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REVIEW

Emerging targets for radioprotection and radiosensitization in radiotherapy

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Abstract Radiotherapy is the biggest force acting behind cancer treatment, yet the vast majority of patients get only modest benefit. The successive failure of targeted therapies in radiotherapy lies in the non-discriminative killing of both normal and cancer cells. However, there is still a reason for optimism due to recent advancement made in cancer biology which unrevealed many new deregulated pathways in cancer and their response towards drug and radiation. In this review, we comprehensively discussed novel and promising druggable target which can be exploited for tumor radiosensitization in addition to normal tissue radioprotection in radiotherapy, for better tumor controllability and patient quality of life. In the last part, we also discussed the radiation countermeasure agents in brief.

Keywords Tumor hypoxia · Drug-radiotherapy · Radioprotection · Chemo-radiotherapy · Cancer-radiotherapy · Cancer radioresistance · Radiosensitization

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Introduction

The three Nobel fundamental discoveries made by Roentgen (x-rays in December 1895), Becquerel (natural radioactivity in March 1896), and Curie (radium 1898) in a short time span astonishes the world scientific community, making everyone into a scientific frenzy. But the era of doom was started soon, when Edison, Tesla, and Gubbe reported radiation-induced (RI) injury in their eyes and skin on March 1896 [1]. Despite the threat, technology was rapidly spreading from bench to bedside and barely 6 months after Roentgen's discovery, radiation was used for the first time in the treatment of gastric cancer and basal-cell carcinoma patients in France, America, and Sweden [2]. In 1902, the first case of RI cancer was reported in the hand of a radiologist [3]. Later, arm of a lab assistant named Clarence Dally was amputated due to RI blistering and subsequently, he died in 1904 [4]. Soon, Curie also reported RI skin erythema and ulceration, and later, she and her daughter Irene both died from RI leukemia. Afterward, Roentgen's wife also joined the list of persons who died, believed to be a consequence of radiation [5]. The fundamental problem in RI damage was due to many reasons, i.e., 1. the lack of knowledge about radiation, 2. instrumentation to measure radiation dose, and 3. undefined unit in those days.

The term "radioprotection" was first used by Dale in 1942 on his articles, arguing that x-ray-induced enzymes/protein inactivity is responsible for RI cell death [6], though later in the 1960s, the concept was superseded by "DNA damage." Subsequently, Patt et al. showed the effect of cysteine in protection against x-rays [7]. Indeed, the first breakthrough report came from Gray et al. while working at Hammersmith Hospital, London, showed that the RI damage could be minimized by 2–3 times in anoxic condition [8]. In the 1960s, many phosphorothioates compounds under the WR series

was synthesized by Akerfeldt at the Walter Reed Army Institute of Research; it was another revolutionary mark in radioprotection [9]. Then from the 1970s onwards, plenty of reports came from multiple investigators dealing with radioprotectors, but due to multiple reasons, only amifostine got a nod from the FDA in 1995 to use as a co-therapy agent in RT for lowering normal tissue damage (fda.gov).

With the demise of cold war hostility in the 1990s, burgeoning incidents of cancer projected to increase by 75 % by 2030 from 2008 figure or reach to 22.2 million [10] in absolute terms as the baby boomer generation of 1970s (record population growth due to improved medical condition) become older. Indeed, the rapid increase in cancer incidence is mainly due to higher life expectancy [11] since the aged are more prone to cancer. Though in reality, the cancer incidents are declining (declined by 3.1 % $year^{-1}$ in men while stable in female between 2009 and 2012 [12]) due to better hygiene, immunization, etc. [11]. The only bad side of the ongoing success in reduction of cancers may be hampered by factors like obesity, stress, and diabetes, etc. or so-called the diseases of civilization. Today, radiation is the centerpiece in cancer treatment; as of 2014, 1.1 million out of 1.67 million total cancer patients received radiotherapy (RT) in the USA alone [13]. Despite extensive uses of RT, the majority of patients only get modest benefits due to collateral damage to normal tissues. In fact, just increasing the cumulative dose by 10–20 % makes the difference between the incomplete and complete eradication of some tumors [14]. Further variables like hypoxia, radio-resistance, faulty repair system, etc. consistently escalate the radiation dose. The dictum *primum non nocere* or "first, do no harm" is the centerpiece in medical profession and means that physicians are duty-bound to access the benefits over the likely harm and try to overcome them later. Hence, aiming for better patient quality of life, radioprotection had evolved from obstacle kilovoltage (1900–1940) to megavoltage (1946–1996) for eliminating severe skin damage, especially when targeting deeper tumor and further adopted much safer computer-assisted (1996–ongoing) 3D conformal RT [15]. In fact, there are a couple of new promising technologies such as stereotaxic ablative body radiotherapy, fine intensity-modulated RT, volumetric modulated arc therapy, dynamic conformal arc therapy, multimodal image-guided RT, concentrating the radiation in tumor via proton particles or carbon ion therapy (Bragg's peak), and recently introduced four-dimensional RT (where respiration is also considered) are rapidly diffusing though expensive over standard or conventional therapy [16]. But due to the ill-defined boundary and complex anatomical location, normal tissue still faced the similar fate as tumor under RT, forcing oncologists to limit the exposure dose; to achieve it, there is a need to either selectively increase the tolerance of normal tissues or radiosensitivity of tumor or both (Fig. 1). Last but not least, need of radioprotectors is also becoming apparent as a radiation countermeasure agent in space science, nuclear energy generation, medical diagnosis/imaging, nuclear fallout, and nuclear terrorism.

Radiosensitization and radioprotection

Here, we are describing promising druggable targets, which can be selectively targeted for normal tissue radioprotection or enhancing the tumor radiosensitivity or both.

Free radical scavengers

Pioneer studies of Biaglow and Bump demonstrated that the irradiation results in depletion of antioxidant pool predominantly glutathione (GSH) and increase in antioxidant enzymes including superoxide dismutase (SOD) following irradiation [17]. SOD2 or Mn-SOD is localized into mitochondrial membrane due to the presence of mitochondrial targeted peptide while SOD1 and SOD3 are localized in the cytoplasm and extracellular spaces, respectively. Delivery of SOD1 construct with mitochondrial targeting peptide or Mn-SOD (not SOD1, SOD3, or even SOD2 without mitochondrial peptide) has shown protection against the RI damage with tumor radiosensitization [17, 18]. Delivering vector construct expressing Mn-SOD is superior over protein form due to the bigger size (222 amino acids) of the latter, making it difficult to internalize by cells. Moreover, protein form is also susceptible to $ONOO^-$ mediated nitration at tyrosine-34, which makes it nonfunctional [19]. Cancer cells under extreme oxidative stress as it diminished the expression of antioxidant enzymes including glutathione peroxidase (GPx) and catalase by epigenetic modification and mutation in promoter region as a mechanism for initiation, progression, and metastasis of cancer (Fig. 2a) [20, 24]. GPx degrades H_2O_2; a product of SOD generated from RI superoxide anion radical (Fig. 2a). Thus, delivering Mn-SOD to tumor-bearing organism causes accumulation of cytotoxic H_2O_2 in the cancer cells following irradiation, while normal cells remain unaffected due to intact GPx (Fig. 2a) [17]. Moreover, cancer cells are more sensitive to Mn-SOD therapy since they are under extreme oxidative stress due to progressive upregulation of Mn-SOD as a mechanism to acquire more aggressive and invasive phenotype/enhanced malignancy (Fig. 2a) [20]. However, it does not mean that inducing ROS (by Mn-SOD) make cancer cells more malignant, as momentary ROS induction is harmful and have killing effects while under persistent ROS, cancer cells survive due to adaptive resistant by upregulating antioxidant system [25]. Delivering Mn-SOD under chemo-radiotherapeutic regime via plasmid-liposome in non-small-cell lung cancer (NSCLC) patients has been shown to reduce RI esophageal toxicity in a phase-I clinical trial [26] and now the phase-II trial is under progress (NCT00618917, clinicaltrials.

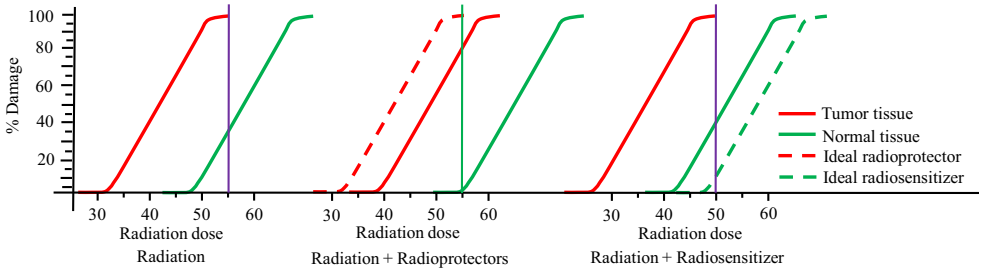

Fig. 1 Present and ideal radioprotector and radiosensitizer for radiotherapy

gov). Therefore expression of SOD, GPx, and catalase are probably useful markers for selecting the patients eligible for co-therapy (Mn-SOD with RT).

Cerium oxide (CeO_2) nanoparticles have shown to protect against the RI lung and gastrointestinal epithelium damage by scavenging free radicals (O_2−, •OH) and upregulation of SOD [27]. The CeO_2 nanoparticles have shown to mimetic catalase and SOD, interestingly the catalase activity lost at acidic pH, resulting in accumulation of H_2O_2 in the presence of superoxide radicals [28]. Cancer thrives in an acidic environment via lactic acid production; hence, using CeO_2 nanoparticles could radiosensitize the tumor via accumulation of H_2O_2 while normal cells remain unaffected due to intact catalase activity. Additionally, modifying the charge of CeO_2 nanoparticles to negative have shown to be preferentially internalized by cancer cells over normal cells [29], which may further potentiate the radiosensitization effect provided that charge modification does not influence the mimetic property.

Nitroxides and its derivatives are recycling free radical scavengers and were shown to reduce the RI damage in different model system by ROS scavenging [30]. Selective targeting of GS-nitroxide (JP4-039) to mitochondria has shown to protect oral mucosa from RI damage in Fancd2$^{-/-}$ mice without affecting tumor radiosensitivity [31]. Tempol is another molecule that has been shown to protect mammalian system against RI cytotoxicity in *in vitro* and *in vivo* (without tumor protection) [32]. Lack of tumor protection by Tempol is due to conversion of Tempol from radioprotective form to non-radioprotective form Tempol-H by bioreduction in hypoxic area. Topical application of Tempol in the scalp has shown to reduce the RT-induced alopecia in brain metastatic cancer patients in a phase I trial [32]. Erker et al. had demonstrated the chemopreventive activity of Tempol in tumor-bearing mice; however, from the study, more questions emerged than answers, like was the effect was due to Tempol or Tempol-H, what was the role of hypoxia, etc. [33]. But still, nitroxide derivatives including Tempol are promising in RT as radiosensitizers, besides radioprotectors, due to its SOD mimetic property which further amplified at acidic pH [20].

Melatonin, a hormone secreted by the pineal gland has shown to protect against RI damage in different biological systems by free radical scavenging and enhancing SOD and GPx activity [34, 35]. However, its efficacy in RT remains disputable. Administration of melatonin (20 mg day^{-1}) in glioblastomas patients undergoing RT has shown a survival

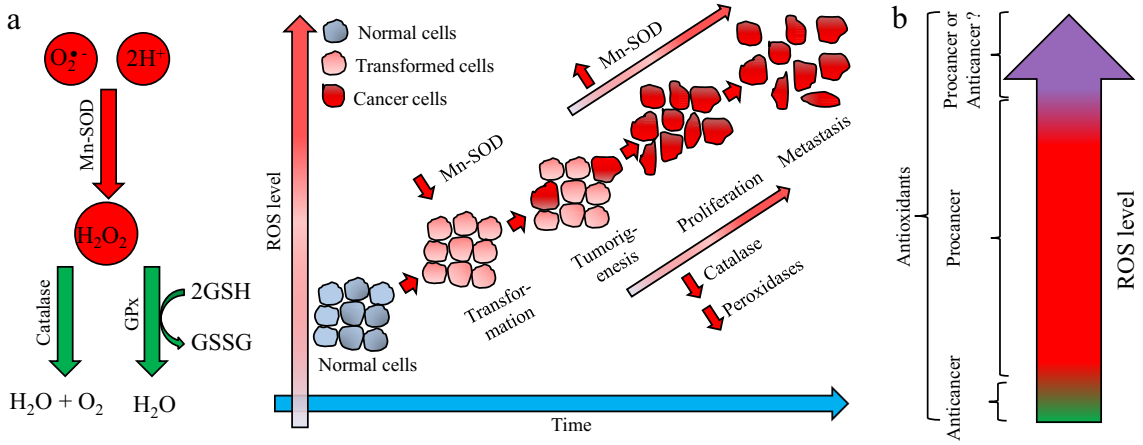

Fig. 2 Reactive oxygen species (ROS) in biological system. **a** Detoxification of ROS in normal cells; enforcement of tumorigenesis by deregulating superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in cells (adapted and modified with permission from Elsevier B.V.©2011 [20]). **b** Role of antioxidant in genomic instability/cancer, a proportional relation between antioxidant supplements and cancer incidents or DNA damage is mostly observed in smokers [21] while an inverse relation was observed in non or passive smokers [22, 23] so on the basis of endogenous ROS level [passive (low-level ROS) vs. non-passive smokers (high-level ROS)] we are proposing that at low level of ROS, antioxidant act as anti-cancer agents while after certain thresholds it behaves as procancer agents, however role of antioxidants at excessive level of ROS such as in radiotherapy is yet to clear

enhancement by 1 year in 6 (14) patients in comparison to only 1 (14) in RT arm [36]. However, in a subsequent phase II clinical trial involving brain metastases patients, melatonin (20 mg day^{-1}) failed to increase the patient survival [37]. Indeed, the clinical trials are ongoing to test the efficacy of melatonin on protection against RT-induced oral mucositis/xerostomia and fatigue in cancer patients (NCT02332928, NCT02430298).

