

REVIEW ARTICLE

Radiation damage and radioprotectants: new concepts in the era of molecular medicine

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ABSTRACT. Exposure to ionising radiation results in mutagenesis and cell death, and the clinical manifestations depend on the dose and the involved body area. Reducing carcinogenesis in patients treated with radiotherapy, exposed to diagnostic radiation or who are in certain professional groups is mandatory. The prevention or treatment of early and late radiotherapy effects would improve quality of life and increase cancer curability by intensifying therapies. Experimental and clinical data have given rise to new concepts and a large pool of chemical and molecular agents that could be effective in the protection and treatment of radiation damage. To date, amifostine is the only drug recommended as an effective radioprotectant. This review identifies five distinct types of radiation damage (I, cellular depletion; II, reactive gene activation; III, tissue disorganisation; IV, stochastic effects; V, bystander effects) and classifies the radioprotective agents into five relevant categories (A, protectants against all types of radiation effects; B, death pathway modulators; C, blockers of inflammation, chemotaxis and autocrine/paracrine pathways; D, antimutagenic keepers of genomic integrity; E, agents that block bystander effects). The necessity of establishing and funding central committees that guide systematic clinical research into evaluating the novel agents revealed in the era of molecular medicine is stressed.

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All life forms on our planet are exposed to cosmic radiation, albeit at a level low enough to allow genetic stability. The Earth's atmosphere forms a transparent shield, allowing in the right amount of radiation to reach soil and water, and supporting life. Experiments in shielded conditions have shown that low-dose ionising radiation is necessary to maintain optimum physiological function [1, 2].

Exposure to higher doses of ionising radiation (above some cGy), however, results in an increased rate of genetic mutations and cell death. A total body acute exposure of mammals to doses of 5–12 Gy results in death due to bone marrow, gastrointestinal, lung or brain damage. Such high exposures occur, however, only after accidental exposure or nuclear weapon usage, or as a medical intervention in the context of bone marrow transplantation for patients with malignancy. Partial exposure of the human body to much higher doses, equivalent to a single acute dose of 16–20 Gy, occurs during radiotherapy of cancer patients. Although in these cases death from radiation tissue damage is rare, late radiation sequelae may severely compromise the quality of life of cancer survivors; therefore, radioprotectants are important in protecting cancer patients during radiotherapy and in preventing remote, sometimes life-threatening, sequelae.

Radioprotectants are also important in minimising the accumulation of genetic mutations in patients receiving

radiotherapy or individuals exposed to non-lethal, but higher than normal, levels of radiation. Levels of exposure to diagnostic radiation during CT scan range from 0.01 to 0.2 Gy, and genetic damage and mutations can be identified in the lymphocytes of individuals undergoing CT [3]. The frequency of deoxyribonucleic acid (DNA) mutation is dose dependent [4, 5] and appears to increase linearly for doses between 0.1 and 5 Gy [6]. The risk of second carcinomas following radiotherapy and/or chemotherapy is not negligible. The lung cancer incidence following breast radiotherapy is as high as 2% [7], and 18% of deaths following successful treatment for Hodgkin's lymphoma are due to secondary carcinomas [8], although chemotherapy is certainly a component of such oncogenesis. It is therefore important to find ways to reduce the cancer risk following radiotherapy, given that cancer survivors account for >3.5% of the total population of developed countries [9]. Whether effective radioprotectants with acceptable side-effect profiles could further decrease the risk of radiation carcinogenesis in young patients undergoing radiological tests or in professional groups receiving a higher than average radiation dose is an issue of current interest. The field of research on radioprotectants is also of great importance in space science [10], given that manned missions are expected to increase in the near future.

Radiation tissue damage

Tissues are well-organised structures composed of cells of epithelial and connective tissue origin. The

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epithelial and haemopoietic bone marrow cell components are continuously regenerating compartments: the doubling time of regenerating clonogenic cells in the crypts of the small intestine is less than 1 day, and the whole intestinal tract epithelial compartment renews entirely within a few days [11, 12]. Conversely, fibroblasts and the endothelial vascular cells that compose the underlying stroma are slowly proliferating cells; consequently, the stroma is a slow-renewing compartment. The endothelial cell turnover in conduit blood vessels *in vivo* is approximately 1 to several years, with only 0.1% of cells actively proliferating in quiescent vessels [13, 14]. Similarly, only 0.3% of submucosal fibroblasts of the intestine are found in the proliferative phase [15]. Under stressful conditions, however, the proliferation rate of these cells can increase dramatically. Neurons, on the other hand, never proliferate.

Owing to this distinct cellular compartmentalisation, we propose that exposure of tissues to ionising radiation has five main effects:

