images of the same anatomical area taken at different times. This approach accounts for changes in size and shape, leading to the adjustment needed (33). If necessary, real-time ART technology can be employed to make adjustments during treatment delivery. Moreover, post-treatment follow-up is essential to evaluate outcomes and monitor patient progress effectively.

5.5 FLASH radiotherapy (FLASH-RT)

FLASH-RT is a novel radiotherapy that delivers high doses of radiation in a short amount of time, with a mean dose rate exceeding 40 Gy/s. This technique enables treatment delivery in less than 200 milliseconds (ms) for electrons and <400ms for protons. It is comparable to conventional radiation (CONV-RT), yet boosts antitumor efficacy and exhibits a protective effect on healthy tissues. FLASH-RT typically uses electron beams generated by linear accelerators (LINACs), but researchers are exploring its application with other types of radiation, such as heavy ions and protons, this last one is very relevant due to their unique properties that may enhance the FLASH effect. Additionally, high-energy X-rays differ from those produced by conventional machines. Standard X-ray machines cannot achieve FLASH conditions; instead, soft X-rays can be generated at high intensities using a synchrotron (34). FLASH-RT is more convenient for patients because it can reduce the number of treatment sessions, resulting in fewer sessions or shorter duration.

An important aspect that distinguishes FLASH radiotherapy, and makes it superior to conventional RT, is its ability to elicit different responses in normal and tumor tissues (35). FLASH-RT is influenced by oxygen concentration, inducing a transient hypoxic state in normal tissues due to the high doses administered in very short times. The brief intervals between radiation pulses facilitate the consumption of oxygen in the tissue and prevent adequate reoxygenation through diffusion. This results in the protection of normal tissues while increasing radioresistance. In contrast, tumors typically exist in low-oxygen environments, but their response to energy delivery through FLASH therapy can be different. Tumor cells may be more susceptible to radiation damage, as they often do not exhibit additional radioresistance associated with decreased oxygen levels. Thus, FLASH-RT may bypass some resistance mechanisms. Furthermore, FLASH-RT

alters reactive oxygen species (ROS) dynamics, generating a greater amount of ROS compared to CONV-RT. This increment in ROS can be eliminated more efficiently in normal tissues than in tumor tissues, thereby enhancing damage in tumor cells (36). Moreover, studies have shown that FLASH-RT minimizes DNA damage in healthy tissues, reducing harm, while the DNA damage generated in tumor cells is comparable to that induced by CONV-RT, indicating that both techniques induce equivalent effects on tumor cells (37, 38).

Another important feature that explains the effects of FLASH therapy is its ability to enhance the immune response and promote a systemic response (34). FLASH therapy preserves vascular damage, protects progenitor and stem cells, and results in a reduction of cytokines involved in the inflammatory process, such as IL-6 and TNF-alpha (39, 40). The evaluation of the immune response following FLASH-RT compared to CONV-RT has shown a significant increase in the population of B cells and CD8+ T lymphocytes, indicating a robust systemic response (41-43). Additionally, FLASH-RT has been found to reduce levels of TGF-beta, a protein that regulates cell proliferation, differentiation, and immunosuppression (44, 45).

These findings suggest that FLASH-RT has the potential to be used in clinics and also in combination with other therapies like immunotherapy, as it can stimulate the immune system and enhance the ability of cells to recognize and destroy cancer cells. Much of the current evidence for FLASH-RT comes from preclinical studies. Ongoing clinical trials are assessing the efficacy and safety of using FLASH-RT. For instance, a prospective phase I study by Mascia et al. investigated proton FLASH therapy for bone metastases, observing therapeutic benefits and reduced toxicity, thereby demonstrating that it is clinically feasible and the necessity for further research (46). Another phase II study is being conducted to evaluate the efficacy of FLASH-RT compared to CONV-RT in patients with localized cutaneous squamous cell carcinoma or basal cell carcinoma. These studies are still in the initial stages and are crucial to obtaining more comprehensive pieces of evidence on FLASH to its implementation in clinical practice. Also, more clinical trials assessing FLASH-RT across various tumors should be prioritized (47, 48).

5.6 Challenges and limitations in the Implementation of Emerging Radiotherapy Technologies

While these technologies in radiotherapy offer promising advances in precision, efficacy, and patient outcomes, there are several shortcomings that can affect their implementation. Most of these techniques are still used to a limited extent, and there is insufficient information regarding the uncertainties associated with these novel therapies. Therefore, comparative and more extensive clinical studies are necessary to generate results that support the effectiveness of these radiotherapies. In addition, clinical trials evaluating the long-term efficacy, safety, side effects, and benefits of these therapies need to be conducted before they can be widely implemented in clinical practice. Despite their potential, there are several limitations that must be addressed, including the high cost of the technologies, the difficulty in predicting the precise radiation dose for tumors and surrounding tissues, the need for specific equipment, and the requirement for qualified and trained staff, factors that can impact the consistency and quality of the therapy delivered (49-51).

In certain therapies, there are specific challenges, as seen in CIRT, which, although precise, is sensitive to patient movement during treatment. Even a small shift can result in the energy delivered at the Bragg Peak affecting nearby healthy tissues. In the case of ART, the process itself can be time-consuming, and the short timeframe for replanning during treatment implementation can lead to errors in the therapeutic process (52, 53). Another significant limitation of ART is the complexity of accurately calculating the cumulative dose delivered during therapy, a critical factor for ensuring both safety and efficacy.