Genistein [38] and fullerenes (hydroxylated) [39] have been shown to reduce the RI lethality in irradiated mice. Pretreatment of mice with genistein (200 mg kg body weight) has been shown to reduce the RI GI damage in mice along with enhancing the radiosensitivity of implanted tumor [38]. Silibinin, a mixture of flavonolignans present in milk thistle (*Silybum marianum*) seed is another promising candidate and can be used as a co-therapy agent. Silibinin has mitigated the RI lung injury and improved the survival of tumor-bearing mice without interfering with tumor radiosensitivity [40]. However, despite enough preclinical evidence, using antioxidants in RT always had a concern over the possibility of protecting tumor from cytotoxic therapeutic-free radicals, and further, may promote the mutagenesis by protecting the extensively damaged cells from apoptosis directly or indirectly by apoptosis inhibition since ROS also act as secondary messengers in apoptosis signaling. The suspicion was not unfounded as in a large (29133 subject) epidemiological study, the incidence of lung cancer was reported to increase dramatically in heavy smokers after vitamin E and β-carotene consumption [21]. In two separate investigations using transgenic mice prone to spontaneous brain cancer and mammary tumor, the fast clearing of the tumor by apoptosis when kept under antioxidant-free diets were shown [41, 42]. In another study supplementing N-acetylcysteine and vitamin E was shown to markedly increase tumor progression and consequently reduce survival in B-RAF and K-RAS-induced lung cancer mouse model [43]. Therefore, it seems that antioxidants may be useful in the reduction of cancer initiation at lower levels of oxidative stress and once the threshold is crossed, they may start behaving as procancer agents (Fig. 2b) [22]. The supplementation of β-carotene was shown to increase DNA damage in smokers while decreased in nonsmokers [23]; therefore, patient lifestyle could be taken into consideration before recommending any antioxidant supplementation, and it is also true for the general public who are taking plenty of antioxidants. In scrutiny of antioxidants one must differentiate between high and low dose of antioxidant supplementation (vital for maintenance of basic function i.e., essential vitamins and antioxidant present in food since numerous epidemiological studies have demonstrated the anti-cancer effects of diet rich in antioxidant substance). In preclinical studies, moderate/intermediate dose of antioxidants were shown to reduce the efficacy of radiation and induce the proliferation of cancer cell [44, 45]. In a clinical trial combining high dose of nutrients i.e., β-carotene, vitamin C, niacin, selenium, coenzyme Q10, and/or zinc with standard therapies (e.g., surgery, chemotherapy, radiation therapy, and hormonal therapy) in nonmetastatic breast cancer patients had been shown to shorten disease-free survival and breast cancer-specific survival in the nutrient-supplemented group than non-supplemented group [46]. In contrast to that, investigation from two separate groups in randomized trial using pentoxifylline and α-tocopherol in patients with NSCLC and melatonin in patients with brain glioblastomas has shown an improvement in survival following RT [36, 46–48]. In a subsequent study, the observed radiosensitization (brain metastasis) or enhanced patient survival with melatonin was not observed in a randomized phase II trial [37]. Moreover interpretation of the clinical results is also quite difficult since doses which limit or induce the cancer growth vary among species and tumor type [44]. Hence, clinical trials are still ongoing to evaluate the efficacy of low and high antioxidant diets in the prevention of RI toxicity (NCT02195960 and NCT00486304). Importantly, β-carotene and α-tocopherol may be given a special consideration in RT with modulating the partial pressures of oxygen (pO_2) since β-carotene changes its behavior from antioxidant to prooxidant with increasing pO_2 while α-tocopherol works as a strong antioxidant at higher pO_2; hence, oxygen pressure-dependent behavior may be exploited in enhancing the radiotherapeutic index [49]. Nevertheless, the post-RT administration of antioxidant such as EGCG, vitamins E and C, β-carotene, etc. has been shown to help in the reduction of RI toxicity in multiple normal organs in different clinical trials [50–52].

Administration of amifostine before RT was shown to protect against the RI mucositis, acute/late xerostomia and dysphagia, and other RI symptoms in normal tissues in NSCLC, diffuse intrahepatic tumor, and bladder cancer patients without compromising the patient's survival [53–56]. Indeed, despite the considerable body of evidence; it disappointed in a few other clinical trials [57, 58]. In fact some serious adverse effects was also noted in some preclinical and clinical studies involving RT [59, 60]. In fact, Amifostine treatment to tumor-bearing mice had shown to reduce the tumor radiosensitivity by inducing the antioxidant enzymes both in normal and tumor tissues [61]. However, it constitutively induces the antioxidant enzymes; therefore, it could be useful in minimizing the effect of occupational and high background radiation exposure [61]. The differential effects of amifostine stem from its metabolism, which allow it to concentrate rapidly in normal tissues instead in tumor, due to the requirement of being dephosphorylation (WR-1065) by alkaline phosphatase, an enzyme highly expressed in the normal cell before being incorporated. Despite being selective, it is not widely used in clinical practice due to the report of adverse side effects such as hypotension, fatigue, etc. [59, 60]; hence, investigations are still ongoing to evaluate its safety/efficacy. Thomas et al. designed a novel glutathione-based pro-drug PB-42 which works on similar mechanism as amifostine shown to increase GSH pool selectively in normal cells and abrogated cisplatin-

induced nephrotoxicity, and the effect was remained unaffected by GSH synthesis inhibitor buthionine sulfoximine (BSO) [62]. Data from previous studies demonstrated that GSH depletion by BSO enhance the tumor radiosensitivity, but severely affects the normal tissues [63]. Therefore, PB-42 may have major clinical significance in RT.

One of the major challenges in RT is how to deplete glutathione selectively from tumor to increase tumor vulnerability without affecting the normal cell radiosensitivity. GSH is synthesized from L-γ-glutamyl-L-cysteine and glycine by glutathione synthetase [64]. L-γ-glutamyl-L-cysteine forms from glutamine and cysteine by γ-glutamylcysteine synthetase (target of GSH synthesis inhibitor BSO), a rate-limiting enzyme [64]. Cancer cells frequently overexpress the glycine decarboxylase (GLDC) as a mechanism to divert the glycine towards DNA synthesis, while normal cells have plenty of glycine [65–67]. Hence, we are hypothesizing that supplementing L-γ-glutamyl-L-cysteine with BSO during RT could protect the normal tissue by increasing GSH pool due to excess glycine while at the same time BSO inhibits the endogenous GSH production in both normal and cancer cells resulting in radiosensitization of cancer.

Targeting hypoxic condition

Historically, hypoxia is the softest target recognized by clinicians due to the physiological difference between hypoxic cancer and normoxic normal cells. Systemic induction of hypoxia by cardiovascular alterations, hemoglobin function manipulating (RSR-13), over-oxygen consumption, reducing the respiration rate or by overexpression of the oxygen-labile subunit of hypoxia-inducible factors (HIF) complex (HIF1/2/3α), result in the induction of pro-angiogenic cytokines, i.e., VEGF and bFGF, known to help in radioprotection. HIF is known to promote neovascularization regulated by prolyl hydroxylase domain (PHD), and it has been observed that inhibition of PHD shown to help in reducing the radiation-induced (RI) lethality by improving the epithelial integrity of the GI tract [68]. However, in RT, deoxygenation may work only in exceptional circumstances where oxygen does not modulate the radiosensitivity [69]. HIF modulation in RT is zero sum game as HIF inhibition has shown to radiosensitize the tumor [70, 71]; hence, the above-mentioned approach is not feasible in RT.

Hypoxia-activated prodrugs (HAPs) are cyclic non-toxic prodrugs which are converted into free radical species by addition of electron (one or two electrons) by reductive enzymes under hypoxic condition causing DNA damage and DNA adducts formation, while under aerobic condition, it again oxidized or converted back to parent molecule after reacting with oxygen or by SOD (an enzyme highly expressed in normal cells). Misonidazole was the first tested drug in RT despite being known to cause neuropathy and used in successive large randomized clinical trial [72]. As anticipated, these trials failed to demonstrate any major clinical outcome in RT but provided proof of principle [73]. Another breakthrough work was done by Zeman et al., using tirapazamine (SR-4233), originally screened as an herbicide in 1972, has shown to induce the selective RI cytotoxicity to hypoxic cells (near ×200), and the rest was history [74]. Tirapazamine acted as a backbone in the development of many improved HAP. Five different chemical moiety, i.e., quinones, aromatic and aliphatic N-oxides, transition metal, and nitro(hetero)cyclic group has shown to change under anaerobic condition [75]. HAPs are classified on the basis of oxygen threshold required for their activation. Class I HAPs (SN-30000, SR-4233, benzotriazine) require a higher threshold for action than class II HAPs (PR-104A, TH-302). Tirapazamine (SR-4233) has shown promising results in preclinical and early clinical studies; however, it was disappointed in phase III clinical trial in combination with RT; hence, currently, it is not in use [76]. Then, focus was shifted towards nimorazole, a less toxic version of misonidazole shown to reduce the locoregional reoccurrence rate without any toxicity to normal tissues following RT in randomized DAHANCA 5 phase III clinical trial; however, it failed to improve survival [73]. The low clinical utility of nimorazole was due to the absence of suitable biomarkers to access the tumor hypoxic condition for screening patients required for trial. In a recent retrospective study classifying patients on the basis of expression of 15 hypoxic signature genes, a statistically better locoregional tumor control and disease-specific survival in patients classified as more hypoxic, than less hypoxic patients was shown [77]. TH-302 (Threshold Pharma) is a novel prodrug of alkylating agent "bromo-isophosphoramide mustard" that showed promising results in preclinical and clinical studies. In two separate investigations, combining TH-302 and radiation with either VEGF-A, or mTOR inhibitor (to inhabit the pro-tumor HIF-mediated angiogenic response under hypoxic condition) has shown to reduce the growth of implanted human sarcomas [78] and renal cell carcinoma (RCC) [79], respectively, in mice model. In fact TH-302, with VEGF-A and radiation combination, the tumor remained dormant for 3 months after cessation of therapy [78]. In another co-treatment study, using TH-302 with radiation showed hypoxic tumor radiosensitization without causing cytotoxicity to normal tissue in rhabdomyosarcoma R1 and H460 NSCLC tumor-bearing animals [80]. In a phase II clinical trial combining TH-302 with doxorubicin, a promising result was shown [81]. Hence, in the future, combination of TH-302 with RT is promising; however, the study must consider utilizing hypoxic cell markers for restricting therapy to only those who have significant numbers of hypoxic cells either it would get similar fate as nimorazole.

Furthermore, reducing pO_2 via limiting the blood supply using vasoconstrictor (i.e., norepinephrine, phenylephrine, epinephrine, etc.) [82] under co-therapy (RT and HAP) regimen certainly reduced the RI toxicity in normal tissues; while

tumor therapeutic response could be enhanced or remains affected, depending on tumor vascularization. Tumors under severe hypoxia have very poor vascularization; hence, further reducing oxygen in the body may not significantly alter the tumor local hypoxic environment hence oxygen enhancement ratio (OER) would remain unaffected while even if its reduced, the reduced OER in tumor further compensated by enhanced HAPs cytotoxicity. However, use of vasoconstrictor must be taken with care since association between tumor blood supply and hypoxia is yet to clear and currently guided by two conflating hypothesis steal and anti-steal effects [83]. Steal effects assumed the fully dilated tumor vessel so vasodilator caused relocating of blood from the tumor to the rest of the body while anti-steal suspected the fully constricted tumor vessel so vasodilator cause relaxation of tumor vessel and reverse the blood flow; so, the vasoconstrictor is only useful under the anti-steal mechanism. Furthermore, currently, no study has been done showing the sensitivity of normal and tumor tissues blood vessels towards vasoconstrictor or vasodilator. Further, we hypothesized that tumor vessels may lose the sensitivity towards vasoconstrictor as an evolutionary feature since they need to maximize the blood supply.

Tumor hypoxia can be considered as oxygen demand surpassing supply. Hence alternatively reducing the oxygen demand as effective as increasing the oxygen to get the same end point (hypoxia elimination) [84]. Metformin is the drug used to treat type II diabetes shown as tumor radiosensitization in multiple preclinical studies [85]. It had been shown to reduce the risk of cancer occurrence when used as a monotherapy agent [86]. However, action mechanism of metformin is not clear and multiple possible explanations are under the current discourse. One explanation is that the metformin reduces the circulating insulin which has mitogenic and prosurvival effects on cells expressing insulin receptors [85]. Another explanation is that the metformin activates the AMPK results in the suppression of mTOR, the main regulator of AKT [86]. The last explanation is the metformin protects the CD8+ tumor-infiltrating lymphocytes from apoptosis [86]. Recently, one fascinating mechanism has been proposed that metformin inhibits the complex-I activity of mitochondrial electron transport chain, and inhibition was anticipated to reduce the oxygen consumption [85]. As expected, metformin has shown to diminish the oxygen consumption in in vitro and reduces the hypoxia in tumor results in tumor growth inhibition and improved survival [85]. Hence, combining metformin with RT seems promising as many cancer patients already received metformin treatment without report of any adverse effects. Recently, few clinical trials have been started to study the synergy of metformin with chemoradiotherapy in NSCLC patients (NCT02186847, NCT02115464).

Nitric oxide (NO) is a widely studied molecule in inducing radiosensitization by modulating the blood flow in poorly vascularized tumor; however, only few reports are available dealing with radioprotection. Increasing NO level by NO donor DEA/NO or blocking the interaction between CD47 and TSP-1, has been shown to reduce the RI mortality in mice model [83, 87]. The role NO was anticipated in CD47, and TSP-1 interaction as both inhibit the NO/cGMP signaling. However, an enhancement in autophagy was found to be the reason behind radioprotection; interestingly, blocking interaction also radiosensitize the implanted melanoma or squamous lung tumors in mice [88, 89]. RRx-001 is a multifaceted anticancer agent, mediates biological action by epigenetic modification through ROS, RNS, etc. [83]. RRx-001 is a NO-donating compounds release NO locally in a biphasic manner due to different metabolism over classical NO agents/donors. Evidence has demonstrated that RRx-001 binds selectively with hemoglobin (at NO binding sites beta-cysteine 93) and glutathione in a rapid and irreversible manner [83]. The glutathione-RRx-001 adduct could increase the oxidative stress, but it is rapidly excreted; while RRx-001-Hemoglobin adduct remains in circulation till the destruction of RBCs. The initial burst of NO following RRx-001 administration is due to replacement of NO from hemoglobin resulting in rapid and transient local vasodilatation [83]. The local vasodilatation allows the flow of oxygenated blood resulting in enhancement of tumor radiosensitivity. The deoxygenated hemoglobin further enhances the NO content by converting the nitrite present in serum at the local site due to its nitrite reductase activity of hemoglobin under hypoxic condition; hence, compensating the missing nitrite synthetase activity of tumor under hypoxic condition [90]. Additionally, the RRx-001 binding with hemoglobin further potentiates the nitrite reductase activity of later enhancing the NO production resulting in better oxygenation and radiotherapeutic efficacy [90]. In an in vitro study, RRx-001 has been shown to augment the DMF by 1.9 times in radio-resistant hypoxic cells [83]. In the extended in vivo study, administration of 5 or 6 mg RRx-001 kg^{-1} body weight shown to synergistically enhance the radiosensitivity of SCCVII syngeneic tumor in mouse after local or whole body irradiation (WBI). Amazingly, RRx-001 also protected the intestinal stem cells or GI tract from lethal (10–15 Gy) WBI [83]. Hence, in view of the promising result, RRx-001 warranted further study in clinical model system.

