Radio Resistance Mechanisms of Cancers: An Overview and Future Perspectives

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Abstract
Radiation therapy is widely applied as a standard curative treatment in cancers and the therapeutic techniques have improved remarkably in recent years. However, repopulation of surviving cancer cells is frequently observed during fractionated radiotherapy, which limits the efficacy of radiotherapy. These surviving cells often acquire radio resistance through the deregulation of survival signaling pathways, DNA damage repair mechanisms, post-transcriptional regulation of miRNAs, and epigenetic modifications. Therefore, advances in our understanding of the mechanisms underlying cellular sensitivity to irradiation may provide novel diagnostic markers and therapeutic targets to improve the efficacy of radiotherapy. In this review, we summarize previous studies that report on the radio resistance of various cancer cells.

Keywords: Radiotherapy; Radio resistance; DNA damage repair; miRNA; Cancer stem cell; Epigenetic modification

Introduction
Radiotherapy (RT) is the standard curative treatment for a number of malignant tumors. At the beginning of the 20th century, radium began to be used in cancer treatment via direct insertion into body cavities. However, the surrounding normal tissue was also injured from ionizing radiation (IR) exposure. With advances in computer technology, radiology developed computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) scans to precisely map targeted tumors in order to reduce the damage to normal tissue. Nowadays, intensity-modulated radiotherapy (IMRT) and proton beam therapy are applied, by aiming photon and proton beams in several directions to efficiently eliminate tumors and to minimize injury to normal organs, which can withstand the treatment and recover after RT. However, despite the improvement of therapeutic techniques, numerous patients are prone to relapse due to the intrinsic resistance of cancer cells to radiation. It is important to uncover the underlying mechanisms and develop new strategies to solve this issue. Radio resistance may arise from a deregulated signaling pathway (e.g., AKT or NF-κB), oncogenic miRNA overproduction, abnormal DNA damage response (Figure 1), or a particular epigenetic modification in cancer subpopulations, such as cancer stem cells (CSCs). Moreover, small populations of cancer cells may survive after RT and repopulate with advanced malignant phenotypes. In this review, we have summarized previous studies that reported on the radio resistance of various cancer cells.

Signaling Pathways Response to RT
Many survival signaling pathways such as the PI3K/AKT signaling pathway are activated after ionizing radiation (IR)-induced damage to protect cells from death. The DNA damage response is induced by the activation of specific sensors and causes cell cycle arrest; DNA repair processes, including single- and double-strand break repair (SSB and DSB repair), participate in and restore the damaged sites. Here, we briefly introduce crucial signaling pathways response to RT, including PI3K/AKT and NF-xB, and the role of the DNA damage repair system in cancer cell radio resistance.

PI3K/AKT signaling pathway
PI3K/AKT activation is one of the most frequent events in multiple tumor types and regulates many cellular processes, including proliferation, invasion, apoptosis, and radio resistance [1-4]. The pathway also contributes to cancer-induced tumor microenvironments, such as angiogenesis and recruitment of inflammatory cells [5,6]. The pathway is activated by upstream receptor tyrosine kinases (RTK), integrins, cytokine receptors, and G-protein-coupled receptors.
Double-strand break repair

DSB is one of the most important DNA damages induced by IR, and today, much evidence has confirmed and intensified the correlation between DSB repair and radio resistance. Two repair mechanisms are involved in DSB, homologous recombination (HR) and non-homologous end joining (NHEJ), that differ in their fidelity and template requirements.

