Strategies to Enhance Radiosensitivity to Heavy Ion Radiation Therapy

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Abstract

Heavy ion radiation therapy has been increasingly used due to several advantages over low linear energy transfer (LET) photon therapy, but further improvement of its therapeutic efficacy would be necessary. In this review, we summarize effective radiosensitizers for heavy ion radiation therapy and mechanisms associated with the radiosensitization. High LET heavy ions induce more complex and clustered DNA damage than low LET radiation. Inhibition of homologous recombination repair or nonhomologous end rejoining and dysfunctional cell cycle checkpoint have been reported to sensitize cancer cells to heavy ions. Radiosensitizing agents, including DNA damage response inhibitors, Hsp90 inhibitors, histone acetylase inhibitors, and nanomaterials have been found to enhance cell killing by heavy ion irradiation through disrupted DNA damage response, cell cycle arrest, or other cellular processes. The use of these radiosensitizers could be a promising strategy to enhance the efficacy of heavy ion radiation therapy.

Keywords: heavy ion radiotherapy; carbon ion; radiosensitizers; DNA repair

Introduction

Heavy ion radiation therapy using charged particles has been increasingly used because it has several advantages over conventional radiation therapy [1]. It leads to more effective tumor cell killing than photon radiation therapy with higher relative biological effectiveness and lower oxygen enhancement ratio [2, 3]. Charged particles allows a highly localized energy deposition, and tumors can be targeted more accurately by adjusting the Bragg peak while minimizing damage in adjacent normal tissues [1, 2]. The National Institute of Radiological Sciences in Japan first began clinical trials on carbon ion radiation therapy in 1994 [4]. Currently, carbon ion beams have been used for the treatment of various tumors, including head/neck, lung, prostate, liver, bone and soft tissue, and other cancers, and the treatment has been successful, giving high local control rate – achieving 90% or higher 5-year local control in some cases and 90% or higher 5-year patient survival for some cancers [2].

Despite the benefits of heavy ion radiation therapy, it is still important to further improve its therapeutic efficacy. Radioresistant tumors, such as malignant mucosal melanoma in the head and neck, had poor response even in heavy ion radiation therapy [5, 6]. Improved efficacy of heavy ion radiation therapy would allow the irradiation dose to be reduced, which could further protect normal tissues near tumors from radiation-induced toxicity. Therefore, strategies to further enhance the therapeutic efficacy of heavy ion radiation therapy need to be investigated.
Although many studies on radiosensitizers for photon radiation therapy have been reported, their radiosensitizing effects on heavy ions might not be warranted due to differences in cellular responses to low versus high linear energy transfer (LET) radiation. For example, gemcitabine radiosensitized human myeloma cell lines with low LET but not high-LET particles [7]. Very recently, strategies to improve the efficacy of heavy ion radiation therapy have been increasingly studied. Here we summarize biological effects of the radiosensitizers for heavy ions and describe their mechanisms to enhance cell death by high energy particles.

**Targets to Radiosensitize Tumors to High-LET Heavy Ions**

DNA damage, especially DNA double-strand breaks (DSBs), refers to the lethal lesions caused by ionizing radiation, and misrepair or failure of DNA DSBs can lead to genomic instability or cell death. Two major pathways for DNA DSB repair are homologous recombination repair (HRR) and nonhomologous end joining (NHEJ), which have been considered as main targets to be altered for radiosensitization. Inhibiting one or both of these repair pathways has been shown to induce radiosensitization in X-irradiated tumor cells [8, 9]. Compared with low LET radiation, high LET heavy ion radiation induces more complex and clustered DNA damage, which makes DNA repair more difficult [3]. Higher numbers of small fragments of DSBs are also produced after exposure to high LET radiation than after low LET radiation [10].

Complex DSBs induced by high LET radiation efficiently activate DNA end resection, which is required for an early step in HRR and could more efficiently trigger HRR after exposure to heavy ions than after X-rays [11]. Zafar et al [12] showed the contribution of HRR to repair DSBs induced by high LET iron ions as well as low LET X-rays using RAD51 (a key protein for HRR) deficient or knockdown cells. High-LET irradiation-induced small double-stranded DNA fragments may affect the binding of Ku protein to DNA, which inhibits Ku-dependent NHEJ repair [10]. The HRR pathway was not affected by iron ion irradiation in the same study. Taken together, HRR is important to repair high LET radiation-induced DNA lesions, and inhibition of HRR is expected to enhance heavy ion irradiation-induced cell death. Deficiency of RAD51 in hamster and human cells enhanced sensitivity to iron ion irradiation [12], and XRCC3-/- cells lacking the HRR pathway were sensitive to proton and carbon ions compared with wild type CHO cells [13]. The importance of the NHEJ pathway in high LET irradiated cells has also been suggested [13–15]. Relative contribution of HRR and NHEJ toward radiosensitization to high LET radiation needs to be further studied.