6.Ionizing radiation effects: biological responses

Radiotherapy is an oncological treatment that utilizes ionizing radiation on the tumor-affected region, aiming to induce DNA fragmentation and triggering the cell death mechanisms as a direct response. Additionally, as an indirect effect, radiation exposure increases intracellular levels of ROS and other free radicals intracellularly, which in turn triggers an enhanced immune response, signaling a threat to cellular homeostasis (54). Furthermore, the bystander effect plays a crucial role in radiotherapy. This phenomenon involves communication between irradiated and non-irradiated cells through signaling molecules such as ROS, cytokines or extracellular vesicles. These signaling molecules can induce a range of cellular responses in neighboring non-irradiated cells, from changes in proliferation to apoptosis (5). In a mouse mammary carcinoma model (TSA), which is refractory to immune checkpoint inhibitors, Vanpouille-Box et al. sought to identify how tumor-directed radiation synergizes with anti-CTLA4 antibody to induce anti-tumor immune responses, particularly against poorly immunogenic TSA tumors.

They found that abscopal responses—tumor regression in non-irradiated tumors—occurred in mice treated with 8GyX3 radiation (8 Gy given over three consecutive days) combined with anti-CTLA4 antibody. This suggests that these combination promotes a systemic anti-tumor immune response, which includes the activation of T cells that target both the irradiated tumors and the non-irradiated tumors, leading to regression of distant, untreated tumors (55).

6.1 DNA Damage

Ionizing electromagnetic radiation can interact directly with cellular components such as DNA, proteins, and lipids. Following the absorption of

energy by the biological medium, electrons are ejected, leading to cellular and tissue damage. This phenomenon, known as the direct effect, accounts for approximately 30% of the biological impact of radiation (56). Additionally, radiation can interact with the medium in which cellular constituents and cells themselves are suspended – water – leading to the production of free radicals (Figure 1B). In this case, the indirect effect occurs, contributing to about 70% of the biological impact of radiation (57). The higher likelihood of the indirect effect is due to the fact that water constitutes a substantial portion of cellular composition, with 32 eV being required for the radiolysis of water. The ejected electron may be captured by another water molecule, and the ions formed (H_2O^+, H_2O^-) interact with neighboring water molecules, ultimately producing free radicals such as hydrogen (H•) and hydroxyl (OH•) radicals. The ejected electron can also be captured by a network of water molecules, forming another free radical, the aqueous electron (e^-aq).

The DNA molecule is a principal target of the cytotoxic effects of ionizing radiation (58). Double-strand breaks (DSBs) in DNA are regarded as among the most severe lesions, often leading to cellular death. It is estimated that cells typically undergo between 20 and 40 DSBs and approximately 1,000 single-strand breaks per gray of radiation (59). The variability in cellular sensitivity or response to radiation is largely attributed to the efficiency and accuracy of the repair mechanisms responsible for addressing radiation-induced damage (60). A particularly important form of damage is the so-called "clustered lesions," which consist of complex alterations involving two or more lesions. These include breaks in the hydrogen bonds that hold the two DNA strands together, oxidation of nitrogenous bases, and the formation of abasic sites on both strands of DNA within a few turns of the double helix. Such concentrated damage in small sections of the DNA molecule is highly refractory to repair and has the potential to induce DSBs (61).

6.2 Membrane damage

Membranes are critical sites for radiation interaction, where radiation engages with structural proteins and lipids, leading to various alterations, including lipid peroxidation. It is proposed that, as a result of lipid peroxidation, the membrane becomes weakened at specific sites, while the majority of the membrane experiences increased rigidity, impairing its overall function (62). In addition to altering the fluidity of the cellular membrane, which can impair proper membrane function and consequently disrupt signal transduction, radiotherapy can affect the function of membrane-associated proteins, lipids and receptors, as well as the membranes of organelles, potentially triggering apoptotic pathways if the damage is too extensive (7)(63). Membrane damage can also contribute to the bystander effect through the release of signaling molecules or extracellular vesicles from irradiated cells. These can alter the membrane properties of neighboring cells and induce responses such as apoptosis (64).

6.3 DNA repair mechanisms

Irradiated cells experience a delay in cell cycle progression, which triggers the activation of genes responsible for DNA repair (65).DNA repair mechanisms are essential for maintaining genomic integrity and cellular survival, especially in response to damage caused by agents such as ionizing radiation (66). Radiation can induce a variety of DNA lesions, including single-strand and DSBs. Cells have different repair systems to address these damages, with nucleotide excision repair and DSBs repair being two of the most critical pathways. Nucleotide excision repair is particularly effective in correcting lesions that distort the DNA double helix, such as thymine dimers, by removing the damaged segment and synthesizing a new nucleotide sequence based on the opposite strand (67). DSB repair is more complex, activating pathways like Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR) (68). NHEJ is a fast and commonly used pathway that directly joins the broken DNA ends, often with the loss of nucleotides, which can lead to mutations. In contrast, HR is a more accurate pathway, as it uses a homologous sequence as a template to repair the break, and is preferentially activated during the S and G2 phases of the cell cycle when a sister chromatid is available (69). The activation of these repair mechanisms is crucial to prevent genomic instability and the development of diseases such as cancer. Failure in proper repair mechanisms can lead to cellular transformation, potentially leading to cancer or activation of cell death pathways (70).The outcome depends on factors such as the radiation dose and the type of cell affected.