Targeting DNA damage repair and cell cycle

Poly ADP ribose polymerase (PARP) is an attractive target which can be exploited in RT. PARP inhibitors have shown to radiosensitize the BRCA1 (breast cancer susceptibility 1) and BRCA2 mutated breast and ovarian cancers [91]. BRCA is known to play a crucial role in the repair of double-strand DNA by homologous recombination (HR) while PARP involves in the repair of single-strand DNA damage via base excision repair [92]. Hence, blocking the base excision repair pathway by PARP inhibitor results in synthetic lethality in

BRCA-deficient or HR-defunct cells, due to accumulation of damage DNA [91]. In normal cell, RI DNA damage could be repaired by HR and non-homologous end joining after PARP inhibition. Interestingly, PARP inhibitor 3-aminobenzamide has also been shown to inhibit the RI irreversible loss of salivary gland fluid secretion by inhibiting the activation of the transient potential melastatin-like 2 resulting in the reduction of RI xerostomia in C57BL/6 mice [93]. Although BRCA1/2 mutation is only limited to breast and ovarian cancers but the mutation in other component of HR pathway such as HR RAD51B (in prostate cancer, acute myeloid leukemia, lipoma, colorectal cancer, non-small-cell lung cancer, pancreatic, and uterine leiomyoma [94, 95]); PTEN (in glioblastoma multiforme, prostate and endometrioid endometrial cancer [96]); RECQL4 (in osteosarcoma, prostate tumor, and basal/squamous cell skin carcinomas [95, 97]); MRE11 (in endometrial carcinomas [98]), EXO-1 (in HNPCC and prostate cancer [99]) and RAD54 and CtIP (in non-Hodgkin's lymphoma and colon cancer [100]); Nbs1 (in melanoma, neck and head cancer, and colorectal cancer [99]); and FANCF (in leukemia and cervical cancer [96]) also makes HR dysfunctional, hence targeting PARP is a promising approach in anti-cancer therapy. Although these mutations may be rare, but if considering >200 proteins critical for the onset and regulation of HR [100] make it attractive to investigate the efficacy of PARP inhibitors in RT. PARP inhibitor has shown to sensitize the endometrial carcinomas expressing defunct MRE11 provided a proof of principle concept [98]. Importantly, defect in HR pathway can be easily detected by DNA methylation-specific microarrays and homologous recombination deficiency test. PARP is also known to act as cofactor for NF-kB; hence, blocking off major survival pathway could further add some therapeutic value [101].

Loss or perturbations of p53 function is the most common feature of human cancers resulting in loss of G1 checkpoint hence cells solely rely on G2 checkpoint regulated by Wee1 following DNA damage [102]. Evidence has shown that inhibition of Wee1 in p53-deficient tumors causes tumor radiosensitization [103]. Additionally, inhibition of Wee1 does not contribute the RI cytotoxicity into normal cells due to the presence of intact G1 blockage [103, 104]. Under chemotherapeutics regimen, Wee1 inhibitors such as PD-166285 and MK-1775 has demonstrated the potential usefulness without causing toxicity to normal cells [104, 105]. Therefore, inhibition of Wee1 is quite attractive in RT. Recently, few clinical trials have been initiated to study the synergy of MK-1775 or AZD1775 under chemoradiotherapy (RT and temozolomide or cisplatin) in glioblastoma multiforme and cervical cancer patients (NCT01849146, NCT01958658).

The key regulator of DDR is ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) protein kinases, have overlapping properties [106]. The ATM monitors all phases of cell cycle in response to double-strand breaks (DSBs) while ATR is responsible for the S and G2 phases of the cell cycle [106, 107]. Normal cells have functional G1 and G2 checkpoints while majority of the cancers are deficient in p53/ATM resulting in loss of G1 checkpoint and therefore slowly dependent on ATR/Chk1 led G2 checkpoint. Hence, it has been hypothesized that inhibiting ATR could result in accumulation of damaged DNA resulting in mitotic catastrophe while normal cells remain protected due to the functional G1 checkpoint; however, initial research mired over the concern of ATR essentiality. ATR disruption has shown to result in chromosomal fragmentation, tumor genesis, and early embryonic lethality [108]. ATR code for vital function; hence, patient with ATR mutation or in rarely occurred Seckel syndrome are naturally hypomorphic (partial loss of protein function). A recent study in ATR mouse model of Seckel syndrome has shown loss of ATR can be reverted by functional p53 [109]. The deletion of p53−/− in ATR-mosaic knockout mice reported to exacerbated tissue degeneration and induced synthetic lethality [110]. Subsequently, these two breakthrough reports worked as guiding path in ATR therapy. VE-821, a novel ATR inhibitor has shown to synergistically enhance the effect of cisplatin in ATM or p53-defective (H23) cell lines, while p53 wild-type normal fibroblast cell line (HFL1) remained unaffected [111]. HeLa cells were radiosensitized by VE-821 having suppressed p53 while normal cells expressing wild-type p53 was unaffected [112]. VE-822 (VX-970, Vertex), an analog of VE-821 having strong ATR inhibition property demonstrated a strong radiosensitizing and chemosensitizing effect in cancer cells both in in vitro and in vivo studies by suppressing the HR without affecting the normal cells [113]. Another ATR inhibitor AZ20 (AstraZeneca), under monotherapy regimen, has shown to reduces the growth of LoVo tumor xenografts in mice [114]. AZD6738 (AstraZeneca) is another improved version of AZ20 induce synthetic lethality in p53 or ATM-deficient cells or tumor in response to chemotherapy and ibrutinib hence could be useful in RT [115]. Caffeine is another ATR inhibitor radiosensitize the cells but it also blocks the ATM; thus it may cause toxicity to normal cells [116]. Additionally, due to extreme replicative stress oncogenic cell becomes more sensitive to ATR inhibition than normal cells, therefore targeting ATR pathway is a promising strategy in RT. Now, phase I clinical trial is underway, investigating the side effects and best dose of VX-970 under RT in brain metastases (NSCLC) and HPV-negative head and neck carcinoma patients (NCT02567422, NCT02589522). ATR inhibition has also shown to induce the synthetic lethality in cell-deficient XRCC1 a protein-involved repair of SSB [117]. Indeed, due to unavailability of the crystal structure of ATR, pose a major challenge in developing selective ATR inhibitor. Additionally, the large size (2644 amino acids, UniProt: Q13535) of ATR makes it difficult to be cloned, expressed, and isolate protein in purified form required for screening

inhibitors in kinase-based assay. The long sequence of ATM also poses a challenge in routine clinical diagnosis, which is further aggravated by missense variants (mostly) spread across the coding sequence as gene does not have any mutational hotspot (NCBI Gene ID: 472). The frequent missense mutation results in the loss or reduction of ATM expression; hence, immunohistochemistry may be a viable tool to inspect the ATM status in cancer patients [118].

ATM is another protein shown to cause synthetic lethality with some specific mutations. ATM kinase inhibitors [119] have shown to radiosensitize the cells carrying Fanconi anemia gene mutations, a common mutated or lost gene in cancer [120]. ATM inhibition resulted in the radiosensitization of bladder cancer cells with DAB2IP gene defects [121], ovarian, endometrial, cervical cancer [122], glioma [119], and glioblastoma stem-like cells [123]. However, despite the fact that currently, no ATM inhibitor is under clinical development over the concern of normal tissue cytotoxicity. Inhibition of ATM by KU-55933 or KU-60019 resulted in persistence DNA damage following irradiation due to defective repair in normal cells [124, 125]. Subsequent study has shown that cells possessing kinase dead (kd) ATM protein is more harmful to the cell than complete loss of ATM gene and, in fact, ATM knockout mice were viable in comparison to embryonic lethality in kd ATM mice [119, 126]. It has been hypothesized that binding of kd ATM to DNA DSBs site causing hindrance in the binding of other protein destined to start alternative repair makes little use of ATM inhibitors in clinical practice. However, brief administration of ATM inhibitor KU59403 shown to chemo-sensitize the human colon cancer xenografts (SW620 and HCT116) in mice without showing any cytotoxicity to normal tissue, further raise the possibility of ATM inhibitor development for RT [127]. The outcome of the study was that ATM inhibitor must be administrated briefly and only with the topoisomerase I (camptothecin) and II inhibitor (etoposide and doxorubicin). However, future investigation must target the ATM either by inhibiting at translation level or by disrupting the interaction between HEAT repeats of ATM and carboxy-terminal FXF/Y motif of Nbs1 [128] with the novel drug instead of targeting kinase activity of ATM.

Inhibitors of cyclin-dependent kinases (CDKs) are the emerging class of the radioprotective agents given the cells is least radiosensitive at mitosis especially during late S (due to HR) and quiescence (G0) phase [129]. Cell enters into cell cycle by D-type/CDK4/6 complex mediated hyperphosphorylation of Rb and subsequent release of E2F to start transcription of gene required for cell division. The entry in cell cycle is inhibited by $p16^{INK4A}$, an inhibitor of CDK4/6 [130]. Cancer cell often deregulates the cell cycle checkpoints as a mechanism to promote the uninterrupted cellular proliferation even in presence of damaged DNA by amplification of CDK4/6 and D-type cyclins, or loss of p16 and correlated with resistance to therapies [130]. Administration of PD0332991 and 2BrIC, an inhibitor of CDK4/6, 4 h prior or 20 h after the irradiation has shown to induce the G1 arrest reversibly in Rb-positive cells resulted in the protection of the bone marrow progenitors cells and all peripheral blood lineages: platelets, erythrocytes, myeloid cells, and peripheral blood lymphocytes [131]. Further, PD0332991 does not protect the Rb-deficient tumors as they develop independently to CDK4/6 [131]. However, it does not mean that it is only effective in patients having Rb-deficient tumors, as CDK inhibitor induces the irreversible blockage causing induction of senescence and apoptosis in cancer cells having functional p53 and mTOR and former act an inhibitor of later, while in normal cells, it induces reversible quiescence, a radioresistant state [14, 132]. Indeed, subsequent studies were not highly conclusive. PD0332991 has shown to be protected against carboplatin-induced hematopoietic injury in Rb-deficient breast cancer mice, but failed to show similar effects in Rb-positive MMTV–HER2 mice [132]. While in another study, combined treatment of PD-0332991 and RT have shown to increase the survival benefit compared with either therapy alone in Rb1-positive glioblastoma intracranial xenografts implanted mice [133]. Treatment with PD-0332991 or 2BrIC have shown to increase the radioresistance of Rb1-positive melanoma cells and immortalized fibroblasts [133]. Hence, with cautions, a clinical trial has been initiated to evaluate the G1T28 (CDK4/6 inhibitor) in reducing the carboplatin-induced hematopoietic injury in SCLC (a common Rb-negative tumor) (NCT02499770). Sorafenib or Nexavar, an FDA-approved drug for liver and kidney cancer, is also a promising candidate; it synergistically reduces the tumor size with radiation without causing normal tissue injury in mice model via acting on at G1-S cell cycle checkpoint and currently under different phase I–II clinical trials involving RT [134].

Inhibiting cell death

The inhibition of RI cell death could be another strategy in radioprotection as p53-deficient mice are shown to be less sensitive to radiation compare to wild type [135]. In subsequent studies, inhibition of p53 by sodium orthovanadate or AS2 had shown to enhance the survival of lethally irradiated mice [136]. Inhibition of the p53 by antisense was also found to be helpful in the radioprotection of mice following TBI (15 Gy) [137]. Therefore, blocking off p53 by its inhibitors nutlin-3 sounds good in radioprotection but given its role in tumor suppression may discourage its uses; however, it has been observed that the temporary and reversible suppression of p53 by genetically or pharmacologically agents found to be helpful in rescuing the large numbers of cell from the clench of apoptosis without increasing the chance of secondary cancer [14]. Many tumors undergo RI apoptosis by the p53-independent pathway, hence blocking p53 in that scenario does not

affect tumor radiosensitivity [138, 139]. Differential phosphorylation of p53 also decides the action of p53 between rescuer (DNA repair) and destroyer (apoptosis) [140]. So, selectively targeting the p53 sites, responsible for apoptosis using novel drug could be another strategy to reduce the RI damage. However, clinical utility of p53 inhibitors are very limited due to p53-independent apoptosis as seen in multiple organs including hematopoietic progenitor cells [138, 141], GI track [142], and endothelial cells (follow ceramide-dependent apoptotic pathway instead of p53-dependent). In fact, p53-deficient mice are more prone to RI GI damage than wild-type [142] and subsequently, targeting ceramide by anti-ceramide antibody, showed to protect against RI GI syndrome [143]. Therefore, it is needed to explore beyond p53.

Peroxisome proliferator-activated receptor (PPAR) is another druggable target that could be exploited in RT. Fenofibrate (Abbott), a PPAR agonist, has shown to reduce RI damage in the peripheral cortex but failed to restore hippocampal-dependent cognitive functions [144], while in another study, it induced the hippocampal neurogenesis by protecting the newborn cells and inhibition of microglial activation following irradiation (10 Gy) in mice [145]. The difference in result seems to be due to species and exposure multiplicity. In the other study, it has been shown to enhance the radiosensitivity of human esophageal carcinoma cell lines (Eca-109 and TE1) [100]. The differential effect of Fenofibrate seems to mediate via halting the cell at G2/M transition following RI DNA damage resulting in mitotic catastrophe in cancer cell due to defunct DNA repair machinery while normal cell successfully repairs the damaged DNA. Rosiglitazone, another promising PPAR agonist, has shown to radiosensitize the implanted A549 lung tumor in CD1 mice by decreasing the expression of NF-kB and TGF-β, without affecting the normal pulmonary tissue [146]. Catalase inhibition has shown to radiosensitize the cancer and normal cells due to the accumulation of cytotoxic H_2O_2 as a byproduct of RI ROS (Fig. 2a) [147, 148]. Exposing the rat primary astrocytes and their cancer counterpart (C6 glioma cells) to different PPAR agonist, i.e., PPAR-α (GW3276 and WY-14643) and PPAR-γ (CP086 or darglitazone, troglitazone, rosiglitazone, CP096, 9cRA) have shown to selectively enhance the catalase expression in normal cells [149]. The maximum difference in expression was observed with CP096 where it was reduced by 38 % in cancer cell while 137 % enhancement in normal cells was observed after 48 h of treatment [149]. Interestingly, the catalase expression was blocked by PPARγ-dominant negative plasmid, indicated altered expression is not due to enhancement of messenger RNA (mRNA) stability instead due to de novo expression [149]. Hence, in view of differential effects of PPAR agonist warranted further studies to elucidate its full potential in RT.

Immunotherapy

Radiotherapy induces a tumor-specific response represented by upregulation of MHCs, pro-inflammatory molecules and their receptors, cell adhesion molecules, dendritic cell maturation, and their cross-presentation ability; hence, radiation can be considered as a perfect in situ vaccine. The role of immune system in tumor response was first suggested in 1979, demonstrating the reduced radiotherapeutic efficacy in mice lacking T cell repository [150]. Following irradiation the upregulation of ligands such as CXCL9, CXCL10, CXCL16, and retinoic acid early inducible (RAE-1) in the tumor takes place [151]. The CXCL9, CXCL10, and CXCL16 enhanced the recruitment of CXCR6+ effector CD8 T and CD4 T_{H1} cells in the tumor, while RAE-1 interacts with NKG2D present in effector T cells resulting in the generation of effective T cell response against tumor [152]. However, inhibitory checkpoint receptor CTLA-4, PD-1, PD-L1, BTLA, TIM-3, CD160, and LAG-3 cause obstruction in the response [153]. Inhibiting the CTLA-4 in RT has shown to induce the successful T cell-mediated anti-tumor response, while only anti-CTLA-4 treatment was failed to show any anti-tumorigenic activity [152, 154]. Since effect was only seen in the single exposure; it is further required to evaluate with fractionation radiation for wider use. Some phase I clinical trial are ongoing to check the efficacy of anti-CTLA-4 in RT. Blocking of PD1 with pembrolizumab in ipilimumab-refractory advanced melanoma patient shown exceptional efficacy under monotherapy resulted in FDA approval in 2014 [84, 155, 156]. Furthermore, targeting CTLA-4 and PD1 has shown a significantly longer progression-free survival in comparison to individual modality in metastatic melanoma patients [157]. In glioblastoma multiforme tumors mice model, combining PD-1 blockade with localized radiation therapy has shown a synergistic effect in survival [158]. The identical therapy in B16-OVA melanoma or 4T1-HA breast carcinoma tumor model has shown a batter tumor control [159]. Combining anti-PD-1 antibody with single (10 Gy) or fractionated (2 Gyx5) RT has shown to promote the anti-tumor T cell response, reducing the local accumulation of myeloid-derived suppressor cells and reduced the IFN-γ mediated induction of PD-1 [160, 161]. The underlying mechanism behind the effects of PD-1 is more or less similar to anti-CTLA-4 therapy and since anti-PD-1 antibodies are milder toxic than anti-CTLA-4 antibodies [84, 156, 162]; therefore, PD-1 seems promising in clinical RT. Further understanding of PD-1 and CTLA-4 pathway is also promising in autoimmunity treatment and tissue transplantation including β cell for treating type 1 diabetes. Moreover, BTLA TIM-3, CD160, and LAG-3 are other promising inhibitory molecules that could be targeted for generating a successful anti-tumor response. Indeed, RT always does not promote anti-tumor response. It also induces a protumor response represented by enhanced immunosuppressive Treg-

cell representation, influx of CD11b+ myeloid cells and M2 macrophage infiltration (Fig. 3) [163–165]. The enhancement in Treg is probably mediated by hypoxia raised from RT-induced disrupted vasculature. The RT-induced influx of myeloid cells into tumors can be inhibited by blocking of HIF1α/CXCL12/CXCR4 [156, 161] or CSF1/CSF1R [156, 162] axes for better radiosensitization of the tumor.