Targeting the cell cycle checkpoint can also lead to enhanced radiosensitivity to high LET radiation. Induction of irreparable DNA damage or an improperly functioning checkpoint causes cell killing or genomic instability, and the combination of these might have more significant biological consequences [16,17]. Therefore, the loss of checkpoint response may allow heavy ion irradiated cells to be released with nonrepaired clustered DNA damages and enter mitosis prematurely before DNA repair is complete [17].

**Radiosensitizers for Heavy Ion Radiation Therapy**

Various agents have been studied for their radiosensitizing effects in heavy ion radiation therapy (Table). Inhibitors of key components in DNA damage response pathways were shown to influence the efficacy of heavy ion radiation therapy. Chemicals regulating multiple signaling pathways were found to enhance cancer cell killing by heavy ion radiation therapy. In recent years, nanotechnology has been applied to develop sensitizers, and several nanomaterials have been tested as radiosensitizer candidates.

**DNA Damage Response Inhibitors**

*Ataxia Telangiectasia Mutated and RAD3-Related (ATR) Inhibitors*

Heavy ion–induced complex DSBs enhance DNA end resection, which generates single-stranded DNA ends during DSB processing. The replication protein A–coated single-stranded DNAs recruit and activate ataxia telangiectasia mutated and RAD3-related (ATR) protein, which regulates G2/M cell cycle checkpoint [18]. The ATR protein plays a pivotal role in detecting DNA damage and regulating DNA repair [19], and, thus, interrupting the ATR signaling pathway may be a strategy for enhancing the lethal effect of cancer cells during heavy ion radiation therapy. The ATR inhibitor VE-821 abrogated G2/M cell cycle arrests and induces micronuclei, leading to enhanced cell death in carbon-ion irradiated cancer cells [20].
DNA-Dependent Protein Kinase Inhibitors

The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) is a key protein in the NHEJ pathway. Currently, several radiosensitization studies with DNA-PKcs inhibitors under heavy ion beams have been reported. The DNA-PKcs inhibitor NU7026 increased carbon ion irradiation–induced H2AX phosphorylation and significantly sensitized human lung cancer cells to carbon ions [14, 21]. This suggests that high-LET radiation may inhibit the NHEJ pathway partially, and NHEJ inhibitors, such as DNA-PKcs inhibitors, could enhance cell death by heavy ion irradiation. Similarly, DNA-PKcs inhibitor M3814 also radiosensitized A549 cells to carbon ions and photons [22]. The radiosensitizing effect was more enhanced under hypoxia than normoxia, which is in contrast to the effect of Ataxia telangiectasia mutated (ATM) inhibitor in the same study. This suggests that combined treatment with DNA-PK inhibitor and carbon ions could be effective strategy to treat hypoxia associated radioresistant cancers.

The multifunctional protein DNA-PKcs is required for mitosis, telomere end capping, or other cellular processes as well as NHEJ [23, 24]. Enhanced radiosensitivity by DNA-PKcs inhibition has found to occur by alternative pathways independent of DNA damage repair as well as NHEJ inhibition. Both NU7026 and NU7441 enhanced radiosensitivity of MCF-7 and H1299 cancer cells to carbon ion irradiation without DNA repair inhibition [25, 26]. Furthermore, NU7026 accelerated telomere loss induced by carbon ion irradiation in MCF-7 cells, accompanied by cellular senescence [25]. Nontoxic concentration of NU7441 sensitized H1299 cancer cells to carbon ions through strong G2/M cell cycle arrest [26]. The role of DNA-PKcs as a multifunctional protein and detailed mechanisms on radiosensitization need to be further investigated; this would provide novel strategies in radiation therapy, including heavy ion therapy.

Poly(ADP-Ribose) Polymerase Inhibitors

Poly(ADP-ribose) polymerase (PARP)-1 plays a key role in single-strand break repair and base excision repair. It detects DNA breaks and promote DNA repair by mediating recruitment of single-strand break repair and base excision repair factors [27], and, thus, PARP-1 inhibition may block the repair of radiation-induced single-strand breaks. In addition, PARP-1 is required for backup NHEJ pathway [28]. In contrast to Ku-dependent NHEJ, PARP-1 dependent complementary backup NHEJ was not affected by heavy ion irradiation [10]. High-LET iron ions induced more cell killing in PARP-1<sup>−/−</sup> cells compared to PARP-1<sup>+/−</sup> cells.