1. Type I early radiation effects/cellular depletion, which involve cellular death and cellular depletion of a tissue followed by a proliferative response of stem cells. At doses used in clinical radiotherapy (<80 Gy), this damage is more evident in the epithelial (or haemopoietic) compartment. This is the early-responding cellular compartment, the depopulation of which results in so-called "acute" or "early" radiation toxicity. Doses higher than those used in radiotherapy may result in complete extinction of such cells. Experimental and clinical data suggest that for this compartment—which has a high α/β ratio—the damage induced depends mainly on the total physical dose and less on the dose per fraction (in cases of fractionated radiotherapy) [16].
2. Type II early radiation effects/reactive gene activation, which involves cellular and tissue dysfunction followed by increased vascular permeability, tissue oedema, production of growth factor and cytokines on behalf of fibroblasts and endothelial cells and chemoattraction of macrophages and other white cells, leading to radiation inflammation. This is an entirely different type of early radiation damage because it is a consequence of reactive activation of genes involved in inflammation, vascular permeability, angiogenesis, apoptosis inhibition and others [17, 18]. This Type II early response of the stromal compartment can persist for weeks or months until it settles. Experimental and clinical data suggest that for this compartment—which has a low α/β ratio—the damage induced strongly depends on the dose per fraction (in cases of fractionated radiotherapy) [16].
3. Type III late radiation effects/tissue disorganisation. If the radiation damage to fibroblasts and vessels (or other cells of connective origin) is high enough, Type II early effects can progress to permanent tissue disorganisation and dysfunction, with proliferation of fibroblasts and vessels, leading to the known clinical signs of oedema, fibrosis and telangiectasia. Severe damage of the vascular component may lead to necrosis. This is a result of an unbreakable vicious cycle of growth factor and cytokine production by fibroblasts, endothelium and immune cells, leading to organ dysfunction that manifests 6 months or even 2–3 years (or more) after irradiation [18].
4. Type IV radiation effects/stochastic effects, in which mutations of the exposed somatic cell genome may accumulate in surviving cells and be passed to offspring. This may occur in blood cells, leading to haematological malignancies, or in all epithelial or connective tissue cells, leading to solid tumours [9]. Moreover, although not supported by strong epidemiological or experimental data, germ cell mutations can be inherited in males and manifested after a number of generations.
5. Type V radiation effects/bystander effects. Although we have been aware of this effect for many decades [19], it has only recently become a target of intensive research to clarify its molecular mechanisms [20, 21]. This effect refers to the radiation damage induced in cells within an organ or the whole body that have not been directly exposed to radiation. This is a result of transmission of molecular signals through gap junctions between cells (local level), or via secreted molecules in the organ environment or in the blood, that reach and damage cells in distant organs (long-range abscopal level). Bystander (non-targeted) and directly irradiated cells undergo a similar type of DNA damage, which includes single- and double-strand breaks, induction of micronuclei, sister chromatid exchange and γ -H2AX foci, in addition to loss of DNA methylation, resulting in increased genomic instability [22, 23]. In this way, Type V effects may significantly contribute to the early and late effects of radiation and, importantly, to the Type V stochastic effects.

Mechanistic classification of radioprotectants

From cell exposure to radiation to the appearance of genetic mutation, cell death or tissue disorganisation, we can identify discrete steps where radioprotective policies can intervene to reverse the destructive process (Figure 1). Exposure of cells to ionising radiation leads to free radical formation that damages both DNA and the cytoplasmic organelles and endoplasmic reticulum. High linear energy transfer (LET) radiation can directly damage DNA strands without affecting the activity of free radicals. A repair process follows, resulting in either the survival of the cell or the triggering of death pathways such as apoptosis. Inadequate survival of cells with mutations leads to carcinogenesis. Damage to late-responding cellular compartments leads to early dysfunction and, eventually, a chronic phase resulting in organ destruction. Each of these steps can be blocked via pharmacological or molecular interference. Classification of the radioprotectants according to their position in the above described process is shown in Table 1.

Type A1 radioprotectants: blockers of oxygen consumption

The first step in radiation interaction with the cell is the formation of free radicals as a result of ionisation of water and other molecules. In 1953, Gray et al [24] showed that the biological effect of radiation is reduced under anoxic conditions. Oxygen-free radicals created

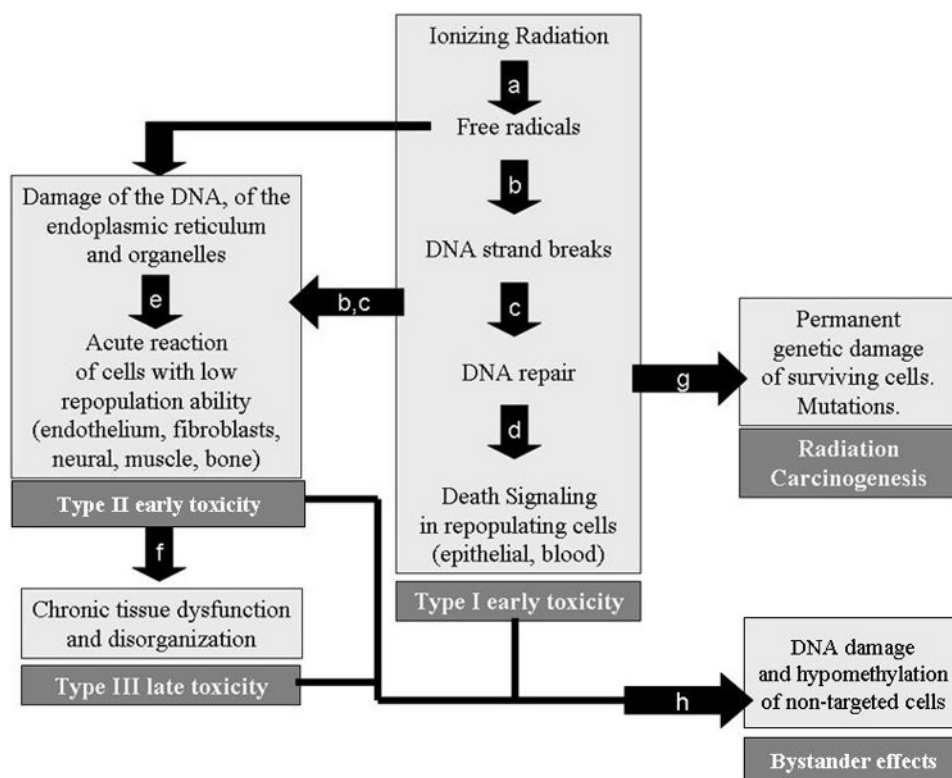


Figure 1. We can interfere in radiation damage by: (a) blocking the formation of free radicals; (b) blocking the free radicals, once formed, with free radical scavengers; (c) enhancing the deoxyribonucleic acid (DNA) repair process; (d) inhibiting death signalling pathways; (e) preventing the acute release of cytokines and growth factors by fibroblasts and endothelium during radiotherapy; (f) breaking the vicious cycle that continuously stimulates fibroblast and endothelium dysfunction; (g) preventing the survival of somatic mutated cells that repopulate the tissue with initiated cells; (h) preventing the DNA damage and hypomethylation of bystander cells.