Radioprotection by growth factors inducers

Hematopoietic growth factors have been used from long to rescue the hematopoietic and progenitor cells following irradiation. Now, it is advanced towards non-hematopoietic factors, i.e., keratinocyte growth factor (KGF). Preclinical studies demonstrated the radioprotective effects of KGF against RI intestinal damage and pneumonia [166, 167]. Interestingly, no tumor cell proliferation was seen, indicates the functional difference in signaling between normal and cancer cells. Palifermin (rKGF-2) reduced the chemoradiation-induced oral mucositis in randomize trial involving head and neck cancer patients and now recommended by FDA for reducing severe mucositis in patient undergoing myeloablative hematopoietic stem cell transplants with TBI [168–170]. However, it has disappointed in other clinical settings hence currently not recommended for wider clinical uses [171, 172]. GM-CSF administration in pre and post-RT has shown to reduce the RI severe oral mucositis in multiple randomized, head and neck cancer patients RTOG trial [173–175]. Interestingly, administration of HSV GM-CSF in squamous cell cancer of the head and neck (SCCHN) patients undergoing chemoradiation therapy has shown to lessen the locoregional recurrence and enhances the survival of two thirds of patients in phase I/II clinical trial [176]. The differential effect of GM-CSF was mediated by tumor oncolysis and immunomodulation and hence due to promising nature, further trial expected to start soon. Erythropoietin has shown to reduce the radiation and tumor-induced anemia without affecting the radiosensitivity of tumor in preclinical studies; however, results from successive clinical studies were not highly encouraging [177–179]. Failure of erythropoietin seems due to inappropriate patients under suitable modality since erythropoietin causes loss of tumor radioresistance due to neovascularization and moreover, the later effect also increases the chemotherapeutic drug diffusibility in tumor result in better tumor control in addition to offering protection to normal tissues from chemoradiation-induced toxicity. Therefore, to avoid failure in future trials, hypoxia must be taken into consideration and only used under chemoradiation settings. In a recent co-treatment study combining erythropoietin with carboplatin has shown to limit the growth of A549 and H838 NSCLC-xenografts in mice [180]. Thrombopoietin is another promising molecule in RT [181, 182]. Velafermin (rhFGF-20) was shown to protect against RI cheek pouch mucositis induced by fractionated radiation [183].

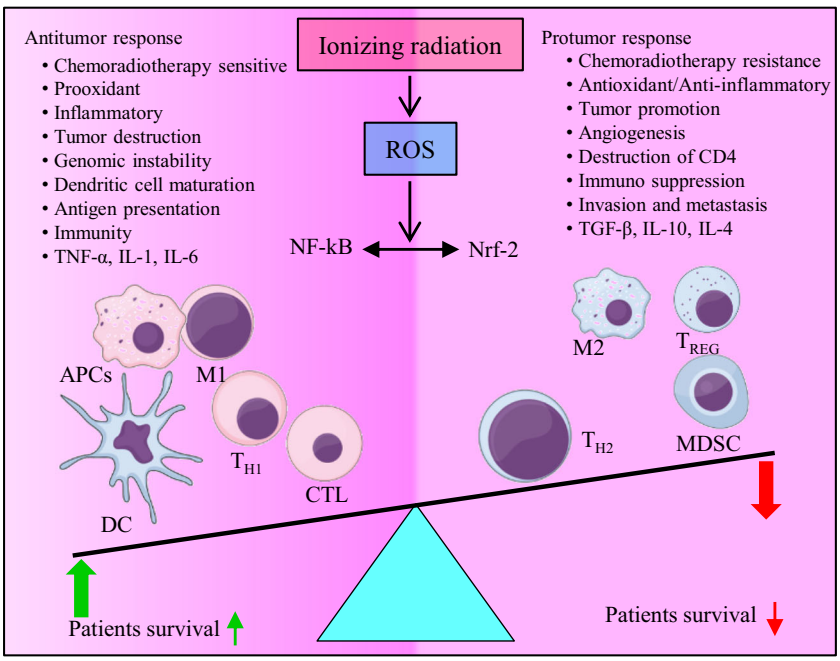

Fig. 3 Radiation and immune response. Radiation generates an oxidative, apoptotic, and inflammatory response that promotes the enhanced antigen presentation by antigen presenting cells (APCs) including dendritic cells (DC) results in cytotoxic T cells (CTL) activation and tumor destruction (*left side*). However, to resist oxidative response, tumor tissues generates a proangiogenic, anti-inflammatory and antioxidative response represented by increase in number of T regulatory (T_{reg}), myeloid-derived suppressor cells (MDSC), Nrf2, M2 macrophages, T_{H1} cells, and its cytokines, i.e., TGF-β, IL-10, IL-4 (*right side*)

Role of inflammation

Radiation has shown to polarize the monocytes and residential macrophages into M1 and M2 macrophages by classical (T_{H1}-based programs) and alternative (T_{H2}-based programs) pathway, respectively, which subsequently participate in inflammation (M1) and tissue reconstruction (M2). Radiation polarizes the macrophages into M1 which subsequently play an important role in generation of anti-tumor immunity operated from NF-kB/TH1 cytokines axis (Fig. 3). Macrophages (M2) polarize in response to RI M1 response or to counteract RI oxidative response (ROS, NF-kB) for homeostasis by upregulating antioxidative (Nrf-2) and anti-inflammatory pathway. M2 macrophage has shown to suppress the DC function and Th1 type adaptive immunity resulting in tumor growth promotion by Nrf-2/TH2 cytokines axis. Furthermore, Nrf-2 induction has demonstrated to shift the metabolic flux towards NADPH regeneration and purine biosynthesis by pentose phosphate pathway for tumor growth [156, 184]. Irradiation potentiates the macrophage to upregulate the IL-1, TNF-α, NO, and a variety of growth factors (PDGF, IGF-1) [185]. Following irradiation tumor becomes hypoxic due to the destruction of the vasculature, result in HIF-1 and HIF-2 mediated infiltration of macrophages [186]. Irradiation to MT1A2 tumor-bearing mice, results in accumulation of MMP9 expressing CD11b+ myelomonocytes at tumor site essential for vascular restoration and tumor growth and selective depletion of them has shown to cause tumor growth inhibitions in pre-irradiated tissue [187, 188]. Other studies also demonstrated a better tumor control following depletion of macrophages (M2) from irradiated tumor-bearing animal [189]. Hence targeting macrophage is promising in RT.

TGF-β is a cytokine strongly induced following irradiation and play a role in tumor survival. Knocking out the Smad3 (S3KO), a downstream signaling intermediate in the TGF- β pathway, in mice has been shown to protect the skin from RI damage as demonstrated by decreased epithelial acanthosis and dermal fibrosis [190]. Inhibition of TGF-β following radiation exposure has shown to help in priming tumor antigens specific T cells, results in better tumor control [191] in addition to radioprotection [192]. TGF-β has also shown to inhibit collagen production in fibroblasts, resulting in fibrosis reduction following RT [193]. However, in the clinical setting, care must be taken since TGF-β also was shown to help in the maintenance of genomic integrity [156, 184, 194].

Protecting the genomic integrity of cells against radiation

The role of radiotherapeutic radiation in the occurrence of secondary cancer (SC) remains controversial and untenable as most of the arguments rely on data extrapolated from World War II atomic bombings life span survivor, where a proportional relation was observed between cancer onset and radiation dose (0.1–2.5 Gy). However, if the frequency of SC calculated using the theoretical model developed from the above-mentioned studies, the chance of SC would be much higher than reported in clinical settings, it means that the risk of cancer caused by radiation is highly extravagated [16]. Interestingly, this linear nonthreshold (LNT) model still widely used by different regulatory agencies [195]. Even the relative risk of SC declined after a certain dose as with increasing dose probability of cell killing is increased or the risk of SC decreased since once cell died it cannot undergo transformation. Therefore, due to uncertainty, radiation is categorized as a weak carcinogen [16]. Although many papers are claiming a relation between RT and SC but in a recent long-term follow-up study involving 12,247 Hodgkin patients does not show any increase in secondary malignancy in comparison to chemotherapy-treated patients [194]. A meta-analysis comprising 762,468 breast cancer patients do not show any link between radiotherapy and second thyroid cancer [196]. Furthermore, in two large cohort studies, no link between RT dose and SC was observed in childhood cancer survivor and atomic bomb survivor or prostate cancer patients and sugary-treated patients [197, 198]. In a recent large pooled cohort of pelvic cancers patients treated with surgery or radiotherapy did not show any significant difference in developing SC [199]. Moreover, even if any relation between both, the variables like tumor microenvironment, genetic predisposition, altered nutrition, immunodeficiency, personal lifestyle, age, and social condition, etc. pose a challenge to draw a conclusive relationship. Furthermore, long-term exposure to low-dose radiation induces cancer via carcinogenesis while direct chromosomal damage and abrupt failure of signaling events in addition to carcinogenesis is responsible for RT-induced SC so both are incomparable. However, it is not a denying fact on the involvement of RT radiation in SC development, though it develops independently to RT dose. Hence, to understand the possibility of developing SC in RT, focus is needed at the individual level rather then on finding a relation between SC and exposure dose as genetic polymorphism is known to play a major role in radiation sensitivity. Recently, Radiogenomics Consortium (supported by NCINIH, USA) has established to work together, share data/samples, perform meta-analyses, identify SNPs, and biomarkers responsible for adverse effects including SC following RT. Response to lymphocytes have shown to act as an interpreter of RI toxicity in patients undergoing RT and could be used for selecting patients for RT [200]. Telomere length is another reliable marker that can be used to predict the RT-induced acute toxicity and SC before RT [201]. This observation has confirmed in a recent large sample clinical study involving childhood cancer survivors where a

relation between less telomere content and treatment-related thyroid cancer was observed [202]. In a large cohort study of patients who has undergone RT, a significant relation between telomere shortening and development of cardiovascular disease [203] was shown. In another cohort study, appearance of RT-induced SC in patients was shown to have a cutoff value for telomere at 6.6 kb in comparison to 9.7 kb where no complication was observed [204]. These studies could serve as a tool for selecting treatment type for better prognosis in cancer patients as prostatectomy could be a better option for those having shorter telomere while brachytherapy or RT for prostate patients having longer telomere length.

Modulation of the cell signaling

Cancer cells require huge quantity of iron for survival, angiogenesis, and metastasis [205]. It is also required for maintenance of the ribonucleotide reductase (RR) activity involved in the conversion of ribonucleotides to deoxyribonucleotides (dNTPs) required for DNA synthesis [206]. Iron chelation has shown to cause hypophosphorylation of the retinoblastoma protein resulting in the reduction of cyclins expression including A, B, and D. Iron chelation by Triapine (3-aminopyridine-carboxaldehyde thiosemicarbazone) (Vion Pharma Ltd.) have shown to radiosensitize the cancer cells [207]. Studies performed over the years using normal cells or animal have demonstrated the reduction in RI damages in the presence of iron chelators since iron is known to participate in hydroxyl radicals generation via Fenton reaction following irradiation [208, 209]. Additionally, due to the difference in iron requirement for normal metabolism, makes cancer cells more sensitive towards iron deprivation than normal cells. Therefore, iron chelating is a quite promising strategy in RT and requires a robust investigation. Triapine is one iron chelator which is undergoing phase I/II clinical trial for evaluation of its synergy with RT and cisplatin combination in cervical or vulvar cancer patients (NCT02595879, NCT02466971, NCT01835171).

Data from earlier studies has demonstrated that radiation induces the expression of NF-kB as a mechanism to reduce the RI lethality via activating prosurvival pathway [210]. LPS has shown to improve the survival following WBI in mice [211]. Flagellin, (an NF-kB inducer from bacterial origin act via TLR-5) has shown to reduce the RI lethality in tumor-bearing mice [212]. However, in contrast to that, subsequent studies have demonstrated that inhibition of NF-kB results in radioprotection. NF-kB inhibition by ethyl pyruvate (EP) or CDDO-TFEA (RTA401) and RTA 408 (Reata Pharma. USA) were found to reduce the RI lethality in zebrafish embryo [213] and GI damage in mice [214], respectively. The EP or CDDO-TFEA (RTA401) was abrogated by proteasomal inhibitor PS-341 (Bortezomib/VELCADE) in zebrafish [213]. The role of RI NF-kB currently guided by two alternative hypothesis revolving around inflammation. Data from burn studies has demonstrated the pro-hematopoietic effects of low to moderate level of inflammation while a high level of inflammation has shown to cause oxidative stress and apoptosis. Therefore, it seems like suppression of RI NF-kB-led inflammation may be the reason behind the later effect while the lower level of inflammation may act as hematopoietic simulative in former studies. Elucidation of the role of RI NF-kB is also quite difficult due to associated embryonic lethality [215]. NF-kB constitutively activated in many cancers as a mechanism to help in the initiation, progression, and invasiveness resulting in poor treatment outcome in preclinical and clinical studies [216]. Inhibition of NF-kB via Icariin or DMAPT has shown to radiosensitize the colorectal cancer and NSCLC, respectively [217]. Furthermore, RTA-408 also showed to radiosensitize the CWR22Rv1, LNCaP/C4-2B, PC3, and DU145 xenografts in mice model in addition to protection of GI tract from RI damage [214]. Therefore, targeting one of major prosurvival pathway in cancer treatment may helpful for better RT outcome.