Homologous recombination

HR is predominant in the S and G2 phases because of the proximity of the homologous template. Recently, studies have discovered that IR-induced DSB activates different damage sensors, such as ataxia telangiectasia mutated (ATM) and BRCA1, that recruit DNA repair complexes to proceeding damage recovery [18-20]. For example, radio resistant subpopulations of breast cancer cells exhibited hyperactivated ATM. Phosphorylated ATM stabilized the interaction between ZEB1 and USP7 to deubiquitylate CHK1, which induced HR and contributed to a radio resistant phenotype [21]. Moreover, glioblastoma CSCs displayed up-regulated phosphorylated DNA damage response proteins and induced G2/M checkpoint activation following IR. Inhibition of ATM kinase by KU-55933 produced potent radiosensitization of the glioblastoma CSCs and effectively abrogated the DSB repair system [22]. Inhibition of ATM-Rad3-related (ATR) by VE-821also led to the abolition of IR-induced G2/M arrest, which further increased DNA damage and inhibition of HR in pancreatic cancer cells [23]. In addition, Shelton JW et al demonstrated that combining RT with a PARP inhibitor, ABT-888 significantly reduced clonogenic ability in vitro and tumor growth in vivo of colorectal cancer cells by impairing the DSB repair system. These findings suggest that combining RT with crucial DSB repair molecular inhibitors may be a novel approach to improve RT therapy.

Non-homologous end joining

NHEJ plays major roles in cell cycle S and M phases by directly recovering the damage sites without the need for a homologous template. Hyper activation of NHEJ imparts radio resistance in many cancer types, including esophageal squamous cell carcinoma, prostate cancer, glioblastoma, lung cancer, cervical cancer, and oral squamous cell carcinoma [24-29]. Beskow et al. [28] analyzed DNA-PKcs, Ku70, and Ku86 expression in 22 samples from cervical cancer patients treated with RT followed by residual tumors, found that relapse tumors represented a higher expression of those NHEJ factors compared with primary tumors. These data suggest that over expression of NHEJ factors may dictate DSB repair and restore the tumor populations in cancers.

MicroRNA System Response To Radiotherapy

MicroRNAs (miRNAs) are small (19–25 nucleotide), noncoding RNA molecules that negatively regulate gene expression by base pairing with 3′-untranslated regions (3′-UTRs) of target mRNAs leading to translational suppression or degradation [30]. Studies have shown that miRNAs play an important role in physiological processes such as proliferation, development, differentiation, metabolism, and the immune system [31-33]. Accumulation evidence suggests that miRNAs emerged as important players in tumorigenesis, acting as oncogenes or tumor suppressors depending on their cancer-related target genes [34]. For example, dysregulation of miRNA cluster miR-17-92 was found in many cancer types, including small-cell lung cancer, retinoblastoma, and oral cancer [35-37]. Kandalam et al. [36] demonstrated that EpCAM regulates the miR 17-92 cluster expression that further influences cell proliferation and invasion in retinoblastoma cells, and when transfected with antagonirs significantly decreased cell viability and induced cell apoptosis. Interestingly, Zhang et al. [37] found that miR-17-20a, two members of the miR-17-92 cluster, represented a negative correlation with TNM and lymphatic metastasis clinically, and over expression of miR17/20a significantly decreased the migratory ability of oral cancer cells by directly targeting 3′-UTR of integrin B8. These findings indicated that the same miRNA may play opposite roles in different cancers, and further discovery of miRNA targets and their relevant pathways are urgently needed. Recently, many studies have observed...
that dysregulation of these miRNAs may influence radiosensitivity in cancers. We summarize the role of miRNAs on radio sensitivity in various types of cancers that use RT as a standard therapy in Table 1. Numerous studies have identified that some miRNAs could act as “radio sensitizers” to strengthen the radio response of cancer cells and may serve as therapeutic targets [38-51]. Those miRNAs are involved in several radiobiological mechanisms, including DNA damage response, autophagy, and survival pathway alterations. For example, Zhang et al. [47] found that miR-205 inhibited DNA damage repair by targeting ZEB1 and Ubc13 in response to IR, and a loss of miR-205 was associated with poor survival of breast cancer patients. Conversely, it has been demonstrated that miR-205 contributed to a radioreistant phenotype by targeting PTEN, and further activated the AKT signaling pathway against IR-induced cell apoptosis in nasopharyngeal carcinoma [52]. That miRNAs cause radiosensitivity when overexpressed in cancer cells has also been extensively studied in various cancer types [53-57]. Many interesting reports provide evidence that different miRNAs affect the radioresponse through their downstream machinery. However, these outcomes may not reflect true radiosensitivity, and clinical validation for those miRNAs on their radioreponse is currently lacking and needs further investigation.