### Table. Radiosensitizers for carbon ion radiation therapy.

| Radiosensitizer | Investigated cancer type | Effects of radiosensitizers | Reference |
|-----------------|--------------------------|----------------------------|-----------|
| VE-821          | Human cervical cancer, human osteosarcoma | Abrogation of G2/M checkpoint, Micronuclei formation | [20] |
| NU7026          | Human lung cancer, human cervical cancer, human breast cancer | Delayed DSB repair, apoptosis, G2/M arrest, telomere length reduction, senescence | [14, 21, 25] |
| M3814           | Human lung cancer         | Radiosensitization of hypoxic cells | [22] |
| NU7441          | Human lung cancer         | G2/M arrest, senescence | [26] |
| AZD2281         | Human pancreatic cancer   | Increase of γ-H2AX, cell cycle arrest | [29] |
| 17-AAG          | Human lung cancer         | RAD51 inhibition, G2 arrest | [33] |
| PU-H71          | Murine osteosarcoma, human lung cancer | RAD51 inhibition, Ku70/DNA-PKcs inhibition, apoptosis, mitotic catastrophe | [37, 38] |
| TAS-116         | Human cervical cancer, human lung cancer | Rad51 inhibition, Ku70/DNA-PKcs inhibition G2/M arrest | [40] |
| SAHA            | Human multiple myeloma, human glioblastoma, Human sarcoma | Rad51 inhibition, delayed DSB repair, Chromatin decondensation, Increase of p21 expression, apoptosis | [48, 50, 51] |
| CHAP31          | Human esophageal squamous cell carcinoma | Decrease of Rad50 and Mre11A expression | [52] |
| AuNPs           | Human cervical cancer     | Production of hydroxyl radical | [55] |
| GBNs            | Human head and neck cancer | Delayed DSB repair | [58] |

Abbreviation: DSB, double-strand break.
with PARP-1 MEF cells. Therefore, PARP-1 inhibition can be a potential strategy to enhance efficacy of heavy ion radiation therapy. Finally, AZD2281 (Olaparib), a well-known PARP-1 inhibitor, was found to sensitize cancer cells to carbon ion and proton irradiation through delayed DNA repair process and cell cycle arrest [29,30].

Other Radiosensitzers to Target Multiple Signaling Pathways

**Heat Shock Protein 90 Inhibitors**

Heat shock protein 90 (Hsp90) is an adenosine triphosphate–dependent chaperone protein that plays a role in regulating the function and activation of proteins. Inhibition of Hsp90 induces degradation and inactivation of client proteins. Since Hsp90 client proteins include oncogenic signaling related proteins (ie, ErbB2, Akt, Raf-1), cell cycle regulator proteins (ie, CDK4, CDK6), and antiapoptotic proteins (ie, survivin, BCL2) [31], inhibition of Hsp90 induces degradation and inactivation of tumor growth–related client proteins and thus has been considered as a strategy to enhance the efficacy of cancer therapy, including radiation therapy. Indeed, Hsp90 inhibitors have higher affinity to tumors than normal cells [32], which leads to tumor selectivity of Hsp90 inhibitors.

The radiosensitizing effects of Hsp90 inhibitors to heavy ions have been studied in the last several years. Treatment with 17-allylamino-17-demethoxygeldanamycin (17AAG) inhibited HRR and intensified G2 cell cycle delay, which resulted in delayed tumor growth after carbon ion irradiation in vivo and in vitro [33]. However, limitations of conventional Hsp90 inhibitors, such as unfavorable toxicity and low solubility, have facilitated the development of novel Hsp90 inhibitors. For example, PU-H71 and TAS-116 were developed as novel inhibitors to overcome the limitations and were suggested as radiosensitzers for heavy ion radiation therapy. Safety, tolerability, and pharmacokinetics of PU-H71 was investigated in patients with solid tumors and non-Hodgkin lymphoma in phase 1 study NCT01581541 [34], and ongoing phase 1 trials (NCT03166085, NCT03373877) are assessing it in combination with chemotherapy drugs. The nanomolar concentration of PU-H71 was enough to regulate client proteins and inhibit tumor cell growth [35]. Treatment with PU-H71 inhibited Rad51/DNA-PKcs expression and enhanced sub-G1 cell proportion and mitotic catastrophe after carbon ion irradiation, which sensitizes human lung tumors and murine osteosarcoma to both X-rays and carbon ions [36–38]. Further, TAS-116 was reported to have less visual impairment than conventional Hsp90 inhibitors in preclinical models [39], and a phase 1 clinical trial is ongoing (NCT02965885). Use of TAS-116 enhanced tumor cell killing by both X-rays and carbon ions [40]. The radiosensitizing effect of this drug was associated with suppression of HRR-/NHEJ related proteins and G2/M cell cycle arrest. Radiosensitization of both PU-H71 and TAS-116 was not observed in human normal cell lines. The selective effect of Hsp90 inhibitors is a great advantage for their use in the clinical setting.