EGFR is the growth factor responsible for radioresistance in many cancers. Targeting EGFR in tumor with cetuximab, gefitinib, and erlotinib in monotherapy had shown promising results. Indeed, in some studies, it has failed due to heterogeneity in EGCR expression in tumor. In phase III clinical trial administering cetuximab in head and neck cancer patients undergoing RT has shown to increase the post-therapy survival to 49 months in comparison to 29 months in RT arm [218, 219]. Despite showing synergy with cisplatin and radiation in sensitization of tumor cells in preclinical studies [220], cetuximab highly disappointed in subsequent clinical trials [221]. Administrating cetuximab in locally advanced rectal cancer patients under chemoradiation regimen does not show any benefits [222]. The failure of study seems to be due to blocking of tumor cell proliferation by cetuximab since proliferating cells are more sensitive to chemoradiotherapy so blocking the target of chemoradiotherapy could be reason behind poor prognosis. Concurrent administration of cetuximab, and cisplatin in stage III/IV head and neck cancer patients undergoing RT in a large ($N = 891$) randomized RTOG trial, failed to demonstrate any disease-free or overall survival [223]. The complete failure of therapy in the above-mentioned case yet to clear, previous studies have suggested the oropharyngeal tumors that are mostly HPV positive likely to benefit from the combination and hence, the study (RTOG 10-16) was carried out. In fact, patients reported acute toxicity, notably mucositis in cetuximab arms. However, despite the outcome and waste of resources, many prospective clinical trials of cetuximab in RT are still undergoing in different treatment settings (NCT02123381, NCT00956007, NCT01614938).

Exploiting the cellular autofluorescence property

Schaue et al. had noticed an increase in cellular autofluorescence at 450 nm following irradiation in different human and murine cell lines and concluded as general phenomena [224]. The conclusions were drawn from the study that autofluorescence is radiation dose-dependent phenomena, and proportional to the level of cell radiosensitivity, i.e., hematopoietic cells have a high level of autofluorescence. The autofluorescence is high in normal cells in comparison to cancer cells. Today, photodynamic therapy is emerging as promising treatment agents in various diseases including cancer [225]. Therefore, we are hypothesizing that using photo-reactivated drug or photodynamic therapy could immensely help in protecting normal tissue by exploiting cellular autofluorescence following irradiation. Today, many photosensitizer drugs such Temoporfin, Porfimer sodium, Methyl aminolevulinate, Hexvix, Talaporfin, or aspartyl chlorine have been used in the treatment of different form of cancer; in fact, a few of them have been shown to improve the RT efficacy [226]. The currently used photosensitizer actively untaken by cancer cells require much longer wavelength (700–850 nm) for excitation then emitted by cell autofluorescence (450 nm). Hence, due to multiple advantages, it is quite promising to develop photo protectors that exclusively reactivated at lower wavelength once the cells are irradiated.

ACE inhibitors

Renin-angiotensin system was discovered in the maintenance of fluid balance and blood pressure. However, subsequent studies have shown that targeting it could be helpful in radioprotection, especially against renal toxicity. Several protease inhibitors such as captopril, enalapril, penicillamine, pentoxifylline, L-158, 809, etc. acting via rennin-angiotensin system has shown to be helpful in the reduction of RI prophylaxis in renal and lung tissues. The use of ACE inhibitors and angiotensin-II receptor blockers have demonstrated a new avenue in the radioprotection especially where the high dose of exposure is imminent; however, in one study, it does not confer protection to the intestinal injury; therefore, it seems that it is offering tissue-specific protection [227]. Inhibition of angiotensin system shown to help in improving the outcome of RT in clinical trials by reducing the radiation pneumonitis [228].

Early growth response 1 (Egr1), known to induce apoptosis by activating the apoptotic genes is another promising transcription factor in radioprotection. Inhibiting EGR 1 with MMA (mithramycin A) in in vivo or knocking out Egr1 in mice (Egr1−/−) have shown to protect the RI GI damage by decreasing the ratio of Bax/Bcl-2 [229]. Egr1 is also known to require for the survival of various cancer including prostate, gastric, and kidney cancers. Earlier studies have shown that targeting Egr1 is helpful in radiosensitization of different cancer cells. Currently, MMA is being investigated for its clinical efficacy (phase II) in the lung, esophageal, neoplasms, and breast cancer patients (NCT01624090).

Radiation mitigation

Radiation mitigators are the agents used in radiation countermeasure following exposure from different sources, i.e., nuclear detonation, terrorist events or accidental exposure that sometimes even happens in clinics during RT or due to mishandling of isotopes [230]. FDA recommended the potassium iodide in 1982 as an emergency radiation countermeasure to protect thyroid gland against radioiodine. However, after 9/11, three more drugs radiogardase (against Cesium-137 and thallium-201), calcium and zinc salt of DTPA (against several transuranic ions, i.e., plutonium, americium, curium, etc.) were added to the list (2003–2004).

Bone marrow is the most radiosensitive and important organ required for survival. In radio-mitigation, the best option is the transplantation or inducing the proliferation of surviving stem and progenitor cells. The 9/11 terrorist attacks dramatically change the trajectory of security initiatives in the USA. Strategic National Stockpile Radiation Working Group in a consensus document and Center for Disease Control and Prevention (CDC) has recommended the bone marrow transplantation [231]. However, salivary gland is the only organ so far which functionality restoration has been demonstrated following irradiation. In an earlier study, many cytokines and hormones had shown to help in radio-mitigation; however, most of them work if they administrated before 24-h post-exposure since most of the cell lost in first 24 h after irradiation from the peak at 4 h [232]. Nevertheless, the US government is keen on mitigator for national stockpiles which can even effectively work after 24 h after exposure. Therefore, mechanism for radioprotection must not lie in apoptosis inhibition. Instead, it must revolve around the hematopoietic cells such as mesenchymal stem cells (MSCs) and bone marrow. Targeting of thrombomodulin-protein C pathway or mobilizing the progenitor's cells after 24 h of irradiation has shown to mitigate the RI lethality in mice [233, 234]. MSCs are other cells that can be used in radio-mitigation. In fact, due to radioresistance over bone marrow and other radioresistant cell lines; currently, MSCs are under intense investigation. Indeed, in few studies, it was also shown to act as a potential source of tumorigenesis in the long term due to the acquisition of some genetic modification like telomerase shortening [235, 236].

Statins are HMG-CoA reductase inhibitors originally developed as lipid control agent has shown to mitigates the RI damages. Simvastatin has been shown to protect the cardiac system from infections in the rat when administered 9 days after irradiation. Therefore, statins could be exploited in both RT and radio-mitigation for RI cardiac problems [237].

Anticoagulants activated protein C is another promising candidate in radioprotection shown to induce the hematopoiesis, resulting in improved survival after administrating as late as 24 h after lethal exposure [233].

Future perspective

Today, RT represents the major anti-cancer modality. However, it failed to demonstrate a significant outcome despite carrying out multiple targeted clinical trials. These studies failed due to various reasons such as drug toxicity, wrong patient selection, lack of information about suitable cancer biomarkers, and heterogeneous disease process. With emergence of molecular biology, cancer biology has made tremendous progress in sorting out the physiological differences between cancer and normal cells. This provides a bedrock for designing novel drugs to selectively enhance the radiosensitivity of tumor in addition to protecting normal tissues. However, every new drug must go through rigorous preclinical studies to understand the basic mechanism and avoid failure in subsequent clinical trial as iniparib, shown to be a PARP inhibitor failed to demonstrate any clinical benefits in phase III trial [238] and further study showed that it is not only a poor inhibitor of PARP but also quite structurally distinct from other PARP inhibitors [239]. Hence, rigorous preclinical studies can avoid resources wastage [223] and discarding of potentially useful targets [73, 77]. The other challenge lies in mice model system as the majority of study for human tumor xenotransplantation is conducted in immunosuppressed mice, and we know that immune system proactively interacts with tumor; therefore, to impart the role of human immune system, humanized mice could serve as a better alternative.

Targeting tumor with monotherapy may give a selective advantage to clones harboring resistant mutant, resulting in the tumor reemergence/relapse [240]. Hence, targeting local environment may be advantageous as it equally affects all cells irrespective of clonal variation. As targeting hypoxia automatically reflected in all cells and make them radiosensitive irrespective of oxygen consumption by individual cells. The tumor is heterogeneous, evolutionary, and plastic in nature which consistently evolves and adopts new conditions until colonized in distant location hence in successful cancer elimination multitargeted therapies acting in synergy are quite promising. In last reduction of tobacco/alcohol/drug consumption, better hygienic-/pollution-free environment, obesity control, immunization, and healthy lifestyle certainly help in winning the war against cancer and may reduce 30–40 % death by cancer. In conclusion, future outcome of RT is entirely dependent on information about continued cancer biomarker identification, cancer type categorization and sorting out the fundamental difference between cancer and normal cells.

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Compliance with ethical standards

Conflicts of interest None.

References

1. Edison TA. Effect of x-rays upon the eye. Nature. 1896;53:1.
2. Thariat J, Hannoun-Levi JM, Sun Myint A, Vuong T, Gerard JP. Past, present, and future of radiotherapy for the benefit of patients. Nat Rev Clin Oncol. 2013;10:52–60.
3. Shah DJ, Sachs RK, Wilson DJ. Radiation-induced cancer: a modern view. Br J Radiol. 2012;85:e1166–73.
4. Sansare K, Khanna V, Karjodkar F. Early victims of x-rays: a tribute and current perception. Dentomaxillofacial Radiol. 2014;40:123–5.
5. Langevin-Joliot H. Radium, Marie Curie and modern science. Radiat Res. 1998;150:S3–8.
6. Dale WM. The effect of x-rays on the conjugated protein d-amino-acid oxidase. Biochem J. 1942;36:80–5.
7. Patt HM, Tyree EB, Straube RL, Smith DE. Cysteine protection against x irradiation. Science. 1949;110:213–4.
8. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol. 1953;26:638–48.
9. Åkerfeldt S, Rönnbäck C, Nelson A. Radioprotective agents: results with S-(3-amino-2-hydroxypropyl) phosphorothioate, amidophosphorothioate, and some related compounds. Radiat Res. 1967;31:850–5.
10. Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. Lancet Oncol. 2012;13:790–801.
11. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev. 2010;19:1893–907.
12. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2015;66:7–30.
13. ASTRO. Meeting program. 2015. https://www.astro.org/Meetings-and-Events/2015-Annual-Meeting/MeetingProgram/Index.aspx.
14. Gudkov AV, Komarova EA. Radioprotection: smart games with death. J Clin Invest. 2010;120:2270–3.
15. Kaplan HS. Hodgkin's disease. Cambridge: Harvard University Press; 1980.
16. Choi WH, Cho J. Evolving clinical cancer radiotherapy: concerns regarding normal tissue protection and quality assurance. J Korean Med Sci. 2015;30.
17. Lattime EC, Gerson SL. Gene therapy of cancer: translational approaches from preclinical studies to clinical implementation. Amsterdam: Elsevier Science; 2013.
18. Epperly MW, Gretton JE, Sikora CA, Jefferson M, Bernarding M, Nie S, et al. Mitochondrial localization of superoxide dismutase is required for decreasing radiation-induced cellular damage. Radiat Res. 2003;160:568–78.
19. Jin C, Qin L, Shi Y, Candas D, Fan M, Lu CL, et al. CDK4-mediated MnSOD activation and mitochondrial homeostasis in radioadaptive protection. Free Radicals Biol Med. 2015;81:77–87.

20. Miriyala S, Spasojevic I, Tovmasyan A, Salvemini D, Vujaskovic Z, St Clair D, et al. Manganese superoxide dismutase, MnSOD and its mimics. Biochim Biophys Acta, Rev Cancer. 2012;1822: 794–814.
21. ATBC. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 1994;330:1029–35.
22. Guo L, Zhu H, Lin C, Che J, Tian X, Han S, et al. Associations between antioxidant vitamins and the risk of invasive cervical cancer in Chinese women: A case-control study. Sci Rep. 2015;5:13607.
23. Welch RW, Turley E, Sweetman SF, Kennedy G, Collins AR, Dunne A, et al. Dietary antioxidant supplementation and DNA damage in smokers and nonsmokers. Nutr Cancer. 1999;34:167–72.
24. Liu Q, Jin J, Ying J, Sun M, Cui Y, Zhang L, et al. Frequent epigenetic suppression of tumor suppressor gene glutathione peroxidase 3 by promoter hypermethylation and its clinical implication in clear cell renal cell carcinoma. Int J Mol Sci. 2015;16: 10636–49.
25. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discovery. 2009;8:579–91.
26. Tarhini AA, Belani CP, Luketich JD, Argiris A, Ramalingam SS, Gooding W, et al. A phase I study of concurrent chemotherapy (paclitaxel and carboplatin) and thoracic radiotherapy with swallowed manganese superoxide dismutase plasmid liposome protection in patients with locally advanced stage III non-small-cell lung cancer. Hum Gene Ther. 2011;22:336–42.
27. Colon J, Hsieh N, Ferguson A, Kupelian P, Seal S, Jenkins DW, et al. Cerium oxide nanoparticles protect gastrointestinal epithelium from radiation-induced damage by reduction of reactive oxygen species and upregulation of superoxide dismutase 2. Nanomedicine. 2010;6:698–705.
28. Wason MS, Colon J, Das S, Seal S, Turkson J, Zhao J, et al. Sensitization of pancreatic cancer cells to radiation by cerium oxide nanoparticle-induced ROS production. Nanomedicine. 2013;9:558–69.
29. Asati A, Santra S, Kaittanis C, Perez JM. Surface-charge-dependent cell localization and cytotoxicity of cerium oxide nanoparticles. ACS Nano. 2010;4:5321–31.
30. Johnke RM, Sattler JA, Allison RR. Radioprotective agents for radiation therapy: future trends. Future Oncol. 2014;10:2345–57.
31. Epperly MW, Shinde A, Berhane H, Rhieu BH, Kalash R, Xu K, et al. Abstract 3340: Intraoral administration of mitochondrial targeted GS-nitroxide (JP4-039) radioprotects the oral mucosa but not orthotopic tumors in Fancd2-/- mice. Cancer Res. 2015;75:3340.
32. Metz JM, Smith D, Mick R, Lustig R, Mitchell J, Cherakuri M, et al. A phase I study of topical Tempol for the prevention of alopecia induced by whole brain radiotherapy. Clin Cancer Res. 2004;10: 6411–7.
33. Erker L, Schubert R, Yakushiji H, Barlow C, Larson D, Mitchell JB, et al. Cancer chemoprevention by the antioxidant tempol acts partially via the p53 tumor suppressor. Hum Mol Genet. 2005;14(12):1699–708.
34. Mihandoost E, Shirazi A, Mahdavi SR, Aliasgharzadeh A. Can melatonin help us in radiation oncology treatments? BioMed Res Int. 2014;2014:12.
35. Vijayalaxmi, Reiter RJ, Tan DX, Herman TS, Thomas Jr CR. Melatonin as a radioprotective agent: a review. Int J Radiat Oncol Biol Phys. 2004;59:639–53.
36. Lissoni P, Meregalli S, Nosetto L, Barni S, Tancini G, Fossati V, et al. Increased survival time in brain glioblastomas by a radioneuroendocrine strategy with radiotherapy plus melatonin compared to radiotherapy alone. Oncology. 1996;53:43–6.
37. Berk L, Berkey B, Rich T, Hrushesky W, Blask D, Gallagher M, et al. Randomized phase II trial of high-dose melatonin and radiation therapy for RPA class 2 patients with brain metastases (RTOG 0119). Int J Radiat Oncol Biol Phys. 2007;68:852–7.
38. Son T, Gong E, Bae M, Kim S, Heo K, Moon C, et al. Protective effect of genistein on radiation-induced intestinal injury in tumor bearing mice. BMC Complementary Altern Med. 2013;13:103.
39. Kepley C, Dellinger A. Fullerenes and their potential in nanomedicine in book Nanoscience and Nanoengineering: Advances and Applications. Abingdon: Taylor & Francis; 2014.
40. Son Y, Lee H, Rho J, Chung S, Lee C, Yang K, et al. The ameliorative effect of silibinin against radiation-induced lung injury: protection of normal tissue without decreasing therapeutic efficacy in lung cancer. BMC Pulm Med. 2015;15:68.
41. Albright CD, Salganik RI, Van Dyke T. Dietary depletion of vitamin E and vitamin A inhibits mammary tumor growth and metastasis in transgenic mice. J Nutr. 2004;134:1139–44.
42. Salganik RI, Albright CD, Rodgers J, Kim J, Zeisel SH, Sivashinskiy MS, et al. Dietary antioxidant depletion: enhancement of tumor apoptosis and inhibition of brain tumor growth in transgenic mice. Carcinogenesis. 2000;21:909–14.
43. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindah lP, Bergo MO. Antioxidants accelerate lung cancer progression in mice. Sci Transl Med. 2014;6:221ra215.
44. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst. 2008;100:773–83.
45. Braunstein MH. Vitamin E: new research. New York: Nova Science Publishers; 2006.
46. Lesperance ML, Olivotto IA, Forde N, Zhao Y, Speers C, Foster H, et al. Mega-dose vitamins and minerals in the treatment of non-metastatic breast cancer: an historical cohort study. Breast Cancer Res Treat. 2002;76:137–43.
47. Misirlioglu CH, Demirkasimoglu T, Kucukplakci B, Sanri E, Altundag K. Pentoxifylline and alpha-tocopherol in prevention of radiation-induced lung toxicity in patients with lung cancer. Med Oncol. 2007;24:308–11.
48. Misirlioglu CH, Erkal H, Elgin Y, Ugur I, Altundag K. Effect of concomitant use of pentoxifylline and alpha-tocopherol with radiotherapy on the clinical outcome of patients with stage IIIB non-small cell lung cancer: a randomized prospective clinical trial. Med Oncol. 2006;23:185–9.
49. Borek C. Antioxidants and radiation therapy. J of Nutr. 2004;134: 3207S–9.
50. Zhao H, Zhu W, Xie P, Li H, Zhang X, et al. A phase I study of concurrent chemotherapy and thoracic radiotherapy with oral epigallocatechin-3-gallate protection in patients with locally advanced stage III non-small-cell lung cancer. Radiother Oncol. 2014;110:132–6.
51. Jacobson G, Bhatia S, Smith BJ, Button AM, Bodeker K, Buatti J. Randomized trial of pentoxifylline and vitamin E vs standard follow-up after breast irradiation to prevent breast fibrosis, evaluated by tissue compliance meter. Int J Radiat Oncol Biol Phys. 2013;85:604–8.
52. Zhao H, Xie P, Li X, Zhu W, Sun X, Chen X, et al. A prospective phase II trial of EGCG in treatment of acute radiation-induced esophagitis for stage III lung cancer. Radiother Oncol. 2015;114: 351–6.
53. Feng M, Smith DE, Normolle DP, Knol JA, Pan CC, Ben-Josef E, et al. A phase I clinical and pharmacology study using amifostine as a radioprotector in dose-escalated whole liver radiation therapy. Int J Radiat Oncol Biol Phys. 2012;83:1441–7.
54. Panteliadou M, Giatromanolaki A, Touloupidis S, Destouni E, Tsoutsou PG, Pantelis P, et al. Treatment of invasive bladder