After irradiation, DSBs in DNA are identified by a sensory system involving several key genes, including ATM (Ataxia Telangiectasia Mutated), BRCA1/2 (Breast Cancer Type 1 and 2), and NBS1 (Nijmegen Breakage Syndrome 1) (71). Following the detection of damage, the ATM protein phosphorylates p53, which subsequently activates p21, leading to cell cycle arrest at the G1/S checkpoint (72). Moreover, ATM also phosphorylates the MDM2 protein, inhibiting its role in p53 degradation (73), thus extending the half-life of p53 in the cell. Upon activation by ATM, CHK2 (Checkpoint Kinase 2) phosphorylates the CDC25A phosphatase (74), leading to its degradation, which delays the cell cycle at the G1/S transition. CHK2 also phosphorylates CDC25B, causing cell cycle arrest at the G2/M transition. The extent of cell cycle delay is influenced by factors such as radiation dose and the particular type of cell involved. In mammalian cells, repair of radiation-induced DNA damage typically occurs within an hour, although this process can take longer in slower-responding normal tissues in vivo (75).The G2/M phase of the cell cycle is particularly sensitive to radiation, largely due to the tight compaction of DNA during this phase (76). This compaction increases the chances of radiation-induced

chromosomal aberrations and cell death, which leads to greater radiosensitivity. The highly compact chromatin structure during G2/M makes it difficult for repair enzymes to access DNA lesions. In contrast, cells in the synthesis (S) phase are less sensitive to radiation, potentially because the replication of genetic material provides a redundant source of information, facilitating more effective DNA repair. Additionally, during the G1/S transition, there is a peak in the phosphorylation activity of DNA-PK (DNA-dependent protein kinase), which plays a key role in repairing DSBs in DNA (77).

6.4 Immune response

Radiotherapy has the potential to induce antitumor immune responses by altering the tumor microenvironment and increasing the immunogenicity of cancer cells. One of the primary mechanisms is the induction of immunogenic cell death (ICD), which releases danger signals known as DAMPs (damage-associated molecular patterns), such as HMGB1, ATP, and calreticulin (78). These signals activate dendritic cells and other antigen-presenting cells, leading to the presentation of tumor antigens to T cells. This process stimulates the production of tumor-specific cytotoxic T cells, which can target tumor cells not only at the irradiated site but also in distant areas, a phenomenon known as the "abscopal effect" (79). Additionally, radiation can induce the expression of MHC-I molecules on tumor cells, enhancing their visibility to the immune system. However, the effectiveness of this immune response may be limited by the presence of immunosuppressive factors within the tumor microenvironment, such as TGF-β and regulatory T cells (Tregs) (80).

Changes in the tumor microenvironment are closely linked to tumor cell growth, invasion, metastasis, and resistance to treatments. Radiation can trigger chronic inflammation, fibrosis, hypoxia, vascular damage, and immunosuppression in the tumor microenvironment, amplifying the pro-inflammatory response (81). Cancer cells secrete proinflammatory factors, including IL-6, IL-1a, TGF-β, and TNF-α, which activate cancer-associated fibroblasts (CAFs) and promote their transformation into inflammatory CAFs (iCAFs) (82). Additionally, radiation induces tumor cells to release high levels of cytokines, which contribute to enhanced radioresistance (83). Radiation further exacerbates tumor hypoxia, reducing oxygen-dependent DNA damage and activating HIF-1-mediated survival pathways (84). It also increases reactive oxygen species (ROS) levels, stabilizing HIF-1 and fostering angiogenesis (85). Consequently, radiation may facilitate tumor survival by creating hypoxic conditions that limit the effectiveness of tumor-eliminating effector immune cells, while simultaneously promoting the activation of immunosuppressive cells, thus contributing to radioresistance.

6.5 Metabolic and bioenergetics effects of radiation on tumors

Ionizing radiation also has significant effects on the metabolism and bioenergetics of tumor cells. Radiation exposure can cause mitochondrial damage, leading to dysfunction in the electron transport chain and increased production of ROS (86). These additional ROS can amplify oxidative stress in tumor cells, contributing to increased genomic instability and DNA damage. Furthermore, radiation can alter glucose metabolism, promoting increased aerobic glycolysis (Warburg effect) (87, 88), which helps tumor cells rapidly obtain energy in a low-oxygen environment. This phenomenon describes how tumor cells preferentially metabolize glucose, even in the presence of oxygen, instead of relying on mitochondrial oxidative phosphorylation (OXPHOS). Cancer cells may rely on aerobic glycolysis because it provides key metabolic intermediates needed for rapid cell proliferation. Producing excess ATP would be less advantageous for cancer cells if essential molecules like FAD+, NADP+, or pyruvate—needed for biosynthetic pathways supporting proliferation—are depleted. Lactate also appears to function as a signaling molecule for cancer cells, while contributing to the acidification of the tumor microenvironment, which can have detrimental effects on healthy cells and immune cells. This acidification can hinder immune responses and promote tumor progression.

7. Radioresistance

Radioresistance is a form of tumor resistance that specifically affects the response to radiotherapy. Multiple mechanisms contribute to radioresistance, including genetic alterations, changes in the TME, and cellular responses that enable tumor cells to repair radiation-induced DNA damage or circumvent cell death pathways. These adaptive processes in the tumor contribute to the failure of radiotherapy, highlighting the need for strategies to overcome resistance and improve treatment outcomes.

7.1 Cell cycle alterations

Eukaryotic cells have cell cycle checkpoints, such as G1/S and G2/M phases, that play crucial roles in regulating the cell cycle to ensure proper cell division, proliferation, and survival. When DNA damage is detected, particularly from ionizing radiation (IR), tumor cells activate these checkpoints to induce cell cycle arrest at specific stages, allowing time for DNA repair and minimizing further damage. This process contributes to radioresistance. For example, the G2/M transition checkpoint is regulated by the ataxia telangiectasia mutated (ATM) protein, a key player in sensing DNA damage and promoting repair. ATM activation leads to the arrest of the cell cycle in the G2 phase, preventing the cell from entering mitosis with damaged DNA and thus aiding in radioresistance (89). Another critical checkpoint regulator is p53, particularly at the G1/S transition. In normal cells, p53 is activated after DNA damage, interrupting the cell cycle in G1 to prevent damaged DNA from entering the S phase (DNA synthesis) and being replicated.