Epigenetic Modification In Radiotherapy

Epigenetic alterations, including DNA methylation, histone modification, and chromatin remodeling are crucial to regulate cell phenotypes without a corresponding change in the DNA sequence. In human cancers, these epigenetic abnormalities affect many neoplasms processes by regulating tumor-suppressor genes or oncogenes. Increasing evidence indicates that epigenetic alternations can regulate radiosensitivity by interfering with DNA repair, cell cycles, programmed cell death, and cancer steness properties [58-62], there by emerging as potential therapeutic targets.

DNA methylation

DNA methylation is one of the most common and most extensively studied epigenetic modifications in many types of cancers, including prostate, glioma, lung, liver, colon, and breast cancers [63-67]. However, a relatively small number of studies have focused on how DNA methylation changes cause cancer cell radio resistance. An analysis of global CpG methylation profiles of radiosensitive H460 and radio resistant H1299 non-small cell lung cancer cell lines by microarray analysis revealed that 747 genes were hypermethylated and 344 genes were hypomethylated in promoter CpG sites [68]. Among the hypermethylated genes, SERPINB5 and S100A6 were highly expressed in the radiosensitive cell line, and repression of these two genes by siRNA could significantly reduce cell death from IR. In contrast, catalase (CAT) and basonuclin 1 (BNC1), hypomethylated in promoter CpG sites, were highly expressed in radio resistant cells. Down-regulation of these two genes also increased the radio sensitivity of H1299 cells, emphasizing the important role of CpG methylation of crucial genes in radiosensitivity regulation.

Histone modification

In addition to DNA methylation, the structure of chromatin can be remodeled by histones that play important roles in gene expression regulation. Translational modifications of histones, such as methylation and acetylation, are through site-specific modifiers, including histone methyltransferase (HMT), histone acetyltransferase (HAT), histone deacetylase (HDAC), and ADP-ribosylaion. Many studies have observed that dysregulation of these modifiers may contribute to cancer progression and therapy failure.