cancer with conformal hypofractionated accelerated radiotherapy and amifostine (HypoARC). Urol Oncol. 2012;30:813–20.
55. Lawrence YR, Paulus R, Langer C, Werner-Wasik M, Buyyounouski MK, Komaki R, et al. The addition of amifostine to carboplatin and paclitaxel based chemoradiation in locally advanced non-small cell lung cancer: long- term follow-up of Radiation Therapy Oncology Group (RTOG) randomized trial 9801. Lung Cancer. 2013;80:298–305.
56. Gu J, Zhu S, Li X, Wu H, Li Y, Hua F. Effect of amifostine in head and neck cancer patients treated with radiotherapy: a systematic review and meta-analysis based on randomized controlled trials. PLoS One. 2014;9:e95968.
57. Movsas B, Scott C, Langer C, Werner-Wasik M, Nicolaou N, Komaki R, et al. Randomized trial of amifostine in locally advanced non-small-cell lung cancer patients receiving chemotherapy and hyperfractionated radiation: radiation therapy oncology group trial 98-01. J Clin Oncol. 2005;23:2145–54.
58. Small Jr W, Winter K, Levenback C, Iyer R, Hymes SR, Jhingran A, et al. Extended-field irradiation and intracavitary brachytherapy combined with cisplatin and amifostine for cervical cancer with positive para-aortic or high common iliac lymph nodes: results of arm II of Radiation Therapy Oncology Group (RTOG) 0116. Int J Gynecol Cancer. 2011;21:1266–75.
59. Koukourakis MI. Amifostine: is there evidence of tumor protection? Semin Oncol. 2003;30(6 Suppl 18):18–30.
60. Rades D, Fehlauer F, Bajrovic A, Mahlmann B, Richter E, Alberti W. Serious adverse effects of amifostine during radiotherapy in head and neck cancer patients. Radiother Oncol. 2004;70:261–4.
61. Grdina DJ, Murley JS, Kataoka Y, Baker KL, Kunnavakkam R, Coleman MC, et al. Amifostine induces antioxidant enzymatic activities in normal tissues and a transplantable tumor that can affect radiation response. Int J Radiat Oncol Biol Phys. 2009;73:886–96.
62. Thomas JP, Geiger PG. Abstract 4443: PB-42: design and characterization of a glutathione pro-drug selectively delivered to normal tissue and not tumor tissue. Cancer Res. 2015;75:4443.
63. Cerutti P. Anticarcinogenesis and radiation protection. New York, Philadelphia: Springer; 2012.
64. Lu SC. Glutathione synthesis. Biochim Biophys Acta. 1830;2013:3143–53.
65. Zhang WC, Shyh-Chang N, Yang H, Rai A, Umashankar S, Ma S, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell. 2012;148:259–72.
66. Shaul YD, Freinkman E, Comb WC, Cantor JR, Tam WL, Thiru P, et al. Dihydropyrimidine accumulation is required for the epithelial-mesenchymal transition. Cell. 2014;158:1094–109.
67. Zamani Z, Arjmand M, Vahabi F, Eshaq Hosseini SM, Fazeli SM, Iravani A, et al. A metabolic study on colon cancer using 1H nuclear magnetic resonance spectroscopy. Biochem Res Int. 2014;2014:7.
68. Taniguchi CM, Miao YR, Diep AN, Wu C, Rankin EB, Atwood TF, et al. PHD inhibition mitigates and protects against radiation-induced gastrointestinal toxicity via HIF2. Sci Transl Med. 2014;6:236ra264.
69. Lagadec C, Dekmezian C, Bauche L, Pajonk F. Oxygen levels do not determine radiation survival of breast cancer stem cells. PLoS One. 2012;7:e34545.
70. Zhong R, Xu H, Chen G, Zhao G, Gao Y, Liu X, et al. The role of hypoxia-inducible factor-1alpha in radiation induced autophagic cell death in breast cancer cells. Tumor Biol. 2015;36:7077–83.
71. Li CY, Li F, Sonveaux P, Dewhirst MW. Inhibition of HIF-1 activation for anti-tumor and anti-inflammatory responses. Google Patents. 2015.
72. Urtasun RC, Chapman JD, Feldstein ML, Band RP, Rabin HR, Wilson AF, et al. Peripheral neuropathy related to misonidazole: incidence and pathology. Br J Cancer Suppl. 1978;3:271–5.
73. Overgaard J, Hansen HS, Overgaard M, Bastholt L, Berthelsen A, Specht L, et al. A randomized double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85. Radiother Oncol. 1998;46:135–46.
74. Zeman EM, Brown JM, Lemmon MJ, Hirst VK, Lee WW. SR-4233: a new bioreductive agent with high selective toxicity for hypoxic mammalian cells. Int J Radiat Oncol Biol Phys. 1986;12:1239–42.
75. Yeh JJ, Kim WY. Targeting tumor hypoxia with hypoxia-activated prodrugs. J Clin Oncol. 2015;33:1505–8.
76. DiSilvestro PA, Ali S, Craighead PS, Lucci JA, Lee YC, Cohn DE, et al. Phase III randomized trial of weekly cisplatin and irradiation versus cisplatin and tirapazamine and irradiation in stages IB2, IIA, IIB, IIIB, and IVA cervical carcinoma limited to the pelvis: a Gynecologic Oncology Group study. J Clin Oncol. 2014;32:458–64.
77. Toustrup K, Sorensen BS, Lassen P, Wiuf C, Alsner J, Overgaard J. Gene expression classifier predicts for hypoxic modification of radiotherapy with nimorazole in squamous cell carcinomas of the head and neck. Radiother Oncol. 2012;102:122–9.
78. Yoon C, Lee HJ, Park DJ, Lee YJ, Tap WD, Eisinger-Mathason TSK, et al. Hypoxia-activated chemotherapeutic TH-302 enhances the effects of VEGF-A inhibition and radiation on sarcomas. Br J Cancer. 2015;113:46–56.
79. Sun JD, Ahluwalia D, Liu Q, Li W, Wang Y, Meng F, et al. Combination treatment with hypoxia-activated prodrug evofosfamide (TH-302) and mTOR inhibitors results in enhanced antitumor efficacy in preclinical renal cell carcinoma models. Am J Cancer Res. 2015;5:2139–55.
80. Peeters SGJA, Zegers CML, Biemans R, Lieuwes NG, van Stiphout RGPM, Yaromina A, et al. TH-302 in combination with radiotherapy enhances the therapeutic outcome and is associated with pretreatment [18F]HX4 hypoxia PET imaging. Clin Cancer Res. 2015;21:2984–92.
81. Chawla SP, Cranmer LD, Van Tine BA, Reed DR, Okuno SH, Butrynski JE, et al. Phase II study of the safety and antitumor activity of the hypoxia-activated prodrug TH-302 in combination with doxorubicin in patients with advanced soft tissue sarcoma. J Clin Oncol. 2014;32:3299–306.
82. Fahl WE. Effect of topical vasoconstrictor exposure upon tumoricidal radiotherapy. Int J Cancer. 2014;135:981–8.
83. Scicinski J, Oronsky B, Ning S, Knox S, Peehl D, Kim MM, et al. NO to cancer: the complex and multifaceted role of nitric oxide and the epigenetic nitric oxide donor, RRx-001. Redox Biol. 2015;6:1–8.
84. Higgins GS, O'Cathail SM, Muschel RJ, McKenna WG. Drug radiotherapy combinations: review of previous failures and reasons for future optimism. Cancer Treat Rev. 2015;41:105–113.
85. Zannella VE, Dal Pra A, Muaddi H, McKee TD, Stapleton S, Sykes J, et al. Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response. Clin Cancer Res. 2013;19:6741–50.
86. Chen YC, Kok VC, Chien CH, Horng JT, Tsai JJ. Cancer risk in patients aged 30 years and above with type 2 diabetes receiving antidiabetic monotherapy: a cohort study using metformin as the comparator. Ther Clin Risk Manag. 2015;11:1315–23.
87. Liebmann J, DeLuca AM, Coffin D, Keefer LK, Venzon D, Wink DA, et al. In vivo radiation protection by nitric oxide modulation. Cancer Res. 1994;54:3365–8.
88. Maxhimer JB, Soto-Pantoja DR, Ridnour LA, Shih HB, Degraff WG, Tsokos M, et al. Radioprotection in normal tissue and