However, in many tumor cells, the G1/S checkpoint is frequently inactivated due to mutations in p53 or other regulatory factors, allowing damaged DNA to be replicated and passed on to daughter cells, facilitating tumor progression and increasing resistance to therapies like radiotherapy (90, 91).

7.2 DNA repair

DNA repair is a critical mechanism in the cellular response to stress caused by ionizing radiation, playing a key role in cell survival following DNA damage. Tumors often exhibit a high capacity to repair DNA breaks induced by radiotherapy, contributing to treatment resistance (92). As mentioned, multiple DNA repair pathways are activated in response to damage, with various sensors detecting and initiating repair processes in tumor cells to overcome radiation-induced harm. These sensors may serve as potential biomarkers for radiotherapy response in cancer patients (92). One important player in DNA damage repair is RAD51, which is commonly upregulated in many tumors, including lung cancer and glioblastoma. RAD51 facilitates the repair of DNA double-strand breaks (DSBs) through the homologous recombination (HR) pathway (Figure 3). By promoting efficient repair, RAD51 contributes to cancer progression and is associated with poor prognosis, as its activity helps tumor cells survive radiotherapy and other DNA-damaging treatments (93, 94). Based on the upregulation of molecules like RAD51 in tumor cells, inhibitors targeting repair pathways have recently been developed to increase tumor sensitivity to radiotherapy. One such class of inhibitors is PARP (poly(ADP-ribose) polymerase) inhibitors have shown efficacy in tumors with deficiencies in the HR pathway, such as those with BRCA1 and BRCA2 mutations, exploiting the concept of synthetic lethality (95).

Additionally, NHEJ inhibitors are being explored for tumors that rely on this pathway to survive radiation-induced damage (96). By suppressing these repair pathways, the accumulation of DNA breaks becomes lethal to tumor cells, enhancing the effectiveness of radiotherapy. Moreover, the identification of DNA repair profiles in individual tumors is emerging as a promising strategy for personalized medicine holding great potential to improve treatment outcomes, as it allows for more precise targeting of vulnerabilities within each tumor's unique DNA repair landscape.

7.3 Pro-survival signaling pathways

Several signaling pathways are involved in regulating key processes such as cell proliferation, survival, apoptosis evasion, and tumor progression. These pathways play a pivotal role in tumor development and contribute to treatment resistance. Dysregulation of pathways like PI3K/Akt and MAPK/ERK can drive uncontrolled cell growth and resistance to therapies. Briefly, the mitogen-activated protein kinase (MAPK) pathway involves a cascade of signaling events, beginning with RAS activation, which then activates RAF. This leads to the activation of MAPK kinase (MEK), ultimately culminating in the activation of extracellular signal-regulated kinase (ERK). The MAPK pathway supports cell proliferation, survival, enhanced migration, stress response, and therapeutic resistance. Its constitutive activation in many cancer types promotes these processes, contributing to tumor growth, metastasis, and resistance to treatments (97). Due to these characteristics, targeted therapy has been developed to inhibit the constitutive activation of the MAPK pathway and sensitize tumors to treatment (98).

The PI3K pathway is another crucial signaling route involved in a variety of cellular functions. Uncontrolled activation of this pathway, through mutations or loss of function in key components, is strongly associated with cancer development and resistance to treatment (99). The diverse functions of the PI3K pathway are mediated through the activation of multiple effectors, such as Akt and the mammalian target of rapamycin (mTOR) (Figure 3). These effectors play a key role in increasing the expression of proteins involved in cell survival (e.g., Survivin) and DNA damage repair (e.g., BRCA1). Additionally, Akt regulates critical processes like DNA replication and cell cycle progression, including the activation of cell cycle inhibitors like p21 (100, 101).

Furthermore, the PI3K pathway plays an essential role in modulating cellular metabolism in both cancer cells and cells in the tumor microenvironment (TME). It facilitates metabolic reprogramming by influencing key pathways, such as lipid metabolism, glucose metabolism, and autophagy, which are crucial for sustaining the high energy demands of rapidly proliferating tumor cells (102, 103). PI3K also promotes cell migration and invasion through the Akt/YAP signaling axis, further contributing to tumor progression and metastasis (104). Similar to the MAPK pathway, the PI3K pathway's dysregulation supports tumor growth and survival, highlighting the potential target of this pathway.

7.4 Tumor metabolism and autophagy

Autophagy is a catabolic process that degrades and recycles cellular components to repurpose the resulting molecules for energy or to form new structures. It plays a crucial role in regulating cellular homeostasis and supporting cell proliferation, survival, and stress response. In cancer cells, autophagy has a dual role, acting either as a tumor suppressor or as a promoter of tumor progression. This makes autophagy a complex factor in cancer biology, influencing tumor growth, therapy responses, and contributing to radioresistance (Figure 3). In glioblastoma, as well as in other cancers, autophagy is induced following radiotherapy, where it plays a cytoprotective role during treatment (105). For example, autophagy-related proteins can regulate radiation-induced G2/M cell cycle arrest, providing cells with time to repair damage before progressing to mitosis (106). Thus, autophagy not only contributes to tumor adaptation by recycling cellular materials but also supports the elevated metabolic demands of rapidly dividing cancer cells.

Another key metabolic alteration that may enhance cancer cell survival but also contribute to radioresistance by providing the substrates needed for DNA repair and cell proliferation is the, previously mentioned, warbug effect. This shift in metabolism also contributes to tumor cells' adaptation and radioresistance by interfering with DNA damage repair and modulating redox homeostasis (107).

