REVIEW

Development of p53-targeting drugs that increase radioresistance in normal tissues

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Abstract : Radiation damage to normal tissues is a serious concern in radiation therapy. Advances in radiotherapeutic technology have improved the dose distribution of the target volumes and risk organs, but damage to risk organs that are located within the irradiation field still limits the allowable prescription dose. To overcome this dose-limiting toxicity, and to further improve the efficacy of radiotherapy, the development of drugs that protect normal tissues but not cancer tissues from the effects of radiation are expected to be developed based on molecular target-based drugs. p53 is a well-known transcription factor that is closely associated with radiation-induced cell death. In radiation-injured tissues, p53 induces apoptosis in hematopoietic lineages, whereas it plays a radioprotective role in the gastrointestinal epithelium. These facts suggest that p53 inhibitor would be effective for radioprotection of the hematopoietic system, and that a drug that upregulates the radioprotective functions of p53 would enhance the radioresistance of gastrointestinal tissues. In this review, we summarize recent progress regarding the prevention of radiation injury by regulating p53 and provide new strategic insights into the development of radioprotectors in radiotherapy. J. Med. Invest. 66 : 219-223, August, 2019

Keywords **:** *radiation, p53, apoptosis, radioresistance, molecular target-based drugs*

INTRODUCTION

Recent progress high precision radiation therapy has been remarkable, and with improvements in dose distribution have resulted in much improved therapeutic effects. The prescription dose has improved by about 1.3 times compared the 4-field box technique (tolerance dose of 60 to 66 Gy) with the latest intensity-modulated radiation therapy IMRT (tolerance dose ~ 80 Gy) (1). However, because the complete segmentation of the target volumes and risk organs is often difficult in clinical settings, risk organs frequently suffer adverse events, especially risk organs that are located in the vicinity of the target lesion. The result is that the prescription dose is still limited by injury to normal tissue. It is especially true that, when the intestinal tract, a radiosensitive tissue, is included in the irradiation field of the pelvic region, adverse events such as diarrhea, intestinal bleeding, ulceration, perforation, intestinal obstruction, and stenosis may occur in the form of early and late effects of intestinal disorders. In addition, because some tumors move with the movement of primary or surrounding organs such as by peristalsis, respiration, and swallowing, a technological innovation for minimizing the damage of normal tissues is also necessary. Now that the physical technology has been optimized, it now becomes imperative to further improve the tolerable dose by new technology. We believe that biological innovations will further improve the present tolerable dose. Therefore, our goal is to develop a normal tissue-selective radioprotective agent that permits a good value of dose reduction factor (DRF; the fold change in irradiation dose to produce a given level of biological effect). We initially set the target value of DRF to 1.3 and more, because a value of 1.3 is excellent protection activity comparable to DRFs achieved by the radioprotective agents developed so far (2, 3).

For radiation protection, particularly important radiosensitive organs are bone marrow and the intestinal epithelium. It is known that bone marrow death occurs with exposures of up to 10 Gy and gastrointestinal death predominates in a dose range from 10 to 50 Gy. These acute radiation syndromes are attributable to radiation sensitive stem cell death in each tissue. p53 is involved in the progression of radiation cell death, but its functions are completely different in each tissue. In bone marrow, it acts as a mediator of radiation-induced apoptosis, but it also acts as a resistance factor for non-apoptotic mitotic death in the intestinal epithelium (4). Thus, it would be necessary to rationally control p53 according to the radiation syndrome being considered.

CONVENTIONAL METHODS FOR PREVENTING RADIATION INJURIES USING ANTIOXIDANTS

Ionizing radiation generates reactive oxygen species (ROS) such as $\cdot OH$ and H_2O_2 through water-splitting reactions in the living body. ROS are highly reactive with respect to cell membranes and DNA, leading to gene mutation and cell death. The use of antioxidants to scavenge ROS is a useful way for preventing radiation injury. In 1948, a few years after the atomic bombs were dropped in Japan, Patt *et al*. demonstrated that the amino acid cysteine, which contains a thiol group, showed radioprotective effect in animal experiments (5). This finding attracted international attention especially from countries involved in the Cold War. Several thiol-containing radical scavengers were subsequently developed as radioprotectors. Among them, amifostine (WR-2721, S-2 [3-aminopropylamino]-ethylphosphorothioic acid; Ethyol) is the most representative one (6).