by the ionising irradiation interaction with intracellular oxygen assault the DNA strands [25]. Dysfunction of mitochondria, the endoplasmic reticulum and other organelles induced by free radicals may also contribute to mutagenesis and death [26, 27]. Reduction of oxygen tension in the body extremities to protect tissues by using tourniquet techniques has been proposed in the past for normal tissue protection [28]. Since the early 1960s, reports have proposed that compounds such as hydroxytryptamine [29] that reduce oxygen consumption could have a role as radioprotectants. Amifostine, the only radioprotectant currently approved for clinical use, has

such a role [30], although its major cytoprotective pathway is its activity as a free radical scavenger. The oxygen tension and saturation of haemoglobin is increased in the venous blood 30 min following injection of the agent; glucose levels drop, showing a shift of normal tissue metabolism to anaerobic pathways [31].

Another approach to reduce the cellular oxygen consumption is amplifying the activity of the hypoxia-inducible factors-1 alpha and 2 alpha (HIF-1 α and HIF-2 α , respectively). These are key transcription factors regulating the expression of a variety of genes involved in glycolysis and angiogenesis [32]. These proteins are constantly degraded by the proteasome pathway; therefore, under normal oxygen tension, their concentration remains low. Under hypoxic conditions, however, degradation is inhibited and HIFs are accumulated in the cytoplasm. Following heterodimerisation, HIFs bind the DNA to the hypoxia response elements (HREs) of a large number of target genes involved in angiogenesis [e.g. vascular endothelial growth factor (VEGF), erythropoiesis and apoptosis/survival regulating proteins (e.g. BNIP3)], switching on their transcription. The *lactate dehydrogenase A* gene (*LDHA*) is under the direct control of HIFs [33], and overexpression of LDHA leads to suppression of oxidative phosphorylation and anaerobic metabolism. Stabilisation of HIFs in normal tissue would reduce oxygen consumption and eventually decrease radiation damage by oxygen species. In fact, this may be a key pathway in the Warburg effect noted in tumours, whereby cancer cells prefer anaerobic glycolysis instead

Table 1. Mechanistic classification of radioprotectants according to their biological activity

- A. Protectants against all types of radiation effects
 1. Blockers of oxygen consumption
 2. Free radical scavengers (endogenous and exogenous)
 3. DNA repair boosters
- B. Protectants against Type I early radiation damage
 1. Death pathway modulators
 2. Growth factors
- C. Protectants against Type II early and Type III late radiation effects
 1. Blockers of radiation inflammation and chemotaxis
 2. Blockers of autocrine/paracrine pathways
- D. Protectants against Type IV stochastic effects
 1. Antimutagenic keepers of genomic integrity
- E. Protectants against Type V bystander effects

DNA, deoxyribonucleic acid.

of mitochondrial oxidative pathways, regardless of the presence of oxygen [34]. Stabilisation of HIFs in normal tissue would reduce oxygen consumption and eventually lead to decreased radiation damage by oxygen species.

Cobalt chloride (CoCl₂), the iron chelator desferrioxamine, and the organomercurial compound mersalyl are important inducers of HIFs, which they achieve by mimicking hypoxia [35–37], probably by reducing the ubiquitination of HIF [38]. Clioquinol, a copper (Cu) (II)/zinc (Zn) (II) chelator, also inhibits the degradation of HIF-1 α and leads to expression of downstream genes in normoxic cells [39]. Isoflurane, a volatile anaesthetic drug, can also upregulate HIF-1 α and enhance HIF-1-responsive genes [40]. Okadaic acid and vanadate also appear to promote HIF-1 α expression via Akt activation [41, 42].

The activity of prolyl-hydroxylase (PHD), the direct sensor of hypoxia, is decreased under hypoxic conditions. Failure of hydroxylation of HIF results in reduced ubiquitination and HIF accumulation. Inhibitors of PHDs have been developed. Tilorone is a low molecular weight antiviral and a potent activator of the HIF pathway in neuronal cell lines, enhancing the expression of downstream target genes [43]. Baicalein suppresses ubiquitination of HIF-1 α by inhibiting the HIF-specific hydroxylases and increasing the target gene transcription in tissue culture cells [44]. A novel PHD inhibitor, FG-4497, which stabilises HIF-1 α and drives the expression of downstream genes, has been shown to protect the intestinal mucosa against chemical insult [45].

Type A2 radioprotectants: free radical scavengers

Generation of free radicals in the nuclear environment leads to a direct reaction with DNA chains and causes strand breakage. Use of free radical scavengers at this stage would reduce the number of free radicals that damage DNA or organelles. All cells contain a certain level of endogenous scavengers, the expression of which can be induced under stressful conditions. Zn, Cu or the mitochondrial manganese (Mn) superoxide dismutase (SOD) are important enzymes converting oxygen radicals to hydrogen peroxide [46]. Glutathione is also an important endogenous scavenger bearing a thiol group [47]. Several compounds can induce the expression of endogenous scavengers, such as *N*-acetyl-cysteine [48]. Amifostine is one of the most potent inducers of MnSOD; levels are maintained high for several days, a phenomenon that may explain the delayed radioprotective activity of amifostine [49]. The expression of endogenous SOD can also be enhanced using adenoviral vectors containing SOD genes [50].