<table>
<thead>
<tr>
<th>Tumor Types</th>
<th>miRNAs</th>
<th>Targets</th>
<th>Functions</th>
<th>RT sensitivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal</td>
<td>miR-24</td>
<td>H2AX</td>
<td>Inhibit DNA damage repair</td>
<td>†</td>
<td>[38]</td>
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<tr>
<td></td>
<td>miR-185-3p, miR-324-3p</td>
<td>WNT2B</td>
<td>Inhibit WNT signaling pathway</td>
<td>†</td>
<td>[39, 40]</td>
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<td></td>
<td>miR-451</td>
<td>RAB14</td>
<td>Inhibit DNA damage repair</td>
<td>†</td>
<td>[41]</td>
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<tr>
<td></td>
<td>miR-205</td>
<td>PTEN</td>
<td>Induce AKT activation</td>
<td>↓</td>
<td>[52]</td>
</tr>
<tr>
<td>Esophageal</td>
<td>miR-381</td>
<td>-</td>
<td>Reduce cell proliferation and migration</td>
<td>†</td>
<td>[42]</td>
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<tr>
<td></td>
<td>miR-21</td>
<td>-</td>
<td>Induce AKT activation</td>
<td>↓</td>
<td>[53]</td>
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<tr>
<td></td>
<td>miR-31</td>
<td>-</td>
<td>Reduce DNA repair genes expression</td>
<td>†</td>
<td>[43]</td>
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<tr>
<td>Lung</td>
<td>miR-15a/16</td>
<td>TLR1</td>
<td>Inhibit activation of NF-κB</td>
<td>†</td>
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<td></td>
<td>miR-511</td>
<td>TRIB2</td>
<td>Induce cell apoptosis</td>
<td>†</td>
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<td></td>
<td>miR-34a</td>
<td>NOTCH1</td>
<td>Inhibit activation of NF-κB</td>
<td>†</td>
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<td></td>
<td>miR-210</td>
<td>NDFUA4</td>
<td>Promote DSBs repair</td>
<td>↓</td>
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<tr>
<td></td>
<td>miR-21</td>
<td>PTEN</td>
<td>Induce cell proliferation and migration</td>
<td>↓</td>
<td>[55]</td>
</tr>
<tr>
<td>Breast</td>
<td>miR-205</td>
<td>ZEB1</td>
<td>Inhibit DNA damage repair</td>
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<td></td>
<td></td>
<td>Ubc13</td>
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<tr>
<td></td>
<td>miR-200c</td>
<td>UBQLN1</td>
<td>Inhibit autophagy</td>
<td>†</td>
<td>[48]</td>
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<tr>
<td></td>
<td>miR-155</td>
<td>RAD51</td>
<td>Impair homologous recombination</td>
<td>†</td>
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<td></td>
<td>miR-95</td>
<td>SGPP1</td>
<td>Induce S1P signaling</td>
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<td>Pancreas</td>
<td>miR-23b</td>
<td>ATG12</td>
<td>Induce autophagy</td>
<td>†</td>
<td>[50]</td>
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<tr>
<td>Rectum</td>
<td>miR-124</td>
<td>PRRX1</td>
<td>Induce cell apoptosis</td>
<td>†</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>miR-21</td>
<td>TP</td>
<td>Induce cell proliferation and reduce apoptosis</td>
<td>↓</td>
<td>[57]</td>
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</table>

Table 1: The role of miRNAs in radiosensitivity regulations in different cancer types.
**Histone methylation**

Depending on the different target sites, methylation or demethylation of histone could promote or prevent transcriptional activity [69]. Histones can be methylated on residues of lysine (K) and arginine (R). Methylation of lysine residues of histone 3 is the most common, including K4, K9, K27, K36, K48, and K79, which regulates gene expression by mono-methylation, di-methylation, and tri-methylation. Common sites associated with methylation for gene activation are H3K4, H3K48, and H3K79, and sites associated with gene repression are H3K9 and H3K27. It has been demonstrated that enhancement of the zeste homolog 2 (EZH2), a HMT that catalyzes H3K27 methylation, was highly expressed in post irradiation recurrent glioblastoma. Its expression was negatively correlated with clinical patient prognosis [70]. Furthermore, attenuated EZH2 activity by treatment with the EZH2 inhibitor significantly restored IR-induced apoptotic populations in radio resistant glioblastoma cells. Interestingly, EZH2 over expression was also associated with malignancy and poor prognosis of a variety of cancers, including lung, breast, prostate, lymphoma, gastric, hepatic, and head and neck squamous cell carcinoma [71-76]. Those results indicated that histone methylation not only contributes to a radioresistant phenotype, but also affects malignant behavior by influencing gene expression in cancers.

**Histone acetylation and deacetylation**

Many reports have demonstrated a significant correlation between histone acetylation and cancer progression [77,78]. Dysregulation of acetylation and deacetylation by HAT and HDAC is coupled to initiation and progress in cancers. Inhibition of HDACs has been preclinically and clinically proven to overcome radio-and chemoresistance of tumors. For instance, vorinostat, a specific HDAC inhibitor, radiosensitized neuroblastoma cells in vitro and in vivo by down-regulation of the DNA repair enzyme Ku-86 [79]. Clinically, vorinostat is the most advanced HDAC inhibitor to have entered clinical trials in cancers, including glioblastoma and gastrointestinal carcinoma [80,81]. However, it showed only modest benefits and it still needs to be confirmed in larger prospective trials.