Cancer cells that survive radiotherapy may undergo metabolic reprogramming or enhance aerobic glycolysis, thereby promoting DNA repair and activating the production of antioxidants like NADPH. These antioxidants help reduce oxidative stress, improve redox balance, and promote cell survival, further supporting tumor repopulation and resistance to treatment. In this way, both autophagy and the Warburg effect are critical contributors to the ability of cancer cells to survive and adapt to therapies, making them important targets for therapeutic strategies aimed at overcoming radioresistance.

7.5 Tumor microenvironment (TME)

The tumor microenvironment (TME) is a complex and dynamic network composed of tumor cells, immune cells, stromal cells, extracellular matrix (ECM), degradative enzymes, various growth factors, cytokines, chemokines and blood vessels that play a crucial role in tumor progression and response to therapies (108). The interactions between tumor cells and the different components of the TME, contribute to the development of radioresistance (109, 110). Among the stromal cells in the TME, cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) are particularly important in regulating tumor progression and treatment outcomes. CAFs secrete pro-tumor cytokines and growth factors that remodel the ECM and promote angiogenesis, creating a more supportive environment for tumor cells to evade radiation-induced damage (111). TAMs, depending on their polarization, can either promote an immunosuppressive "cold" TME or drive immune activation in a "hot" TME. RT has the potential to convert a cold TME into a hot one by enhancing immune cell infiltration. However, this shift is delicate, and the overall therapeutic response can vary depending on specific characteristics of the tumor's microenvironment (112).

One of the key components of the TME involved in radioresistance is cancer stem cells (CSCs). These cells possess robust DNA repair mechanisms and enhanced resistance to cell death, allowing them to survive radiotherapy and drive tumor regeneration. CSCs can exist in a dormant state within the TME, protected from radiation-induced damage, and play a significant role in tumor relapse following RT. Targeting CSCs with combination therapies that simultaneously address both the tumor microenvironment and the unique repair mechanisms of these cells holds promise for overcoming radioresistance (113). Another important component of the tumor microenvironment is extracellular vesicles (EVs). These are nanostructures with a double membrane secreted by cells into the extracellular space, carrying various molecules, including microRNAs, DNA, proteins, lipids, and noncoding RNAs (114). EVs can transfer these molecules to recipient cells, influencing tumor progression and the response to therapy (115). One of the key mechanisms by which EVs mediate radioresistance is through the transfer of miRNAs, which can regulate critical pathways involved in cell survival, apoptosis, and DNA repair. For instance, EVs containing miR-142-5p from tumor cells can target and downregulate pathways like HGF/c-Met and EGF/EGFR, both of which are involved in tumor growth, survival, and radiation resistance (116). By inhibiting these pathways, EVs can enhance the radiosensitivity of tumor cells, as shown in nasopharyngeal carcinoma models. Similarly, EVs carrying miR-503-3p from radioresistant oral squamous cell carcinoma cells can suppress radiation-induced apoptosis by targeting BAK, a key protein in the mitochondrial apoptotic pathway. This inhibition of apoptosis contributes to enhanced survival of tumor cells post-irradiation, promoting radioresistance (117).Overall, these vesicles are not only important for understanding the mechanisms behind treatment resistance but also represent potential therapeutic targets or delivery systems for overcoming radioresistance in cancer treatment.

A key characteristic of the TME that contributes to resistance is hypoxia, a condition commonly found in rapidly growing solid tumors. Hypoxia not only promotes aggressive tumor behaviors, such as increased metastatic potential, but also increases resistance to RT (118). Tumor cells in hypoxic regions (1% oxygen, or 7.5 mmHg oxygen) require higher doses of radiation to induce the same level of damage seen in normoxic tissues (8% oxygen, or 60 mmHg oxygen) (119). Under hypoxic conditions, the transcription factor HIF-1α becomes stabilized, driving several pathways that contribute to radioresistance (120). HIF-1α activates the expression of vascular endothelial growth factor (VEGF), which protects tumor blood vessels from radiation-induced damage and ensures continued nutrient and oxygen supply to tumor cells. This supports tumor growth and survival even under harsh conditions (121, 122). Additionally, hypoxia leads to metabolic reprogramming, including increased glycolysis, which further supports tumor cell proliferation and resistance to RT (Figure 3) (123).

In addition to traditional cell death pathways like apoptosis and necrosis, new forms of regulated cell death, such as necroptosis and ferroptosis, are gaining attention as mechanisms by which tumors evade RT-induced damage (124). Necroptosis, unlike apoptosis, involves membrane rupture and the release of intracellular contents, and may be regulated by proteins like RIPK1, RIPK3, and MLKL (125). These alternative pathways offer potential targets for combination therapies designed to overcome radioresistance.

In summary, the TME plays a multifaceted role in radioresistance through mechanisms such as CSC survival, hypoxia-driven signaling, metabolic

reprogramming, and immune modulation. Understanding and targeting these components, especially in combination with RT, offers promising strategies to enhance treatment efficacy and prevent tumor recurrence.