Antioxidative agents should be administered prior to exposure to radiation, since ROS production occurs at the moment of exposure. Although this type of agent is unsuitable for use in

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unanticipated exposure situations such as the Fukushima and Chernobyl nuclear disasters, it has a great utility when used in a timely manner. The US Food and Drug Administration (FDA) currently approves amifostine as the only radioprotector aimed at protecting salivary glands in radiation therapy to head and neck cancers, but the dosage is strictly limited due to a variety of side effects (*e.g.* nausea, vomiting, and hypotension) (3, 6). In addition, most antioxidantive agents, including amifostine, also have a disadvantage, in that they increase radioresistance in cancer tissue due to their non-specific protective effects.

EFFICACY OF p53 REGULATION IN CANCER TREATMENT

In recent years, some protective agents have been developed that enhance radioresistance by controlling the molecular mechanism of cell death without relying on antioxidant activity. The p53 regulatory agent selectively protects normal tissue cells from radiation damage, which have, as a matter of course, normal p53 function. In contrast, the p53 regulatory agent does not protect many cancer cells from radiation injury, because nearly half of all types of cancer have lost their p53 function by oncogenic suppression or mutation. Therefore, the p53 regulatory agent is expected not only to function as a radioprotector in nuclear disasters but also as a reducing agent for alleviating the side-effects associated with cancer treatment by overcoming the dose-limiting toxicities of radiation therapy and chemotherapy (Figure 1). Although the risk of promoting carcinogenesis as a disadvantage of p53 suppression is often pointed out, several studies have shown that this is not a concern by using genetically modified mice. These studies have reported that the genetic impairment of the apoptosis-inducing activity of p53 does not increase carcinogenic risk, and its function as a tumor suppresser is independent from that of inducing apoptosis, cell cycle arrest, and senescence (7-9). Hence, transient pharmacological p53 regulation is an ideal strategy for protecting normal tissues in cancer therapy.

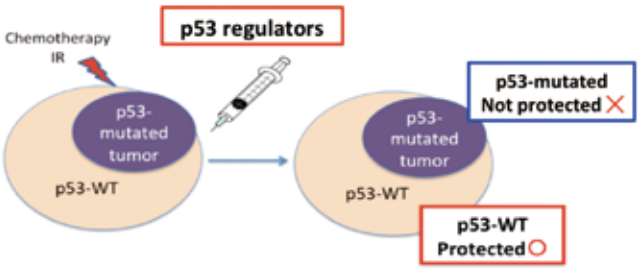

Figure 1 Specific protection of normal tissues during cancer treatment. A mutation in the *TP53* gene that encodes p53 is found in nearly half of all human cancers. This is a major cause of differences in the biological response to various stimuli including radiation between normal and cancer tissues. The p53 regulator permits normal tissues having wild p53 to be specifically protected against DNA damage-induced cytotoxicity, and is expected to function as a protective agent for the normal tissues during radiotherapy and chemotherapy.