Most of the well-known Hsp90 inhibitors target N-terminal of Hsp90 and block the binding of adenosine triphosphate molecules. In this event, a heat-shock response can be produced, followed by induction of Hsp70 [41, 42]. This protein plays a role in the antiapoptotic pathway, and it can reduce the efficiency of tumor cell killing by Hsp90 inhibitors. Co-treatment with Hsp70 inhibitors or the use of C-terminal Hsp90 inhibitors, not inducing Hsp70, could be another strategy to enhance radiosensitizing effects in cells exposed to heavy ions [43–45].

**Histone Deacetylase Inhibitors**

Histone deacetylases (HDACs) are known to regulate chromatin modification and gene transcription by controlling histone acetylation level. Recent studies have found that HDACs are directly/indirectly involved in DNA damage response. Both HDAC1 and HDAC2 are recruited to DNA damage sites, lead H3K56 hypoacetylation, and regulate the recruitment of NHEJ-related proteins [46]. The HDAC inhibitors repressed the expression of DNA repair proteins, such as Ku70/80, DNA-PKcs, and RAD51 [47]. In addition, one of the well-known HDAC inhibitors, Suberoylanilide hydroxamic acid (SAHA) disrupted RAD51-dependent HRR by blocking the recruitment of RAD51 to DNA damage sites [48]. Indeed, HDAC inhibition can regulate transcription of genes related to various cellular process, which induces cell cycle arrest and/or apoptosis [49].

Furthermore, HDAC inhibitors have been shown to enhance radiosensitization with carbon ions through disrupting DNA repair and related signal pathways. Use of SAHA enhanced cell death of human glioblastoma and sarcoma cell lines after carbon ion irradiation, by delaying DNA repair and increasing p21 expression [50, 51]. The radiosensitizing effect of SAHA was observed similarly in both carbon ion-irradiated and X-irradiated cells. The treatment of cyclic hydroxamic-acid-containing peptide 31(CHAP31) inhibited expression of RAD50 and MRE11 and radiosensitized human esophageal squamous cell
carcinoma to carbon ions *in vitro* and *in vivo* [52]. Several HDAC inhibitors have been approved in clinical use for cancer therapy [53], and the use of the HDAC inhibitors would be a promising strategy for heavy ion radiation therapy.

**Nanoparticles**

Nanoparticles with high-Z elements have been considered as a strategy to improve targeting of tumors and enhance efficacy of radiation therapy [54]. Charged particles can activate nanoparticles, and radicals are produced by interaction of electrons emitted by nanoparticles. The emitted electron and reactive oxygen species clusters may lead to complex damage and enhance cell death by charged particles. Gold nanoparticles enhanced cell killing of HeLa cells by carbon ions and X-rays [55]. Schuermann et al [56] has summarized pathways to the clinical implementation of gold nanoparticles as radiosensitizers. Gadolinium-based nanoparticles amplified the induction of single-strand breaks and DSBs by helium ion or carbon ion, mediated by water radicals [57], and enhanced carbon ion-induced cell death in 3 head and neck tumor cell lines, accompanied by an increased number of unrepaired DSBs [58].

**Conclusion**

Heavy ion radiation therapy has received great attention due to the high relative biological effectiveness, low oxygen enhancement ratio, and accurate targeting of tumors. Despite the impressed advantages, there is a need to further improve the therapeutic efficacy of heavy ion radiation therapy in order to control radioresistant tumors and/or protect normal tissues surrounding tumors. Various radiosensitizers have been studied so far. Sensitizers to target DNA damage responses or cell cycle checkpoints enhanced cell killing induced by heavy ions, and these treatment would allow practitioners to reduce the irradiation dose while still having similar therapeutic effects by heavy ions. Accordingly, the use of radiosensitizers described in this report could be promising strategies to enhance efficacy of heavy ion radiation therapy, and further validation studies would accelerate their clinical applications.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Conflicts of Interest:** The authors have no conflicts of interest to disclose.

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