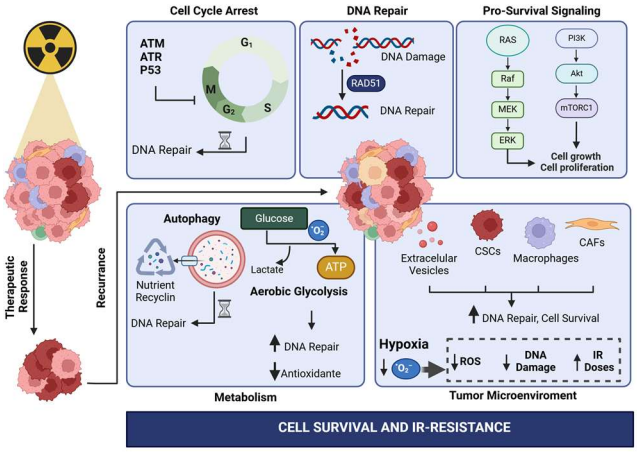

Figure 3. Mechanisms of Radioresistance in Cancer. The multifactorial mechanisms contributing to radioresistance in cancer, highlighting cellular and microenvironmental adaptations. Cell Cycle Arrest and DNA Repair: Cancer cells arrest the cell cycle at critical checkpoints (e.g., G1/S or G2/M) to allow time for DNA repair mechanisms to resolve radiation-induced damage. Enhanced DNA repair pathways are often upregulated in tumors, contributing to increased survival. Pro-Survival Signaling: Constitutive activation of pro-survival pathways (e.g., RAS/RAF, PI3K/AKT) enhances resistance by promoting cell survival and mitigating apoptotic responses to radiation. Altered Metabolism and Autophagy: Cancer cells rewire metabolism, utilizing aerobic glycolysis (Warburg effect) to generate antioxidants that reduce reactive oxygen species (ROS) induced by radiation. Autophagy further supports survival by recycling nutrients and damaged organelles, facilitating DNA repair and adaptation to metabolic stress. Tumor Microenvironment (TME): Cells in the TME, such as cancer stem cells, tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), and extracellular vesicles (EVs) modulates radiation response by enhancing DNA repair, altering immune responses, and promoting tumor survival. Hypoxia: Hypoxic regions in tumors reduce radiation efficacy by lowering ROS production, decreasing DNA damage, and necessitating increased radiation doses for effective treatment. Source: The authors (2025) by Biorender.

8. Innovations in Enhancing Radiotherapy Outcomes

8.1 The Role of Nanoparticles as Radiosensitizers

Nanoparticles have emerged as promising radiosensitizers due to their unique physicochemical properties, which enhance the precision and effectiveness of radiotherapy (126, 127). By optimizing radiation absorption and promoting targeted cellular interactions, these engineered nanoparticles improve radiotherapy outcomes, maximizing tumor cell damage while minimizing exposure to surrounding healthy tissues. Various nanoparticle-based radiosensitization strategies have been explored, and these can generally be divided into three major categories: metallic, inorganic non-metallic and organic nanoparticles.

Metal-based nanoparticles, such as gold and silver, are especially effective due to their high atomic numbers, which allow for greater localized radiation dose deposition in tumor cells (128). This enhanced absorption intensifies the radiation dose delivered to cancerous cells while minimizing damage to surrounding healthy tissues, ultimately improving the overall efficacy of the treatment. Metallic nanoparticles, particularly those with high atomic numbers (e.g., gold, silver, platinum), can generate ROS when exposed to ionizing radiation. Upon interaction with X-rays or gamma rays, these nanoparticles eject high-energy electrons from the metal atoms. These photoelectrons then interact with surrounding molecules, especially water, leading to water ionization and the subsequent formation of ROS. The generation of ROS promotes cell death by directly damaging key molecules, such as DNA, resulting in significant DNA breaks. Since cancer cells typically have less efficient or dysfunctional DNA repair systems compared to normal cells, they are particularly vulnerable to DNA damage caused by ROS during radiotherapy. In addition to DNA damage, ROS also interacts with other vital molecules, including proteins and lipids. This can lead to the inactivation of essential enzymes, damage to structural proteins, and degradation of cell membranes, all of which compromise cell integrity and eventually result in cell death. The excessive ROS generated by nanoparticles can overwhelm the cancer cells' ability to cope with oxidative stress. While cancer cells can develop resistance to radiotherapy by enhancing their DNA repair mechanisms or by overexpressing antioxidant proteins that neutralize ROS, boosting ROS levels beyond the threshold of these defenses can increase the radiosensitivity of resistant tumors (129). This helps to overcome radioresistance, ultimately improving treatment outcomes for more aggressive or resistant cancers.

Multidisciplinary studies have highlighted the potential of gold nanoparticles (AuNPs) as effective radiosensitizers, with extensive research conducted across various tumor models (130, 131). AuNPs are among the most widely investigated and are considered ideal for radiotherapy due to their high X-ray absorption properties. Beyond their direct effects, where they produce high local ionization in tumor tissues, AuNP-mediated radiosensitization also results from tumor microenvironment (TME) modulation. For instance, cell uptake rates of AuNPs differ within the TME, with CAFs showing a higher uptake than normal fibroblasts (131). This selective uptake leads to increased dose enhancement in CAFs, evidenced by elevated DNA damage in these cells, further contributing to the therapeutic effects of AuNPs in radiotherapy.

Other metals like silver are also largely explored for their radiosensitizing potential. Like gold nanoparticles, silver nanoparticles (AgNPs) have a high atomic number, making them efficient absorbers of X-rays, generating ROS and inducing cancer cell death by oxidative stress and DNA damage. AgNPs can be functionalized with tumor-targeting ligands, allowing selective accumulation within tumor tissues and increasing cancer cell death (132). AgNPs show promising potential in enhancing the radiosensitivity

of hypoxic tumors, particularly hypoxic glioma cells, where they demonstrate greater radiosensitizing effects compared to cells under normoxic conditions (133). The underlying mechanism for this enhanced radiosensitivity involves the promotion of apoptosis and destructive autophagy pathways. Hypoxic cells exhibit a higher uptake capacity for AgNPs, which may account for the increased effectiveness in these low-oxygen environments, offering a valuable approach to targeting the radioresistant nature of hypoxic tumor cells.