The dephosphorylated thiolic form of amifostine (WR-1065) is a potent exogenous free radical scavenger with established clinical position in the protection against platinum toxicities and the prevention of radiation xerostomia [51]. In the recent European Society for Medical Oncology recommendations, the potential value of amifostine in the protection of radiation proctitis and oesophagitis is highlighted [52]. Experimental studies have shown important protection of cells against *hypoxanthine-guanine phosphoribosyl transferase* (HPRT) gene mutations after exposure to radiation [53]. Amifostine can be administered as a simple subcutaneous injection,

has an acceptable tolerance profile and is a selective protectant of normal tissues in various malignancies, as shown in a large number of randomised studies [54]. More recently, an oral nanoparticle formulation of WR-1065 has been tested in pre-clinical models [55].

Fullerenes are crystal forms of carbon molecules that are neither graphite nor diamond. They consist of a spherical, ellipsoid or cylindrical arrangement of dozens of carbon atoms. The addition of a hydroxy moiety on each carbon of a 60-fullerene gives 60-fullerenol, a water-soluble molecule with potent free radical scavenger activity. In a study involving rats, the administration of a high dose of this compound resulted in radioprotection against total body irradiation (TBI) of 8 Gy, similar to that provided by amifostine [56]. However, the tolerance of the drug and its selectivity remains unknown. Cerium oxide (CeO₂) nanoparticles have also been shown to protect the gastrointestinal epithelium and lung tissue against radiation damage in mice by acting as free radical scavengers and increasing the endogenous production of SOD [57, 58].

Nitroxides have also been tested as free radical scavengers. The lead compound of oxidised forms of nitroxides, tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), has shown radioprotective efficacy in experimental models [59]. The unique quality of the drug to have paramagnetic properties allows the study of the accumulation and clearance using MRI, which may be useful in optimising the administration time before radiotherapy to prevent tumour protection [60].

Type A3 radioprotectants: DNA repair boosters

Immediately following the formation of DNA strand breaks, a complex machinery involving a large number of enzymes is activated to enable rapid and accurate repair of the damage, so that the cell can survive with unaltered genomes. The recent application of fluorescent immunohistochemical techniques with antibodies recognising the phosphorylated form of (phosphorylated histone 2AX) that appears immediately after the formation of a double-strand break enables us to easily investigate the rate of repair and how putative radioprotectants affect the process [61].

Oxidation of the WR-1065 thiolic product of amifostine in cells leads to a disulphidic form, WR-33278, with DNA repair activity. An effect of the molecule on the polyamine-induced compaction and aggregation of DNA has been reported [62] as decreasing DNA strand break accumulation. WR-33278 has structural similarities to the polyamines putrescine, spermidine and spermine [63], and is transported at the same velocity by the polyamine transport system. Polyamines are integral components of the cell chromatin structure and are involved in DNA repair mechanisms [64–66]. Inhibitors decreasing their intracellular concentration result in enhanced irradiation cell killing and mutagenic effect. In supercoiled plasmid DNA experiments, WR-33278 has protected DNA even following neutron exposure, by scavenging of hydroxyl radicals and by reducing the accessibility of radiolytic attack sites via the induction of packaging of DNA in liquid crystalline condensates [67]. Enhancement of the topoisomerase I-mediated unwinding of

supercoiled DNA is a process mediated by WR-33278 [68]. Both WR-1065 and WR-33278 molecules have the ability to remove the platinum adducts from DNA [69].

Resveratrol (3,4-trihydroxy-trans-stilbene) is a poly-ethanol extract from red grapes that is available as a dietary supplement; several studies have shown that it increases life expectancy in experimental animals and prevents carcinogenesis. The addition of resveratrol during radiotherapy of mice resulted in a reduction of chromosomal aberrations [70]. A possible mechanism by which resveratrol acts as a radioprotectant is the stimulation of expression of sirtuins (silent information regulator 2), a class of proteins that exhibit histone deacetylase or mono-ribosyltransferase activity accelerating DNA repair [71].

Direct molecular interference in DNA repair machinery by targeting multiple enzymes involved in the process may also prove critical in accelerating the restoration of DNA damage. Oxoguanine DNA glycosylase (OGG1) initiates base excision repair of oxidised purine bases and exhibits 8-OH-G glycosylase activity [72]. Although this enzyme does not interfere with DNA double-strand breaks and cell death, it seems to have an important antimutagenic effect, as shown in cell lines exposed to ultraviolet light [73]. OGG1 seems also to prevent oxidative damage of mitochondrial DNA [74]. Butin, a 7,3',4'-trihydroxydihydroflavone, protects cells against hydrogen peroxide-induced damage of DNA by enhancing the transcriptional activity of OGG1 and the expression of phosphorylated Akt, a regulator of OGG1, which makes the compound a candidate antimutagenic agent [75].

A key enzyme of base excision repair is the human apurinic/apyrimidinic endonuclease (HAP1/APE1); the mitochondria-targeted APE1 exhibits robust DNA repair activity. Overexpression vectors of mitochondria-targeted truncated APE1 and full-length APE1 significantly protect normal cells from oxidative stress [76]. Poly-(adenosine diphosphate-ribose) polymerase (PARP-1) is a nuclear enzyme that is possibly involved in DNA base excision repair. PARP-1 is involved in suppressing imprecise repair of endogenous DNA damage, leading to deletion mutation during ageing [77]. PARP-1 amplifies a signal for rapid recruitment of repair factors, enabling efficient restoration of genome integrity [78]. Experiments in PARP-1^{-/-} mice have revealed the importance of the enzyme in the survival of intestinal epithelial stem cells following irradiation [79]. A role of the enzyme in the repair of DNA double-strand breaks following irradiation has also been postulated [80]. Boosting the activity of such enzymes by gene therapy techniques could have an important role in the prevention of radiation mutagenesis or toxicity.