SEARCHING FOR CHEMICAL COMPOUNDS THAT REGULATE RADIATION-INDUCED APOPTOSIS

In our initial attempts to identify chemical compounds that regulate radiation-induced apoptosis, we found that sodium orthovanadate (vanadate) acts on both the p53-mediated transcription-dependent and -independent pathways, and protects mice from total-body 12 Gy-irradiation, which is a superlethal dose that kills bone marrow stem cells, and also produces severe, but not lethal effects itself, including damage to the intestines, a process that is generally referred to as gastrointestinal death (10). Vanadate was originally identified as an inhibitor of the induction of p41, which was detected in irradiated human T-cell leukemia MOLT-4 cells using a two-dimensional electrophoretic analysis (11). In subsequent experiments we found that p41 is a caspase-mediated cleavage product of a nuclear protein, called SETβ (p42) (12). However, vanadate had no direct inhibitory effect on caspase activity. Further analyses of the apoptotic pathway upstream of caspase activation revealed that vanadate inhibits p53. It is known that p53 acts as a transcription factor in an early response to radiation. In addition, it was also found that vanadate induced a conformational change in p53 to an inactive form without affecting the phosphorylation and accumulation of p53 after DNA damage, resulting in the loss of DNA binding activity and the transcriptional activity of p53 target genes. Furthermore, the use of cell lines having different *TP53* status and a p53 knockdown cell line revealed that the anti-apoptotic action of vanadate was required for p53, leading us to conclude that the target molecule of vanadate is p53 (13). Moreover, vanadate was superior to other known p53 inhibitors, such as pifithrin α (PFTα) (14) and PFTμ (15) in suppressing p53-dependent apoptosis, presumably because of its wide spectrum of anti-p53 activities that inhibited not only the transcription-dependent pathway but the transcription-independent pathway as well, in which p53 directly acts on mitochondrial Bcl-2 family molecules. An excellent radioprotective activity was also demonstrated by *in vivo* radioprotection assays where vanadate rescued 60% of mice that had been treated with total-body 12 Gy-irradiation, a procedure that killed all of the control mice that had been treated with vehicle or a representative p53 inhibitor, cyclic PFTα, within 12 days. This result clearly indicated that vanadate was the first p53 inhibitor capable of rescuing gastrointestinal death induced by total-body irradiation (TBI) (10). In addition, a follow-up study revealed that vanadate functions, not only as a protective agent, but also as a mitigative agent when administered "after" the mice were exposed to radiation (16). These results suggest that the blockade of both p53 pathways is an excellent strategy for maximizing radioprotective effects by inhibiting the action of p53.

However, according to reports by other investigators, the inhibition of apoptosis is effective for the protection of TBI-induced bone marrow death, but it is not effective for protection against gastrointestinal death. In addition, it was also reported that increasing p53 is effective for protection against gastrointestinal death (4). These conclusions were arrived at based on a series of an abdominal irradiation experiments called sub-total-body irradiation (SBI), designed to avoid bone marrow death by shielding the bone marrow of the mouse fore leg with lead. Indeed, an innate immune agonist CBLB502 (17), which activates the p53 antagonist NF-κB, and also vanadate showed limited efficacy against gastrointestinal death by around 12 Gy. These results suggest that the efficacy of p53-suppressing radioprotectors may be limited to exposure below levels of about 12 Gy, in which the hematopoietic syndrome and gastrointestinal syndrome are combined. Therefore, in the case of a dose to the abdominal region that exceeds 13 Gy, enhancing the anti-cell death function

of p53 seems to be appropriate. It has also been shown that p21 (*Cdkn1 a*)-deficient mice are susceptible to gastrointestinal death by abdominal irradiation. Moreover, super p53 mice, which have one more copy of *Trp53*, show resistance to intestinal death by abdominal irradiation when compared with wild type mice. Taken together, the p53-p21 pathway is quite important for radioresistance of the intestinal epithelium (4, 18). Clinically, severe bone marrow suppression is not assumed in radiation therapy, except for bone marrow transplantation, which is performed by TBI with a chemotherapeutic agent. Therefore, we decided to search for a new radioprotective agent, with the goal of protecting risk organs in the abdominal and pelvic region against radiotherapy.