Hypoxia-inducible factor-1 (HIF-1) is frequently upregulated in the hypoxic microenvironment of solid tumors, contributing significantly to treatment resistance and cancer progression. Silver nanoparticles have been shown to inhibit HIF-1 activation by reducing HIF-1α protein accumulation and suppressing the expression of HIF target genes, such as VEGF-A and GLUT1 (134). Given the roles of HIF-1 and VEGF-A in promoting angiogenesis, AgNPs also demonstrate anti-angiogenic effects limiting the blood supply to the tumor, which can complement the effects of radiotherapy (134-136). These combined effects of AgNPs hold promise for complementing radiotherapy by targeting the hypoxia-driven pathways in cancer. Although non-metallic nanoparticles lack the high atomic numbers of metallic counterparts, they can still enhance radiotherapy through various mechanisms beyond ROS generation. The structure and surface chemistry of carbon-based nanoparticles, such as carbon nanotubes, graphene, and fullerenes, enable them to generate ROS, thereby increasing oxidative stress in cancer cells. Additionally, carbon nanotubes, in particular, can generate hyperthermia due to their unique physical properties. They can absorb light energy, especially near-infrared (NIR) light, and efficiently convert it into thermal energy. This localized heating damages cancer cells, making them more vulnerable to radiation and enhancing the overall effectiveness of radiotherapy (137).

Nanoparticles have gained much interest as radiosensitizers due to their feasibility of modulation of physicochemical properties, such as the core material, surface charge, size, and shape for tumor targeting (138-140). Nanoparticles can passively accumulate in tumors due to the enhanced permeability and retention (EPR) effect, a phenomenon where the leaky blood vessels of tumors and high interstitial pressure allow nanoparticles to penetrate more easily than normal tissues. However, a key advantage of nanoparticles is their ability to be engineered with targeting ligands, such as antibodies or peptides that bind specifically to cancer cells. This active targeting mechanism directs the nanoparticles to selectively attach to tumor cells expressing the corresponding antigen, significantly increasing their concentration at the tumor site. By reducing exposure to healthy tissues, this targeted approach enhances the treatment's efficacy and minimizes potential side effects.

Another approach involves encapsulating radiosensitizers within liposomes—spherical vesicles composed of lipid bilayers. Liposomes can effectively deliver radiosensitizing agents, such as chemotherapeutic drugs or ROS-generating molecules, directly to cancer cells (141, 142). Once inside the tumor, these agents are released, enhancing the cancer cells' sensitivity to radiation. Encapsulation within liposomes reduces the systemic toxicity of the radiosensitizers, enabling higher concentrations to be delivered to the tumor while minimizing adverse effects on healthy tissues.

Recent advances in nucleic acid therapeutics have paved the way for innovative cancer treatments, with nanoparticles emerging as a highly effective platform for targeted gene delivery. Nanoparticles can transport therapeutic nucleic acids to tumor cells to modulate key gene expressions, especially within DNA damage response pathways, thereby enhancing the cells' sensitivity to radiation. For example, Zetrini et al. demonstrated that RAD50 silencing using RAD50-siRNA-loaded nanoparticles increased radiation sensitivity in triple-negative breast cancer cells (143).

A substantial body of literature has investigated the use of aptamers—also known as chemical antibodies— to deliver cytotoxic molecules directly to cancer cells in a tumor-targeted manner, aiming to enhance the efficacy of radiotherapy. This approach has been demonstrated in both preclinical models (144-147). Additionally, aptamers can be conjugated to nanoparticles to improve tumor targeting. Ghahremani et al. showed that gold nanoclusters functionalized with the AS1411 aptamer, which binds to the overexpressed nucleolin on cancer cell surfaces, significantly enhanced nanoparticle uptake and radiotherapy efficacy in 4T1 breast tumor-bearing mice (149). Expanding on this approach, a programmable, sequential therapeutic strategy using multifunctional fusogenic liposomes loaded with gold-containing Auranofin (AUR) and embedded with multivalent-gated aptamer assemblies (ACP) and PD-L1 aptamers within the lipid membrane has been developed to overcome the intrinsic radio-immunotherapeutic resistance of solid tumors (150).These liposomes selectively bind to PD-L1-overexpressing melanoma cells and fuse with their cytoplasmic membrane, enhancing targeted AUR delivery to melanoma cells. This targeted strategy amplifies the immunogenic cell death induced by ionizing radiation, potentiating a more effective therapeutic response against melanoma. By ensuring delivery and uptake of nucleic acids within tumor cells, this approach maximizes the therapeutic impact, potentially enabling the use of lower radiation doses while maintaining efficacy. Aptamers have also been employed to enhance the efficacy of radio-immunotherapy, addressing the challenge posed by the insufficient activation and impaired effector functions of T cells within the immunosuppressive tumor microenvironment, which significantly diminish the immunostimulatory effects of radiotherapy. He et al. developed a multifunctional nanoradiosensitizer that integrates molecularly engineered aptamer precursors, which self-assemble into PD-L1/PD-1

bispecific aptamer-based T cell engagers, encapsulated within cisplatin-loaded liposomes (151). This innovative strategy aims to induce pronounced immunogenic cell death (ICD) of tumor cells via cisplatin-mediated radiosensitization while simultaneously enhancing T cell-mediated recognition and elimination of tumor cells following exposure to ionizing radiation.