Type B1 radioprotectants: inhibitors of death signalling pathways

The DNA damage and, presumably, the endoplasmic reticulum and mitochondria damage induced by radiation-free radicals immediately trigger the pro-apoptotic cell response [81]. The *p53* gene, as a guardian of genomic integrity, is rapidly upregulated, producing the wild type *p53* protein, which is considered as the first step of

apoptosis. The *gap1* phase cell-cycle arrest that follows (first death check point) provides the necessary time for the DNA repair process to occur. If the cell considers the repair to be effective then proliferation follows; if not, the cell progresses to the second death check point (G2 arrest), followed by the release of mitochondrial caspases and death. The B-cell lymphoma 2 (*bcl-2*) family of proteins, being the guardians of this latter check point, can regulate the cell decision towards survival [82]. The anti-apoptotic activity of *Bcl-2* is prominent in cancer cells [83].

Blockage of the *p53* function will break the apoptotic pathway and prevent death, ignoring sometimes important DNA damage. Sodium orthovanadate (Na_3VO_4) and Pifithrin- α (imino-tetrahydrobenzothiazol-tolylethanone hydrobromide) are potent *p53* inhibitors. The administration of these compounds in mice receiving lethal TBI (8 Gy) significantly enhances survival and protects the gastrointestinal mucosa from radiation damage [84]. Antisense oligonucleotides blocking the *p53* upregulated modulator of apoptosis (PUMA) protein, a *p53* downstream protein which is important for the apoptotic process, also protect mice against 15 Gy of TBI and allow regeneration of the intestinal progenitor cells, preventing the gastrointestinal syndrome [85]. Such *p53* blocking approaches seem important for the prevention of Type I early radiation toxicities due to restriction of cellular and stem cell depletion of epithelial tissues. However, one should keep in mind that by blocking apoptosis a higher percentage of cells with mutations will survive, because the *p53* guardian gene will not be allowed to eliminate such cells. In this way, Type I toxicities may be prevented, but Type IV stochastic effects would increase the enhancement of radiation carcinogenesis.

An important group of proteins controlling apoptosis at the post-*p53* level is the *Bcl-2* family of proteins. *Bcl-2* and *Bcl-extra large* (*Bcl-xl*) are anti-apoptotic, while *bcl-2* associated protein X (*Bax*) and *bcl-2* homologous antagonist/killer protein are apoptosis-inducing proteins. Pre-treatment of mice with small-molecule inhibitors of glycogen synthase kinase 3 β (GSK-3 β), SB216763 and SB415286, shows a significant reduction of *Bax* expression and *Bcl-2* upregulation in intestinal crypt cells after radiation with 4–8 Gy compared with radiation alone, and reduces the apoptotic rate of these cells and protects mice against gastrointestinal radiation syndrome [86]. Viral proteins such as the Human Papilloma Virus 16E5 protein seem to have anti-apoptotic effects by stimulating the ubiquitin–proteasome-mediated degradation of the *Bax* protein [87], and may become models for the development of anti-apoptotic radioprotectants. The angiotensin receptor blockers pioglitazone and TAK-491 also have anti-apoptotic functions; they increase Akt and extracellular signal-regulated kinase (ERK) activity and inhibit *Bax* activation [88].

Nuclear factor kappa light-chain enhancer of activated B cells (NF- κ B) is an important transcription factor regulating the expression of a variety of genes including *Bcl-2* and *Bcl-xl*, as well as the expression of the inhibitors of apoptosis proteins (IAPs) [89, 90]. NF- κ B is usually found as a complex with an NF- κ B inhibitor, keeping NF- κ B inactive in the cytoplasm. Activation of specific receptors such as the Toll-like receptors leads to activation of the NF-kappa-B essential modulator/inhibitor of nuclear factor kappa-B kinase-interacting

proteins that induce degradation of the NF- κ B inhibitor protein, releasing the NF- κ B protein that shifts into the nucleus [91]. Flagellin is a bacterial protein extracted from *Salmonella enterica* that binds to the TLRs. CBLB502 is an engineered derivative containing the TLR-5-activating domains of flagellin [92]. Administration of CBLB502 before 13 Gy of TBI in mice results in preservation of the normal crypt cell proliferation rate and significantly protects against death, showing a more potent effect than that of amifostine [92]. CBLB502 also protects monkeys exposed to 6.5 Gy TBI. On the other hand, direct inhibitors of NF- κ B activity, including ethyl pyruvate and the synthetic triterpenoid CDDO-TFEA (RTA401), not only protect against but also mitigate radiation toxicity when given 1–2 h post-exposure to zebrafish embryos [93]. Inhibitors of kappa B ($I\kappa$ B) kinase inhibitors also improve the overall survival of lethally irradiated zebrafish embryos. These may imply a complex role of NF- κ B in radiation sensitivity.

An important pathway controlling death and survival of cells is the autophagic machinery. Although manipulation of autophagy with specific inducers and blockers alters the radiosensitivity of cancer cells [94], there are no data on the role of these in the prevention or treatment of normal tissue radiation damage [95]. Preliminary (unpublished) data from our group suggest that radiation leads to aberrant autophagosome formation and accumulation, implying an important role of autophagy as a stress response pathway to radiation. Studies investigating the phenomenon and assessing the eventual impact on radioprotection are ongoing.

Type B2 radioprotectants: growth factors

Depopulation of the early responding component of tissues (epithelial and blood cells) induces accelerated proliferation of the relevant stem cells, in order to balance the cellular loss. The pick of this Type I early radiation toxicity appears 15–20 days after exposure to radiation. Specific growth factors acting directly on the stem cell population of epithelial or haematological tissues enhance the rate of repopulation and toxicity healing.