delayed tumor growth by blockade of CD47 signaling. Sci Transl Med. 2009;1:3ra7.
89. Soto-Pantoja DR, Miller TW, Pendrak ML, DeGraff WG, Sullivan C, Ridnour LA, et al. CD47 deficiency confers cell and tissue radioprotection by activation of autophagy. Autophagy. 2012;8:1628–42.
90. Fens MH, Larkin SK, Oronsky B, Scicinski J, Morris CR, Kuypers FA. The capacity of red blood cells to reduce nitrite determines nitric oxide generation under hypoxic conditions. PLoS One. 2014;9:e101626.
91. Verhagen CVM, de Haan R, Hageman F, Oostendorp TPD, Carli ALE, O'Connor MJ, et al. Extent of radiosensitization by the PARP inhibitor olaparib depends on its dose, the radiation dose and the integrity of the homologous recombination pathway of tumor cells. Radiother Oncol. 2015;116:358–65.
92. Alagpulinsa D, Ayyadevara S, Yaccoby S, Shmookler Reis R. Dinaciclib, a CDK inhibitor, impairs homologous recombination and sensitizes multiple myeloma cells to PARP inhibition. Blood. 2014;124:479.
93. Liu X, Cotrim A, Teos L, Zheng C, Swaim W, Mitchell J, et al. Loss of TRPM2 function protects against irradiation-induced salivary gland dysfunction. Nat Commun. 2013;4:1515.
94. Nowacka-Zawisza M, Wiśnik E, Wasilewski A, Skowrońska M, Forma E, Bryś M, et al. Polymorphisms of homologous recombination RAD51, RAD51B, XRCC2, and XRCC3 genes and the risk of prostate cancer. Anal Cell Pathol. 2015;2015:9.
95. Mohaghegh P, Hickson ID. DNA helicase deficiencies associated with cancer predisposition and premature ageing disorders. Hum Mol Genet. 2001;10:741–6.
96. Cerbinskaite A, Mukhopadhyay A, Plummer ER, Curtin NJ, Edmondson RJ. Defective homologous recombination in human cancers. Cancer Treat Rev. 2012;38:89–100.
97. Su Y, Meador JA, Calaf GM, De-Santis LP, Zhao Y, Bohr VA, et al. Human RecQL4 helicase plays critical roles in prostate carcinogenesis. Cancer Res. 2010;70:9207–17.
98. Koppensteiner R, Samartzis EP, Noske A, von Teichman A, Dedes I, Gwerder M, et al. Effect of MRE11 loss on PARP-inhibitor sensitivity in endometrial cancer in vitro. PLoS One. 2014;9:e100041.
99. Bartosova Z, Krejci L. Nucleases in homologous recombination as targets for cancer therapy. FEBS Lett. 2014;588:2446–56.
100. Helleday T. Homologous recombination in cancer development, treatment and development of drug resistance. Carcinogenesis. 2010;31:955–60.
101. Castri P, Lee YJ, Ponzio T, Maric D, Spatz M, Bembry J, et al. Poly(ADP-ribose) polymerase-1 and its cleavage products differentially modulate cellular protection through NF-kappaB-dependent signaling. Biochim Biophys Acta, Mol Cell Res. 2014;1843:640–51.
102. Nagasawa H, Li CY, Maki CG, Imrich AC, Little JB. Relationship between radiation-induced G1 phase arrest and p53 function in human tumor cells. Cancer Res. 1995;55:1842–6.
103. Bridges KA, Hirai H, Buser CA, Brooks C, Liu H, Buchholz TA, et al. MK-1775, a novel Wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. Clin Cancer Res. 2011;17:5638–48.
104. Pappano W, Zhang Q, Tucker L, Tse C, Wang J. Genetic inhibition of the atypical kinase Wee1 selectively drives apoptosis of p53 inactive tumor cells. BMC Cancer. 2014;14:430.
105. Leijen S, Beijnen JH, Schellens JH. Abrogation of the G2 checkpoint by inhibition of Wee-1 kinase results in sensitization of p53-deficient tumor cells to DNA-damaging agents. Curr Clin Pharmacol. 2010;5:186–91.
106. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461:1071–8.
107. Fokas E, Prevo R, Hammond EM, Brunner TB, McKenna WG, Muschel RJ. Targeting ATR in DNA damage response and cancer therapeutics. Cancer Treat Rev. 2014;40:109–17.
108. Brown EJ, Baltimore D. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev. 2000;14:397–402.
109. Murga M, Bunting S, Montana MF, Soria R, Mulero F, Canamero M, et al. A mouse model of ATR-Seckel shows embryonic replicative stress and accelerated aging. Nat Genet. 2009;41:891–8.
110. Ruzankina Y, Schoppy DW, Asare A, Clark CE, Vonderheide RH, Brown EJ. Tissue regenerative delays and synthetic lethality in adult mice after combined deletion of Atr and Trp53. Nat Genet. 2009;41:1144–9.
111. Reaper PM, Griffiths MR, Long JM, Charrier JD, Maccormick S, Charlton PA. Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. Nat Chem Biol. 2011;7:428–430.
112. Fujisawa H, Nakajima NI, Sunada S, Lee Y, Hirakawa H, Yajima H, et al. VE-821, an ATR inhibitor, causes radiosensitization in human tumor cells irradiated with high LET radiation. Radiat Oncol. 2015;10:175.
113. Fokas E, Prevo R, Pollard JR, Reaper PM, Charlton PA, Cornelissen B, et al. Targeting ATR in vivo using the novel inhibitor VE-822 results in selective sensitization of pancreatic tumors to radiation. Cell Death Dis. 2012;3:e441.
114. Foote KM, Blades K, Cronin A, Fillery S, Guichard SS, Hassall L, et al. Discovery of 4-{4-[(3R)-3 Methylmorpholin-4-yl]-6-[1-(methylsulfonyl)cyclopropyl]pyrimidin-2-y l}-1H-indole (AZ20): a potent and selective inhibitor of ATR protein kinase with monotherapy in vivo antitumor activity. J Med Chem. 2013;56:2125–38.
115. Kwok M, Davies N, Agathanggelou A, Smith E, Oldreive C, Petermann E, et al. ATR inhibition induces synthetic lethality and overcomes chemoresistance in TP53 or ATM defective chronic lymphocytic leukemia cells. Blood. 2015.
116. Sarkaria JN, Busby EC, Tibbetts RS, Roos P, Taya Y, Karnitz LM, et al. Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. Cancer Res. 1999;59:4375–82.
117. Sultana R, Abdel-Fatah T, Perry C, Moseley P, Albarakti N, Mohan V, et al. Ataxia telangiectasia mutated and Rad3 related (ATR) protein kinase inhibition is synthetically lethal in XRCC1 deficient ovarian cancer cells. PLoS One. 2013;8:e57098.
118. Weber AM, Ryan AJ. ATM and ATR as therapeutic targets in cancer. Pharmacol Ther. 2015;149:124–38.
119. Beumer JH, Fu KY, Anyang BN, Siegfried JM, Bakkenist CJ. Functional analyses of ATM, ATR and Fanconi anemia proteins in lung carcinoma : ATM, ATR and FA in lung carcinoma. BMC cancer. 2015;15:1–10.
120. Beumer JH, Fu KY, Anyang BN, Siegfried JM, Bakkenist CJ. Functional analyses of ATM, ATR and Fanconi anemia proteins in lung carcinoma. BMC Cancer. 2015;15:1–10.
121. Zhang T, Shen Y, Chen Y, Hsieh JT, Kong Z. The ATM inhibitor KU55933 sensitizes radioresistant bladder cancer cells with DAB2IP gene defect. Int J Radiat Biol. 2015;91:368–78.
122. Teng P, Bateman NW, Darcy KM, Hamilton CA, Maxwell GL, Bakkenist CJ. Pharmacologic inhibition of ATR and ATM offers clinically important distinctions to enhancing platinum or radiation response in ovarian, endometrial, and cervical cancer cells. Gynecol Oncol. 2015;136:554–61.
123. Carruthers R, Ahmed SU, Strathdee K, Gomez-Roman N, Amoah-Buahin E, Watts C, et al. Abrogation of radioresistance in glioblastoma stem-like cells by inhibition of ATM kinase. Mol Oncol. 2015;9:192–203.
124. Choi S, Gamper AM, White JS, Bakkenist CJ. Inhibition of ATM kinase activity does not phenocopy ATM protein disruption: implications for the clinical utility of ATM kinase inhibitors. Cell Cycle. 2010;9:4052–7.

125. White JS, Choi S, Bakkenist CJ. Transient ATM kinase inhibition disrupts DNA damage-induced sister chromatid exchange. Sci Signaling. 2010;3:ra44.
126. Yamamoto K, Wang Y, Jiang W, Liu X, Dubois RL, Lin CS, et al. Kinase-dead ATM protein causes genomic instability and early embryonic lethality in mice. J Cell Biol. 2012;198:305–13.
127. Batey MA, Zhao Y, Kyle S, Richardson C, Slade A, Martin NMB, et al. Preclinical evaluation of a novel ATM inhibitor, KU59403, in vitro and in vivo in p53 functional and dysfunctional models of human cancer. Mol Cancer Ther. 2013;12:959–67.
128. You Z, Chahwan C, Bailis J, Hunter T, Russell P. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. Mol Cell Biol. 2005;25:5363–79.
129. Liao Y, Feng Y, Shen J, Hornicek F, Duan Z. The roles and therapeutic potential of cyclin-dependent kinases (CDKs) in sarcoma. Cancer Metastasis Rev. 2015;35:1-13.
130. Barton KL, Misuraca K, Cordero F, Dobrikova E, Min HD, Gromeier M, et al. PD-0332991, a CDK4/6 inhibitor, significantly prolongs survival in a genetically engineered mouse model of brainstem glioma. PLoS One. 2013;8:e77639.
131. Johnson SM, Torrice CD, Bell JF, Monahan KB, Jiang Q, Wang Y, et al. Mitigation of hematologic radiation toxicity in mice through pharmacological quiescence induced by CDK4/6 inhibition. J Clin Invest. 2010;120:2528–36.
132. Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. J Natl Cancer Inst. 2012;11:3913.
133. Indovina P, Pentimalli F, Casini N, Vocca I, Giordano A. RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. Oncotarget. 2015;6:17873–90.
134. Hsu FT, Chang B, Chen JCH, Chiang IT, Liu YC, Kwang WK, et al. Synergistic effect of sorafenib and radiation on human oral carcinoma in vivo. Sci Rep. 2015;5:15391.
135. Komarova EA, Chernov MV, Franks R, Wang K, Armin G, Zelnick CR, et al. Transgenic mice with p53 responsive lacZ: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity in vivo. EMBO J. 1997;16:1391–400.
136. Morita A, Ariyasu S, Wang B, Asanuma T, Onoda T, Sawa A, et al. AS-2, a novel inhibitor of p53-dependent apoptosis, prevents apoptotic mitochondrial dysfunction in a transcription-independent manner and protects mice from a lethal dose of ionizing radiation. Biochem Biophys Res Commun. 2014;450:1498–504.
137. Vavrova J, Rezacova M. Importance of proapoptotic protein PUMA in cell radioresistance. Folia Biol. 2014;60:53–6.
138. Lee CL, Blum JM, Kirsch DG. Role of p53 in regulating tissue response to radiation by mechanisms independent of apoptosis. Transl Cancer Res. 2013;2:412–21.
139. Roberts M, Saffie R, Salmons H, Ghoto M, Schneider J, Forrester J. Abstract 346: differentiation induced apoptosis in AML cells: the role of p73 in p53-independent versus p53-mediated apoptosis. Cancer Res. 2014;74:346.
140. Wang Z, Sun Y. Targeting p53 for novel anticancer therapy. Transl Oncol. 2010;3:1–12.
141. Yu H, Shen H, Yuan Y, XuFeng R, Hu X, Garrison SP, et al. Deletion of Puma protects hematopoietic stem cells and confers long-term survival in response to high-dose gamma-irradiation. Blood. 2010;115:3472–80.
142. Komarova EA, Kondratov RV, Wang K, Christov K, Golovkina TV, Goldblum JR, et al. Dual effect of p53 on radiation sensitivity in vivo: p53 promotes hematopoietic injury, but protects from gastro-intestinal syndrome in mice. Oncogene. 2004;23:3265–71.
143. Rotolo J, Stancevic B, Zhang J, Hua G, Fuller J, Yin X, et al. Anti-ceramide antibody prevents the radiation gastrointestinal syndrome in mice. J Clin Invest. 2012;122:1786–90.
144. Greene-Schloesser D, Payne V, Peiffer AM, Hsu FC, Riddle DR, Zhao W, et al. The peroxisomal proliferator activated receptor (PPAR) α agonist, fenofibrate, prevents fractionated whole-brain irradiation-induced cognitive impairment. Radiat Res. 2014;181:33–44.
145. Ramanan S, Kooshki M, Zhao W, Hsu FC, Riddle DR, Robbins ME. The PPARalpha agonist fenofibrate preserves hippocampal neurogenesis and inhibits microglial activation after whole-brain irradiation. Int J Radiat Oncol Biol Phys. 2009;75:870–7.
146. Mangoni M, Sottili M, Gerini C, Bonomo P, Bottoncetti A, Castiglione F, et al. A PPAR-gamma agonist attenuates pulmonary injury induced by irradiation in a murine model. Lung Cancer. 2015;90:405–9.
147. Epperly MW, Melendez JA, Zhang X, Nie S, Pearce L, Peterson J, et al. Mitochondrial targeting of a catalase transgene product by plasmid liposomes increases radioresistance in vitro and in vivo. Radiat Res. 2009;171:588–95.
148. Bechtel W, Bauer G. Catalase protects tumor cells from apoptosis induction by intercellular ROS signaling. Anticancer Res. 2009;29:4541–57.
149. Khoo NK, Hebbar S, Zhao W, Moore SA, Domann FE, Robbins ME. Differential activation of catalase expression and activity by PPAR agonists: implications for astrocyte protection in anti-glioma therapy. Redox Biol. 2013;1:70–9.
150. Slone HB, Peters LJ, Milas L. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. J Natl Cancer Inst. 1979;63:1229–35.
151. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105:256–65.
152. Ruocco MG, Pilones KA, Kawashima N, Cammer M, Huang J, Babb JS, et al. Suppressing T cell motility induced by anti–CTLA-4 monotherapy improves antitumor effects. J Clin Invest. 2012;122:3718–30.
153. Madureira P, de Mello RA, de Vasconcelos A, Zhang Y. Immunotherapy for lung cancer: for whom the bell tolls? Tumor Biol. 2015;36:1411–22.
154. Slovin SF, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol. 2013;24:1813–21.
155. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014;384:1109–17.
156. Schaue D, McBride WH. Opportunities and challenges of radiotherapy for treating cancer. Nat Rev Clin Oncol. 2015;12:527–40.
157. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23–34.
158. Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys. 2013;86:343–9.
159. Sharabi AB, Nirschl CJ, Kochel CM, Nirschl TR, Francica BJ, Velarde E, et al. Stereotactic radiation therapy augments antigen-specific PD-1-mediated antitumor immune responses via cross-presentation of tumor antigen. Cancer Immunol Res. 2015;3:345–55.
160. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, McKenna C, Jones S, Cheadle EJ, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. Cancer Res. 2014;74:5458–68.