In recent years, aptamers have emerged as a promising therapeutic alternative to antibody-based therapies. Ausejo-Mauleon et al. utilized an aptamer targeting TIM-3, which is expressed in both tumor cells and cells of the adaptive and innate immune systems, as a strategy to potentiate the effects of radiotherapy (152). Blocking TIM-3 with oligomeric aptamers combined with radiotherapy significantly increased overall median survival in glioma models by promoting the expansion of myeloid populations and T cells within the TME and inducing immune memory. This new class of drugs could serve as an alternative to existing immune checkpoint blockade therapies, such as anti-CTLA-4 and PD-L1 antibodies, which are among the most successful immunotherapy strategies for many cancer patients.

Nanoparticles offer multifunctional capabilities, allowing for precise targeting, enhanced local radiation deposition, and modulation of the tumor microenvironment, making them an invaluable tool for improving the efficacy of radiation therapy. Despite their promising radiosensitizing effects, concerns remain about their biocompatibility and potential toxicity, particularly with prolonged exposure.

8.2 AI and Machine Learning in Radiotherapy Planning

Machine learning techniques, often referred to as artificial intelligence (AI), are impacting the first and the most important step in RT, the segmentation of target volumes and organs at risk (OARs). RT necessitates a precise spatial description of target volumes and OARs to deliver a highly conformal radiation dose to tumor cells while minimizing exposure to healthy tissues. Target volumes and OARs are delineated and segmented from medical images generated by computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). A large volume of annotated imaging data is generated through these advanced medical imaging modalities. Oncologists review the imaging data slice by slice to determine the treatment plan, not only selecting the Regions of Interest (ROI) and OAR, but also defining margins to account for movement and uncertainties throughout the treatment process. This procedure has been relying on contouring guidelines developed to standardize contour delineation (153). As it remains a manual process, it can be influenced by the preferences and styles of individual planners, resulting in variability in the quality of radiation therapy plans and potentially leading to suboptimal patient outcomes (154).

AI algorithms facilitate the automatic segmentation of tumors and OAR, allowing for more accurate delineation compared to manual methods (155, 156). These AI-driven tools, particularly deep learning algorithms, are trained on extensive datasets generated to recognize patterns in medical imaging modalities (CT, MRI, PET), resulting in highly accurate tumor and tissue identification. However, radiation oncologists need to review and refine the AI-generated contours to ensure precision and accuracy in the treatment planning process.

As previously discussed, radiotherapy treatment plans often require adjustments during therapy to accommodate changes in tumor size, shape, or patient anatomy. This process is known as adaptive radiotherapy (ART). AI plays a critical role in enhancing the precision of ART by analyzing real-time imaging data, such as CT or MRI scans, to dynamically adjust radiation doses. AI-driven ART allows the treatment to be continuously tailored to the patient's evolving anatomy, ensuring more accurate tumor targeting while minimizing radiation exposure to surrounding healthy tissues and organs.

One of the advantages of automatic segmentation is the improved contouring accuracy and reduction in inter-observer variability (157). Additionally, AI assistance in segmentation can lead to a reduction in re-contouring time, thereby saving considerable time for radiation oncologists. Prostate cancer is one of the primary beneficiaries of enhanced precision through AI-assisted adaptive radiation therapy. The contour of prostate cancer is particularly challenging because of the proximity with the rectum and bladder which are constantly changing volume. Current evidence shows that automatic segmentation techniques show good agreement and repeatability compared with manual segmentation in patients with prostate cancer (158, 159). A significant challenge for radiation oncologists is accounting for tumor movement caused by the patient's breathing. Tumors, particularly those in the chest and abdomen, such as lung, liver, or breast cancers, shift position during radiation delivery. This makes it difficult to precisely target the cancer cells without affecting the surrounding healthy tissues. To compensate for breathing-induced motion, radiotherapy plans often incorporate larger margins around the tumor, which increases the risk of irradiating nearby healthy tissues and organs. Additionally, tumor movement can lead to inconsistent radiation coverage, resulting in underdosing in some parts of the tumor and overdosing in adjacent healthy areas. AI plays a crucial role in reducing the need for large safety margins by accurately tracking tumor movement (160, 161). It can analyze large datasets of imaging to detect patterns of tumor motion caused by breathing. During radiotherapy, AI can automatically adjust the radiation beam in response to these movements, ensuring continuous and precise targeting of the tumor without requiring constant manual intervention. By enabling real-time tumor tracking, AI improves the effectiveness of treatment, enhances radiation precision, and reduces side effects for the patient.

Although the use of AI in enhancing radiotherapy dose planning is promising, AI-supported adaptive

radiotherapy remains an emerging field. Researchers are actively exploring which types of cancer would benefit most from adaptive radiotherapy compared to traditional approaches, as well as determining how frequently treatments should be adjusted.

9. Conclusion

In conclusion, while radiotherapy remains a cornerstone in cancer treatment, its effectiveness is often compromised by challenges such as tumor heterogeneity, radioresistance, and the complex tumor microenvironment. Advances in technology have improved the precision and delivery of radiation. However, while this increased precision holds great promise, further studies are still needed to fully understand the biological effects of these new technologies, their benefits, and potential adverse outcomes. This deeper understanding is crucial, especially when considering the challenge of overcoming radioresistance, which remains a critical obstacle. A comprehensive understanding of the biological mechanisms driving resistance—particularly the roles of hypoxia, cancer stem cells, and DNA repair pathways—is essential for the development of more effective treatments. Emerging innovations, such as multifunctional nanoparticles and the integration of artificial intelligence in radiotherapy planning, offer promising strategies to enhance treatment outcomes. Ongoing research into these technologies is vital for optimizing treatment protocols, minimizing side effects, and ultimately improving survival rates for cancer patients.

Acknowledgment

The authors used artificial intelligence tools, Grammarly and ChatGPT (OpenAI;2024) in order to verify grammar use and writing style. Figures were designed using Biorender.

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