Haematopoietic growth factors such as erythropoietin (EPO) and granulocyte (G) or granulocyte-macrophage (GM) colony-stimulating factor (CSF) have an established position in the treatment of anaemia and neutropenia following chemotherapy or radiochemotherapy [96]. GM-CSF may also have a role in the healing of acute radiation mucositis [97]. Second-generation thrombopoietin (TPO) mimetic agents such as romiplostim and eltrombopag are important agents activating the TPO receptor [98] and have been approved for the treatment of immune thrombocytopenic purpura. The keratinocyte growth factor palifermin has been approved for the treatment of severe oral mucositis that occurs after high-dose chemotherapy and radiotherapy followed by stem cell rescue [99].

Becaplermin, a recombinant platelet-derived growth factor B-chain homodimer, has shown important activity in wound healing and seems also to have an important role in osteogenesis [100]. Intracoronary infusion of recombinant human (rh) VEGF, a potent angiogenic factor, induces revascularisation and improves angina in patients with stable exertional angina [101]. Repeated local application

of the rhVEGF telbermin in chronic diabetic foot ulcer has been applied in Phase I trials [102]. An eventual role of such topical gel formulations [103] to accelerate healing of severe radiation mucositis is postulated as VEGF over-expression, and has been shown to occur during the healing of oesophageal acute radiation damage [97].

A peptide derived from the receptor-binding domain of fibroblast growth factor (FGF)-2, FGF-P is a potent mitogen that promotes stem cell renewal, progenitor cell differentiation and epithelial proliferation. 5-day administration of this peptide in mice immediately after sub-TBI (to avoid death by myelosuppression) shows enhanced proliferation of crypt cells of the small intestine, preservation of the gastrointestinal function and prevention of weight loss, suggesting an eventual role of this approach in preventing acute gastrointestinal radiation syndrome [104]. An FGF-1:FGF-2 chimeric growth factor with universal FGF receptor specificity significantly protects the small intestine crypts when given intraperitoneally to BALB/c mice prior to whole-body irradiation [105]. Velafermin, the recombinant FGF-20, reduces lethality of TBI in mice by increasing expression of nuclear factor-erythroid 2 p45-related factor 2 and of MnSOD, and by activating the ERK and Akt signal transduction pathways [106].

Type C1 radioprotectants: blockers of radiation inflammation and chemotaxis

Following irradiation, the slowly proliferating cell compartments of tissues suffer a reactive upregulation and downregulation of genes, changing the functional profile of the cells [17]: an event that results in early Type II radiation toxicity. Agents targeting this effect may be extremely useful in reducing acute vascular dysfunction and leukocyte chemotaxis and infiltration during radiation pneumonitis and colitis, as well as in preventing acute breast, laryngeal/pharyngeal or even brain oedema. Type C1 radioprotectants could block the appearance of Type II toxicities when given during or immediately after the end of a radiotherapy course. These are also likely to act prophylactically against the subsequent development of Type III toxicities by preventing the establishment of autocrine/paracrine pathways. Moreover, they may have a role in the treatment of Type III toxicities by interfering in immunological pathways together with Type C2 radioprotectants.

Blockers of the leukocyte chemotaxis may be useful in the avoidance of the early inflammatory response that leads to vasodilation and oedema. Anti-inflammatory interleukins (ILs) such as IL-10 suppress tumour necrosis factor α (TNF α) and inhibit the expression of endothelial adhesion molecules [e.g. intercellular adhesion molecules (ICAMs)], preventing leukocyte extravasation [107, 108]. Endogenous IL-10 limits Ang II-mediated oxidative stress and vascular dysfunction [109]. Inhibitors of the IL-6 pro-inflammatory cytokine, which is considered to be a major cause of lymphocytic alveolitis, may be important blockers of this phase [110]. In studies of radiation brain injury, neutralising antibodies against either IL-1 β or TNF α prevented the expression of ICAM-1, and thus of leukocyte migration [111].

Recombinant IL-10 (TenovilTM; Schering Corporation, Kenilworth, NJ) has passed the clinical phase of experimentation in patients with Crohn's disease, who have

shown an excellent tolerance profile for 12 week subcutaneous administration [112]. Dekavil, a recently synthesised immunocytokine produced by the fusion of IL-10 with the fibronectin-8 antibody recognising fibronectin and tenascin-C, is being tested as an inhibitor of collagen-induced arthritis [113]. Given the fact that fibronectin and tenascin-C are upregulated by radiation [114], blocking approaches would be of interest in the prevention and treatment of radiation tissue fibrosis via multiple immunomodulatory and fibroblast targeting pathways. Airway-directed gene transfer of IL-10 using viruses also seems to be an interesting approach. The recombinant Sendai virus seems to be an efficient IL-10 gene transfer approach to the airway epithelium [115], with eventual applications in radiation pneumonitis. Noradrenaline elicits anti-inflammatory actions in the central nervous system (CNS), inducing IL-10 production and signalling in the CNS, which may protect against neurodegeneration. The noradrenaline reuptake inhibitor reboxetine combined with the α_2 -adrenoceptor antagonist idazoxan induces a profound increase in IL-10 in the brain [116], and could be tested as a neuroprotector during radiotherapy or in the treatment of radiation CNS syndrome. Compounds such as Y-40138 [117], the peroxisome proliferator-activated receptor PPAR α ligand WY14643 [118] and the anti-tumour alkyl-lysophospholipid analogue edelfosine [119] are potent inducers of IL-10.

Infliximab, an anti-TNF α agent, reduces the infiltration of the synovium by TNF α -producing inflammatory cells [120]. Antibodies against the IL-6 receptor such as tocilizumab have a potent anti-inflammatory effect by blocking leukocyte adhesion to the endothelium [121, 122]. The novel calcineurin/nuclear factor of activated T cells blocker A-285222 reduces IL-6 production by arteries [123]. Baicalein, a component of *Scutellaria radix*, blocks I κ B α degradation followed by downregulation of IL-6 [124]. Anakinra, an IL-1 receptor antagonist, is also an effective agent that inhibits inflammatory pathways and exhibits potent antirheumatoid activity [125].