REGULATION OF p53 FUNCTION BY ZINC CHELATION

We focused on the degenerative action of vanadate on p53 that showed the highest radioprotective effect among the p53 inhibitors tested (10). It is known that this denaturation action of p53 is caused by the dissociation of zinc ions (19) or substituting zinc with other metal ions (20) in zinc ion binding sites that are located between the DNA-binding domain of p53 and target DNA (Figure 2). Thus, a zinc (II)-chelating agent was considered as the first candidate for use as a new radioprotective agent. However, there are no reports showing that zinc (II) chelators inhibited p53-dependent apoptosis. In addition, there were concerns regarding the influence of the wide range of chelating toxicity. Therefore, we first attempted to search for zinc chelators (tridentate to hexadentate ligands) that inhibited p53-dependent apoptosis, in an attempt to show the p53 denaturation effect with low cytotoxicity. As a result, we found that Bispicen, a tetradentate ligand, had excellent p53 inhibitory effects and apoptosis inhibiting effects (21). Although Bispicen was able to inhibit p53-dependent apoptosis in cultured cells, it did not show protective effect against total-body irradiated mice, presumably due to its chelating toxicity.

8-HQ DERIVATIVES REGULATE p53 FUNCTIONS AND SUPPRESS RADIATION INJURIES

Therefore, we next searched for 8-hydroxyquinoline (8-HQ)-based radioprotective agents. The characteristic feature of 8-HQ is that the ligands are bidentately coordinated to the zinc (II) ion (Figure 3A). These bidentate chelating agents generally have a low chelating activity, and would be expected to show a low toxicity to living cells and organisms. They were also expected to affect the DNA-binding activity of p53 by virtue of being accommodated into the niche between p53 and the target DNA without depriving p53 of a zinc ion. We investigated whether the synthesized 8-HQ derivative had an inhibitory effect against p53-dependent radiation-induced apoptosis, and found that several compounds showed anti-apoptotic effects (22). Among them, AS-2 (5,7-bis(*N*-methylaminosulfonyl)-8-hydroxyquinaldine) protected mice from an exposure of 8 Gy, which induces bone marrow death (Figure 3 B, C) (23). However, most of the 8-HQ derivatives, including AS-2, that we have examined so far, except for 5-chloro-8-quinolinol (5CHQ), showed radioprotective efficacies against bone marrow death, but had no effect on gastrointestinal death. The limited radioprotective efficacies appeared to be the limit of radioprotection by p53 inhibition, as predicted by results reported by Kirsch, *et al*. (4). However, we found that 5CHQ had a unique radioprotective activity during our evaluation of a series of 8-HQ derivatives. 5CHQ enhances the p53-mediated induction of antiapoptotic p21 and suppresses the induction of proapoptotic PUMA, which consequently leads to the prevention of p53-dependent cell death (2). The protective effect of 5CHQ on the mice bone marrow death after 7.5 Gy-TBI had a limited effect, with half of the mice being rescued. However, its pharmacological effect of enhancing p21 induction was considered to be effective for protecting against gastrointestinal damage. Therefore, we performed SBI to avoid bone marrow death, and examined the radioprotective effect on the acute gastrointestinal syndrome. As expected, 5CHQ showed a significant protective effect on the mice gastrointestinal death after SBI, and its DRF was 1.3, which has reached the original target value for radioprotection (Figure 4) (2).

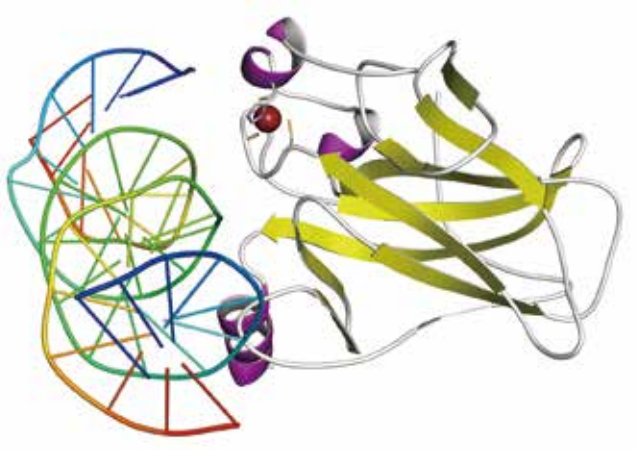

Figure 2 Structure of the p53/DNA complex created from the Protein Data Bank (PDB code : 1 TUP) (24) using a molecular viewer, Molmil (25). The p53 molecule contains a zinc ion (red sphere) locating at the binding site to target DNA. Three cysteines (yellow) residues and one histidine (blue) residue constitute a zinc-finger structure (represented by stick bond model) in the DNA binding domain of the p53 molecule (represented by ribbon model).