161. Deng L, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR, et al. Irradiation and anti–PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest. 2014;124:687–95.
162. Chow LQ. Exploring novel immune-related toxicities and endpoints with immune-checkpoint inhibitors in non small cell lung cancer. Am Soc Clin Oncol Educ Book. Meeting. 2013;1:280–285.
163. Zhang D, Chen Z, Wang D, Wang X. Regulatory T cells and potential inmmunotherapeutic targets in lung cancer. Cancer Metastasis Rev. 2015;34:277–90.
164. Yazdani Y, Mohammadnia-Afrouzi M, Yousefi M, Anvari E, Ghalamfarsa G, et al. Myeloid-derived suppressor cells in B cell malignancies. Tumor Biol. 2015;36:7339–53.
165. Hu H, Hang JJ, Han T, Zhuo M, Jiao F, Wang LW. 2016. The M2 phenotype of tumor-associated macrophages in the stroma confers a poor prognosis in pancreatic cancer. Tumor biol. 2016. doi:10.1007/s13277-015-4741-z.
166. Farrell CL, Bready JV, Rex KL, Chen JN, DiPalma CR, Whitcomb KL, et al. Keratinocyte growth factor protects mice from chemotherapy and radiation-induced gastrointestinal injury and mortality. Cancer Res. 1998;58:933–9.
167. Chen L, Brizel DM, Rabbani ZN, Samulski TV, Farrell CL, Larrier N, et al. The protective effect of recombinant human keratinocyte growth factor on radiation-induced pulmonary toxicity in rats. Int J Radiat Oncol Biol Phys. 2004;60:1520–9.
168. Hensley ML, Hagerty KL, Kewalramani T, Green DM, Meropol NJ, Wasserman TH, et al. American Society of Clinical Oncology 2008 clinical practice guideline update: use of chemotherapy and radiation therapy protectants. J Clin Oncol. 2009;27:127–45.
169. Henke M, Alfonsi M, Foa P, Giralt J, Bardet E, Cerezo L, et al. Palifermin decreases severe oral mucositis of patients undergoing postoperative radiochemotherapy for head and neck cancer: a randomized, placebo-controlled trial. J Clin Oncol. 2011;29:2815–20.
170. Le QT, Kim HE, Schneider CJ, Murakozy G, Skladowski K, Reinisch S, et al. Palifermin reduces severe mucositis in definitive chemoradiotherapy of locally advanced head and neck cancer: a randomized, placebo controlled study. J Clin Oncol. 2011;29:2808–14.
171. Schuette W, Krzakowski MJ, Massuti B, Otterson GA, Lizambri R, Wei H, et al. Randomized phase II study of palifermin for reducing dysphagia in patients receiving concurrent chemoradiotherapy for locally advanced unresectable non-small cell lung cancer. J Thorac Oncol. 2012;7:157–64.
172. Vitale KM, Violago L, Cofnas P, Bishop J, Jin Z, Bhatia M, et al. Impact of palifermin on incidence of oral mucositis and healthcare utilization in children undergoing autologous hematopoietic stem cell transplantation for malignant diseases. Pediatr Transplant. 2014;18:211–6.
173. Ryu JK, Swann S, LeVeque F, Scarantino CW, Johnson D, Chen A, et al. The impact of concurrent granulocyte macrophage-colony stimulating factor on radiation-induced mucositis in head and neck cancer patients: a double blind placebo-controlled prospective phase III study by Radiation Therapy Oncology Group 9901. Int J Radiat Oncol Biol Phys. 2007;67:643–50.
174. Hoffman KE, Pugh SL, James JL, Scarantino C, Movsas B, Valicenti RK, et al. The impact of concurrent granulocyte-macrophage colony-stimulating factor on quality of life in head and neck cancer patients: results of the randomized, placebo-controlled Radiation Therapy Oncology Group 9901 trial. Qual Life Res. 2014;23:1841–58.
175. Masucci G, Broman P, Kelly C, Lindahl S, Malmberg L, Reizenstein J, et al. Therapeutic efficacy by recombinant human granulocyte/monocyte-colony stimulating factor on mucositis occurring in patients with oral and oropharynx tumors treated with curative radiotherapy: a multicenter open randomized phase III study. Med Oncol. 2005;22:247–56.
176. Harrington KJ, Hingorani M, Tanay MA, Hickey J, Bhide SA, Clarke PM, et al. Phase I/II study of oncolytic HSVGM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. Clin Cancer Res. 2010;16:4005–15.
177. Lövey J, Bereczky B, Gilly R, Kenessey I, Rásó E, Simon E, et al. Recombinant human erythropoietin alpha improves the efficacy of radiotherapy of a human tumor xenograft, affecting tumor cells and microvessels. Strahlenther Onkol. 2008;184:1–7.
178. Debus J, Drings P, Baurecht W, Angermund R. Prospective, randomized, controlled, and open study in primarily inoperable, stage III non-small cell lung cancer (NSCLC) patients given sequential radiochemotherapy with or without epoetin alfa. Radiother Oncol. 2014;112:23–9.
179. Blohmer JU, Paepke S, Sehouli J, Boehmer D, Kolben M, Wurschmidt F, et al. Randomized phase III trial of sequential adjuvant chemoradiotherapy with or without erythropoietin Alfa in patients with high-risk cervical cancer: results of the NOGGO-AGO intergroup study. J Clin Oncol. 2011;29:3791–7.
180. Doleschel D, Rix A, Arns S, Palmowski K, Gremse F, Merkle R, et al. Erythropoietin improves the accumulation and therapeutic effects of carboplatin by enhancing tumor vascularization and perfusion. Theranostics. 2015;5:905–18.
181. Angiolillo AL, Davenport V, Bonilla MA, van de Ven C, Ayello J, Militano O, et al. A phase I clinical, pharmacologic, and biologic study of thrombopoietin and granulocyte colony-stimulating factor in children receiving ifosfamide, carboplatin, and etoposide chemotherapy for recurrent or refractory solid tumors: a children's oncology group experience. Clin Cancer Res. 2005;11:2644–50.
182. Wang C, Zhang B, Wang S, Zhang J, Liu Y, Wang J, et al. Recombinant human thrombopoietin promotes hematopoietic reconstruction after severe whole body irradiation. Sci Rep. 2015;5:1–12.
183. Ara G, Watkins BA, Zhong H, Hawthorne TR, Karkaria CE, Sonis ST, et al. Velafermin (rhFGF-20) reduces the severity and duration of hamster cheek pouch mucositis induced by fractionated radiation. Int J Radiat Biol. 2008;84:401–12.
184. Kim MR, Lee J, An YS, Jin YB, Park IC, Chung E, et al. TGFβ1 protects cells from gamma-IR by enhancing the activity of the NHEJ repair pathway. Mol Cancer Res. 2015;13:319–329.
185. Russell JS, Brown JM. The irradiated tumor microenvironment: role of tumor-associated macrophages in vascular recovery. Front Physiol. 2013;4:157.
186. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest. 2010;120:694–705.
187. Ahn GO, Brown JM. Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. Cancer Cell. 2008;13:193–205.
188. Ahn GO, Tseng D, Liao CH, Dorie MJ, Czechowicz A, Brown JM. Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. Proc Natl Acad Sci U S A. 2010;107:8363–8.
189. Shiao SL, Ruffell B, DeNardo DG, Faddegon BA, Park CC, Coussens LM. TH2-polarized CD4(+) T cells and macrophages limit efficacy of radiotherapy. Cancer Immunol Res. 2015;3:518–25.
190. Arany PR, Flanders KC, DeGraff W, Cook J, Mitchell JB, Roberts AB. Absence of Smad3 confers radioprotection through modulation of ERK-MAPK in primary dermal fibroblasts. J Dermatol Sci. 2007;48:35–42.

191. Vanpouille-Box C, Diamond JM, Pilones KA, Zavadil J, Babb JS, Formenti SC, et al. TGFbeta is a master regulator of radiation therapy-induced antitumor immunity. Cancer Res. 2015;75: 2232–42.
192. Pietraszkiewicz H, Shaw J. UTL-5g lowers levels of TGF-β and TNF-α elevated by lung irradiation and does not affect tumor-response to irradiation. Am J Biomed Sci. 2014;6:157–65.
193. Park JH, Ryu SH, Choi EK, Ahn SD, Park E, Choi KC, et al. SKI2162, an inhibitor of the TGF-beta type I receptor (ALK5), inhibits radiation-induced fibrosis in mice. Oncotarget. 2015;6: 4171–9.
194. Koshy M, Rich SE, Mahmood U, Kwok Y. Declining use of radiotherapy in stage I and II Hodgkin's disease and its effect on survival and secondary malignancies. Int J Radiat Oncol Biol Phys. 2012;82:619–25.
195. Scott BR, Walker DM, Tesfaigzi Y, Schollnberger H, Walker V. Mechanistic basis for nonlinear dose-response relationships for low-dose radiation-induced stochastic effects. Nonlinearity Biol Toxicol Med. 2003;1:93–122.
196. Grantzau T, Overgaard J. Risk of second non-breast cancer after radiotherapy for breast cancer: a systematic review and meta-analysis of 762,468 patients. Radiother Oncol. 2015;114:56–65.
197. Zelefsky MJ, Pei X, Teslova T, Kuk D, Magsanoc JM, Kollmeier M, et al. Secondary cancers after intensity modulated radiotherapy, brachytherapy and radical prostatectomy for the treatment of prostate cancer: incidence and cause-specific survival outcomes according to the initial treatment intervention. BJU Int. 2012;110: 1696–701.
198. Doi K, Mieno MN, Shimada Y, Yonehara H, Yoshinaga S. Meta-analysis of second cancer risk after radiotherapy among childhood cancer survivors. Radiat Prot Dosim. 2011;146:263–7.
199. Wiltink LM, Nout RA, Fiocco M, et al. No increased risk of second cancer after radiotherapy in patients treated for rectal or endometrial cancer in the randomized TME, PORTEC-1, and PORTEC-2 trials. J Clin Oncol. 2014;58:6693.
200. Ozsahin M, Crompton NE, Gourgou S, Kramar A, Li L, Shi Y, et al. CD4 and CD8 T-lymphocyte apoptosis can predict radiation-induced late toxicity: a prospective study in 399 patients. Clin Cancer Res. 2005;11:7426–33.
201. Shim G, Ricoul M, Hempel WM, Azzam EI, Sabatier L. Crosstalk between telomere maintenance and radiation effects: a key player in the process of radiation-induced carcinogenesis. Mutat Res, Rev Mutat Res. 2014;760:1–17.
202. Gramatges MM, Liu Q, Yasui Y, Okcu MF, Neglia JP, Strong LC, et al. Telomere content and risk of second malignant neoplasm in survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. Clin Cancer Res. 2014;20:904–11.
203. M'Kacher R, Girinsky T, Colicchio B, Ricoul M, Dieterlen A, Jeandidier E, et al. Telomere shortening: a new prognostic factor for cardiovascular disease post-radiation exposure. Radiat Prot Dosim. 2015;164:134–7.
204. Mirjolet C, Boidot R, Saliques S, Ghiringhelli F, Maingon P, Crehange G. The role of telomeres in predicting individual radiosensitivity of patients with cancer in the era of personalized radiotherapy. Cancer Treat Rev. 2015;41:354–60.
205. Bystrom LM, Guzman ML, Rivella S. Iron and reactive oxygen species: friends or foes of cancer cells? Antioxid Redox Signaling. 2014;20:1917–24.
206. Zhang C, Liu G, Huang M. Ribonucleotide reductase metallocofactor: assembly, maintenance and inhibition. Front Biol (Beijing, China). 2014;9:104–13.
207. Yao K, Patel R, Ferris G, Oleinick NL. Triapine enhances radiosensitivity of HPV negative head and neck squamous cell carcinoma cells. Int J Radiat Oncol Biol Phys. 2015;93:E513–4.
208. Kumar IP, Goel HC. Iron chelation and related properties of Podophyllum hexandrum, a possible role in radioprotection. Indian J Exp Biol. 2000;38:1003–6.
209. Persson HL. Radiation-induced lysosomal iron reactivity: implications for radioprotective therapy. IUBMB Life. 2006;58:395–401.
210. Egan LJ, Eckmann L, Greten FR, Chae S, Li ZW, Myhre GM, et al. IkappaB-kinasebeta-dependent NF-kappaB activation provides radioprotection to the intestinal epithelium. Proc Natl Acad Sci U S A. 2004;101:2452–7.
211. Riehl TE, Newberry RD, Lorenz RG, Stenson WF. TNFR1 mediates the radioprotective effects of lipopolysaccharide in the mouse intestine. Am J Physiol Gastrointest Liver Physiol. 2004;286: G166–73.
212. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. 2008;320:226–30.
213. Daroczi B, Kari G, Ren Q, Dicker AP, Rodeck U. Nuclear factor kappaB inhibitors alleviate and the proteasome inhibitor PS-341 exacerbates radiation toxicity in zebrafish embryos. Mol Cancer Ther. 2009;8:2625–34.
214. Alexeev V, Lash E, Aguillard A, Corsini L, Bitterman A, Ward K, et al. Radiation protection of the gastrointestinal tract and growth inhibition of prostate cancer xenografts by a single compound. Mol Cancer Ther. 2014;13:2968–77.
215. Beg AA, Baltimore D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. Science. 1996;274: 782–4.
216. Izzo JG, Malhotra U, Wu TT, Ensor J, Luthra R, Lee JH, et al. Association of activated transcription factor nuclear factor kappab with chemoradiation resistance and poor outcome in esophageal carcinoma. J Clin Oncol. 2006;24:748–54.
217. Zhang Y, Wei Y, Zhu Z, Gong W, Liu X, Hou Q, et al. Icariin enhances radiosensitivity of colorectal cancer cells by suppressing NF-kB activity. Cell Biochem Biophys. 2014;69:303–10.
218. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006;354:567–78.
219. Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, et al. Radiotherapy plus cetuximab for ocoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol. 2010;11:21–8.
220. Fan Z, Baselga J, Masui H, Mendelsohn J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-diamminedichloroplatinum on well established A431 cell xenografts. Cancer Res. 1993;53:4637–42.
221. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. Cancer Res. 1999;59:1935–40.
222. Debucquoy A, Machiels JP, McBride WH, Haustermans K. Integration of epidermal growth factor receptor inhibitors with preoperative chemoradiation. Clin Cancer Res. 2010;16:2709–14.
223. Ang KK, Zhang Q, Rosenthal DI, Nguyen-Tan PF, Sherman EJ, Weber RS, et al. Randomized phase III trial of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III to IV head and neck carcinoma: RTOG 0522. J Clin Oncol. 2014;32:2940–50.
224. Schaue D, Ratikan JA, Iwamoto KS. Cellular autofluorescence following ionizing radiation. PLoS One. 2012;7:e32062.
225. Zhang LJ, Yan YJ, Liao PY, Margetic D, Wang L, Chen ZL. Synthesis and antitumor activity evaluation of a novel porphyrin derivative for photodynamic therapy in vitro and in vivo. Tumor Biol. 2015;37:1–11.

226. Nyst HJ, Wildeman MA, Indrasari SR, Karakullukcu B, van Veen RLP, Adham M, et al. Temoporfin mediated photodynamic therapy in patients with local persistent and recurrent nasopharyngeal carcinoma after curative radiotherapy: a feasibility study. Photodiagn Photodyn Ther. 2012;9:274–81.
227. Moulder JE, Fish BL. Angiotensin converting enzyme inhibitor captopril does not prevent acute gastrointestinal radiation damage in the rat. Radiat Oncol Invest. 1997;5:50–3.
228. Bracci S, Valeriani M, Agolli L, De Sanctis V, Maurizi Enrici R, Osti MF. Renin-angiotensin system inhibitors might help to reduce the development of symptomatic radiation pneumonitis after stereotactic body radiotherapy for lung cancer. Clin Lung Cancer. 2015;17:189–197.
229. Zhao DY, Jacobs KM, Hallahan DE, Thotala D. Silencing Egr1 attenuates radiation-induced apoptosis in normal tissues while killing cancer cells and delaying tumor growth. Mol Cancer Ther. 2015;14:2343–52.
230. Allen B, Marcu L, & Bezak E. Biomedical physics in radiotherapy for cancer. Clayton: CSIRO PUBLISHING; 2012.
231. Prasad KN. Radiation injury prevention and mitigation in humans. Abingdon: Taylor & Francis; 2012.
232. Moding EJ, Kastan MB, Kirsch DG. Strategies for optimizing the response of cancer and normal tissues to radiation. Nat Rev Drug Discovery. 2013;12:526–42.
233. Geiger H, Pawar SA, Kerschen EJ, Nattamai KJ, Hernandez I, Liang HP, et al. Pharmacological targeting of the thrombomodulin-activated protein C pathway mitigates radiation toxicity. Nat Med. 2012;18:1123–9.
234. Singh VK, Wise SY, Fatanmi OO, Beattie LA, Ducey EJ, Seed TM. Alpha-tocopherol succinate- and AMD3100-mobilized progenitors mitigate radiation combined injury in mice. J Radiat Res. 2014;55:41–53.
235. Christensen R, Alsner J, Brandt Sorensen F, Dagnaes-Hansen F, Kolvraa S, Serakinci N. Transformation of human mesenchymal stem cells in radiation carcinogenesis: long-term effect of ionizing radiation. Regener Med. 2008;3:849–61.
236. Cruet-Hennequart S, Drougard C, Shaw G, Legendre F, Demoor M, Barry F, et al. Radiation-induced alterations of osteogenic and chondrogenic differentiation of human mesenchymal stem cells. PLoS One. 2015;10:e0119334.
237. Lenarczyk M, Su J, Haworth ST, Komorowski R, Fish BL, Migrino RQ, et al. Simvastatin mitigates increases in risk factors for and the occurrence of cardiac disease following 10 Gy total body irradiation. Pharmacol Res Perspect. 2015;3:e00145.
238. O'Shaughnessy J, Schwartzberg L, Danso MA, Miller KD, Rugo HS, Neubauer M, et al. Phase III study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin in patients with metastatic triple negative breast cancer. J Clin Oncol. 2014;34:3840–3847.
239. Patel AG, De Lorenzo SB, Flatten KS, Poirier GG, Kaufmann SH. Failure of iniparib to inhibit poly(ADP- Ribose) polymerase in vitro. Clin Cancer Res. 2012;18:1655–62.
240. Barber LJ, Davies MN, Gerlinger M. Dissecting cancer evolution at the macro-heterogeneity and micro heterogeneity scale. Curr Opin Genet Dev. 2015;30:1–6.