Irradiation of mice lung results in a sharp increase of lung tissue messenger (m) ribonucleic acid (RNA) for ICAM-1/cluster of differentiation 54 (CD54), vascular cell adhesion molecule and P-selectin and E-selectin in the pulmonary endothelium some hours after irradiation, which is sustained at a high level for several days and returns to normal within 1 week [126]. Trans-endothelial migration into the alveoli and interstitium is thereby facilitated. Anti-CD54 blocking antibodies lead to attenuation of inflammatory cell infiltration in the irradiated lung and ICAM-1 knockout mice show no lung inflammatory response to radiation [127]. ICAM-1 is induced in the mouse CNS following irradiation, suggesting a role in leukocyte migration and brain vascular inflammation [128]. Pravastatin, a new hydroxymethylglutaryl coenzyme A reductase inhibitor, exerts persistent anti-inflammatory and antithrombotic effects on irradiated endothelial cells [129] by inhibiting the overproduction of monocyte chemoattractant protein 1, IL-6 and IL-8 and the enhanced expression of ICAM-1. It seems to be an important antifibrotic agent in radiation-induced enteropathy [130], because it inhibits Type I collagen and fibronectin accumulation, modulating the secretory phenotype of mesenchymal cells. It also seems to prevent or reduce radiation-induced skin damage [131].

Type C2 radioprotectants: blockers of autocrine/paracrine pathways

Protracted gene activation and stromal cell functional deregulation leads to the establishment of autocrine/paracrine loops, stimulating fibroblast and endothelial proliferation and leading to structural disorganisation and organ failure (Type III radiation toxicity). Identification of the key molecules involved in this cycle and of specific inhibitors would allow the breakage of the loop, interruption of damage progression and, possibly, reversal of the process. Type C2 radioprotectants could be useful during the immediate post-radiotherapy period to prevent the establishment of late radiation sequelae or could be used in the treatment of the already established Type III toxicities.

Lung hypoxia is a common event, with maximum levels reached within 6 months following irradiation, as shown in experimental studies applying pimonidazol localisation appraisal [132]. Blockage of the prolyl- and asparaginyl hydroxylase activity and subsequent accumulation of hypoxia-inducible factors 1 and 2 is, therefore, expected to dominate Type III radiation toxicities. A variety of genes, including VEGF, EPO and lactate dehydrogenase-A (which is involved in angiogenesis, glycolysis and apoptosis regulation), are under the direct transcriptional control of HIFs [32]. Because VEGF induces VEGF receptors in fibroblasts, myofibroblasts and endothelial cells [133, 134], blockage of the VEGF-related pathway by VEGF tyrosine kinase inhibitors (vatalanib, pazopanib and cediranib) may also lead to cessation of the autocrine or paracrine loops acting on fibroblasts and endothelial cells. Blockage of VEGF attenuates pulmonary fibrosis induced by bleomycin [135]. The TNP-470 anti-angiogenic compound suppresses the proliferation of myofibroblasts in experimental models by reducing the expression of VEGF [136]. Specific HIF inhibitors have been developed, such as PX-478 and YC-1 [137, 138], which, apart from finding a position in the treatment of cancer, may prove to be useful tools in the prevention and treatment of late radiation sequelae.

Transforming growth factor beta (TGF β) is a key cytokine in the fibrotic process [139] because it induces phenotypic modulation of human lung fibroblasts to myofibroblasts and stimulates collagen protein synthesis [140, 141]. TGF β gene expression increases rapidly within 14 days after irradiation [142]. Following thoracic irradiation, elevated plasma TGF β -1 levels are noted in patients [143]. Rats treated with an adenoviral-mediated soluble TGF β Type II receptor showed markedly reduced fibroproliferative changes following irradiation [144]. SMAD inhibitors such as naringenin downregulate expression and phosphorylation of SMAD protein in fibroblasts, blocking TGF β signalling [145]. A small molecular weight molecule, halofuginone, inhibits the TGF β signalling pathway by increasing the inhibitory SMAD-7 expression and significantly lessens radiation-induced fibrosis [146]. Relaxin, a potent antifibrotic agent and TGF β inhibitor, also seems to exploit the SMAD pathway [147]. Specific inhibitors of the activin receptor-like kinase activity of the TGF β -1 receptor, such as SB-525334, exhibit antifibrotic activity in lungs [148, 149]. The SB203580 and WP631 SMAD inhibitors abrogate excessive proliferation of human lung fibroblasts induced by

γ -rays [150]. Pirfenidone, a novel drug approved for the treatment of idiopathic pulmonary fibrosis, reduces fibroblast proliferation and collagen production [151, 152]. Pentoxifylline also exerts antifibrotic activity by downregulating TGF β [153].

Platelet-derived growth factor (PDGF) isoforms have an important role in stimulating the proliferation and migration of myofibroblasts during fibrosis. PDGF action is directed towards PDGF α and β receptors with tyrosine kinase activity, present on the surface of stimulated fibroblasts [154]. Radiation induces expression of PDGF and phosphorylation of PDGF receptors that persists for a long period (26 weeks) even in the late phase of radiation-induced fibrosis [83]. PDGF receptor tyrosine kinase inhibitors reduce radiation-induced fibroblast and endothelial cell activation [155]. Administration of imatinib or SU9518 (an agent that blocks PDGF receptors) results in prolongation of survival of mice receiving 20 Gy to the lungs, and reduces the radiomorphological signs of lung fibrosis. Imatinib also inhibits bleomycin-induced lung fibrosis [156]. In a recent study, administration of imatinib (100 mg kg⁻¹) daily after 18 Gy whole lung irradiation of mice until euthanasia significantly reduced lung fibrosis [157, 158].