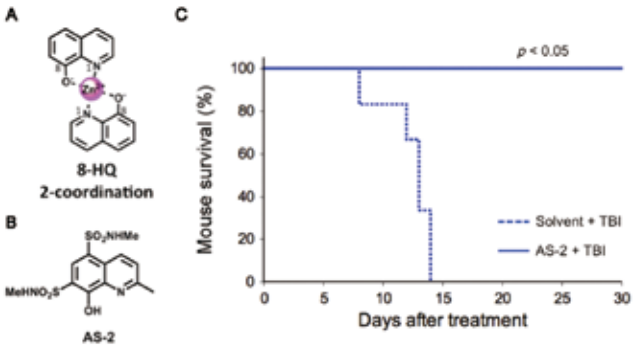

Figure 3 Radioprotective effects of AS-2, a derivative of 8-HQ, on radiation-induced hematopoietic injury. A, 8-HQ and zinc-ion complex formation. B, Structural formula of AS-2. C, Thirty-day survival rates in mice after total-body X-irradiation. Female ICR mice (8 wks) received a single intraperitoneal injection of AS-2 (80 mg/kg) 30 min before 8 Gy of total-body X-irradiation. AS-2 rescued 100% of the mice from bone marrow death caused by the irradiation. (Modified form ref. 23)

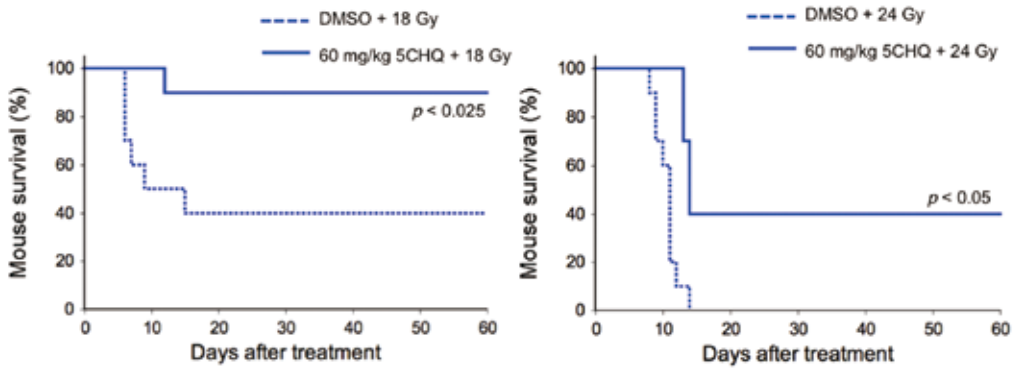

Figure 4 5CHQ protects mice from gastrointestinal death induced by a high dose of abdominal γ-irradiation. Female ICR mice (8 wks) received a single intraperitoneal 5CHQ injection (60 mg/kg) 30 min before the irradiation. Mice treated with 5CHQ showed a significantly higher survival rate at day-30 after the irradiation compared with mice that had been treated with DMSO. (Modified form ref. 2)

CONCLUSION

In this review, we summarize the efficacy of regulating p53 for preventing radiation injuries. Among the p53 regulators examined by the authors, 5CHQ was found to have a quite unique property in that it increased p53 functions that are associated with radioresistance without inhibiting the activity of p53. This modulator may become a seed compound for developing new agents that assist cancer treatment in radiotherapy and chemotharpy. Further studies will clearly be needed to clarify detailed pharmacological mechanism of 5CHQ and to assess potential clinical applications by using animal tumor models.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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