**Cancer Stem Cell Population Response To Radiotherapy**

CSCs are defined as a small population within a tumor that possesses the capacity to self-renew and to generate the heterogeneous lineages of cancer cells [82]. This definition implies that cancer can only be cured if all CSCs are eliminated by anticancer therapy. There is increasing evidence that these CSCs are resistant to radiation therapy, and this property contributes to the poor therapeutic outcome of cancer patients [83,84]. For example, CD133-positive glioblastoma (GBM) cells, considered to be CSCs, presented high tumorigenic and radio resistant properties with strong STAT3 phosphorylation [85]. Treatment of GBM-CD133+ cells with the STAT3 inhibitor suppressed stemness expression and facilitated the differentiation of GBM-CD133+ cells into GBM-CD133- cells. Therefore, inhibiting STAT3 signaling could induce apoptosis and radiosensitivity. A similar result was also observed in HNSCC [86]. Chen et al. [86] demonstrated that CD44, ALDH1, and phosphorylated STAT3 were highly expressed in high-grade HNSCCs, and CD44+ALDH1+ cells displayed greater tumorigenicity, sphere formation ability, radio resistance and epithelial- mesenchymal-transition. Notably, xenotransplant animal experiments showed that a STAT3 inhibitor combined with RT significantly reduced tumorigenesis and metastasis, and emerged as a potential radio sensitizer in anti-CSC therapy. In addition to STAT3 signaling, numerous studies have shown that CSCs can be protected by many intrinsic and extrinsic mechanisms, including enhanced DNA repair, activation of survival signaling pathways (such as PI3K/ AKT and WNT/ β- catenin), and tumor microenvironments (e.g., growth factors and cytokines) [22, 87-92]. It was also discovered that subsets of CSCs in some human and murine breast tumors contained lower ROS levels than corresponding non-tumorigenic cells. CSCs in these tumors generated less DNA damage after irradiation because of their high expression levels of free radical scavenging systems. Treatment with a ROS scavenger inhibitor significantly decreased CSCs clonogenicity and resulted in radio sensitization [93]. Furthermore, it was also found that breast CSCs (BCSCs) expressed high levels of a free radical scavenger, a phenomenon HAT was independent of hypoxia and was maintained when cells were re-oxygenated [94]. Since then, a number of experimental studies and clinical research have focused on the improvement of loco-regional control after IR treatment through correction of tumor hypoxia; this study may explain why correcting hypoxia has not significantly improved clinical outcomes. The emerging knowledge suggests that targeting CSCs is a potential therapeutic strategy to overcome radio resistance after RT.

**Summary and Future Directions**

Tumor biology has developed over decades and significant improvements to cancer therapies have increased patients’ life spans. RT is an effective approach for local tumor control. However, resistance to irradiation remains one of the major hurdles for successful control of the disease. The radio response of a tumor is the key factor in determining the therapeutic effect, which is related to tumor radio sensitivity and radio resistance. Several biological processes are involved in radio response regulation, including activation of survival signaling pathways, DNA damage responses and repair mechanisms, miRNA regulation, and epigenetic modification. Our summaries here demonstrated that some classical pathways played important roles in modulating the radio response, particularly PI3K/AKT and NF-κB pathways. MiRNA is a class of short, non-coding ribonucleic acid molecules that influenced tumor metastasis and radio resistance. We highlighted the crucial miRNAs in radio sensitivity regulation through the post-transcriptional repression of target genes to control DNA damage repair, survival, autophagy, or apoptosis. DNA methylation and histone modification also played important roles in the dynamic processes of a tumor acquiring a radio resistant phenotype. The parallel growth in our understanding of genetics and epigenetics may provide novel diagnostic markers and therapeutic targets to improve the efficacy of RT. Moreover, much evidence indicated that CSCs have the capacity to regenerate heterogeneous cell populations after IR and generate radio resistance. Uncovering the intrinsic and extrinsic mechanisms that control the maintenance of CSCs is crucial for developing novel therapeutic strategies to eliminate CSCs, and further eradicate cancers without apparent recurrence in the future.

**References**