TNF α is produced by activated macrophages and released in large amounts during the fibrotic process [159]. It stimulates fibroblast proliferation, secretion of extracellular matrix proteins and secretion of pro-inflammatory cytokines such as IL-1 and IL-6. Thoracic irradiation of mice results in lung infiltration by TNF α , producing macrophages. This reaction settles within 4 months, suggesting that TNF α probably has a role in the early phase of radiation pneumonitis [160]. Blockage of TNF α activity with recombinant TNF α receptor administration results in resolution of lung fibrotic lesions [161]. TNF receptor 1 inhibition using antisense oligonucleotides preserves lung function following local fractionated irradiation of lung mice [162]. Inhibitors of TNF α such as infliximab, a chimeric monoclonal immunoglobulin G1 (IgG1) antibody against the tumour necrosis factor used in Crohn's disease and rheumatoid arthritis, downregulate both basic FGF (bFGF) and VEGF in the serum of patients [163]. Administration of infliximab in a patient with lung fibrosis and pulmonary hypertension associated with advanced systemic sclerosis has resulted in stabilisation of lung function tests and pulmonary arterial pressures that progressively worsened after cessation of therapy [164]. Anti-TNF α treatment protects normal brain vasculature against radiation [165].

The combination of pentoxifylline and vitamin E in the prevention and treatment of fibrotic lesions has been tested in clinical trials. Pentoxifylline inhibits TNF α [166] and leukotriene synthesis, and reduces inflammation. Prolonged treatment with this combination appears to reduce radiation skin fibrosis [167]. Other clinical trials on breast, lung and pelvic tissue fibrosis have provided inconclusive results [168–171].

Hepatocyte growth factor (HGF) is a tyrosine kinase, a product of the *c-Met* gene, with angiogenic properties. HGF appears to be important in reparative lung response following injury [172]. Activation of NF- κ B by HGF has been reported and abrogation of NF- κ B activity by I κ B α repressors have resulted in loss of HGF/scatter factor-mediated protection of renal cells against doxorubicin

[173]. HGF promotes the proliferation of ECV304 cells and inhibits radiation-induced apoptosis of endothelial cells [174]. Intramyocardial injection of adenoviral vectors transferring the HGF gene protect against experimental radiation-induced heart disease [175]. Continuous infusion of HGF in mice attenuates bleomycin-induced lung damage; furthermore, administration of HGF after establishment of bleomycin fibrosis reverses the fibrotic process and accelerates the proliferation of alveolar epithelial cells [176]. HGF plasmids, when injected into the liver and transferred by electroporation, significantly protect rats against radiation-induced liver damage [177]. Retinoic acid, an active metabolite of vitamin A that acts on specific receptors on cells, prevents fibrosis by counteracting the activity of TGF β and stimulating HGF promoter activity and HGF receptor phosphorylation [178].

bFGF activates the ERK mitogen-activated protein kinase pathway and induces activator protein-1 binding, a nuclear factor that regulates the expression of a variety of genes involved in fibrosis [179]. bFGF promotes fibroblast growth and differentiation, and is produced by endothelial cells within hours following radiation exposure [180, 181]. Serum concentrations of bFGF (together with TNF α and IL-6) are consistently higher in patients receiving lung irradiation [182]. Blockage of bFGF may be useful in the treatment of radiation fibrosis only after the completion of radiotherapy, because blockage of this pathway during irradiation may worsen Type I radiation toxicity.

There is some limited evidence for the protective effect of angiotensin-converting enzyme inhibitors, especially captopril and an angiotensin II Type 1 receptor blocker, on radiation-induced pulmonary injury [183]. Finally, cyclo-oxygenase selective inhibitors may also have a role in preventing radiation pneumopathy [184].

Type D radioprotectants: keepers of genomic integrity

Patients undergoing radiotherapy are exposed to inhomogeneous low-dose whole-body radiation (ranging from 0.004% to 1% of the dose fraction [185, 186]) as a consequence of the scattering of the radiation outside the portals used; in addition, varying volumes of normal tissues receive high doses ranging from 10% to 85% of the total fraction delivered to the tumour. The estimated overall incidence of secondary radiotherapy-induced carcinoma is 1–2% [187], which is low compared with the benefit of cure rates, but significant considering that 3.5% of the general population are cancer survivors [9]. Diagnostic radiology, like CT scans, also delivers a dose in the range of 2 cGy to large body areas [188] and the increasing application of CT scanning in medicine highlights worries regarding potential increased radiation carcinogenesis in the general population [189]. Type D radioprotectants are therefore important in decreasing the overall cancer incidence induced by medical applications. Such agents may also be useful in protecting, for example, radiologists, nuclear industry workers [190], aircraft crew [191] and astronauts [10] against occupational exposure.

Experimental studies show that Type I radioprotectants such as amifostine are important in decreasing the mutation load, given that incorrectly repaired DNA double-strand breaks are considered to be the major cause of radiation mutagenesis [192]. However, no study has yet to assess their efficacy in protecting against secondary carcinomas in a series of patients undergoing radiotherapy and, given the 1–2% excess rate expected within 10–20 years, it is unlikely that such data will ever be available. Administration of 100 mg kg⁻¹ of amifostine in mice has been shown to reduce cyclophosphamide-induced mutagenesis at the HPRT by fivefold [193]. Mutagenesis of splenic T lymphocytes at the HPRT locus following whole-body irradiation of neutrons (single doses, 50–150 cGy) and ⁶⁰Co photons (single doses, 250–750 cGy) also declined when amifostine administration (400 mg kg⁻¹) preceded irradiation, by factors of 1.4 and 2.4, respectively [194]. Amifostine has also been shown to reduce the frequencies of radiation-induced altered hepatocyte foci in Sprague Dawley® (Charles River, Wilmington, MA) rats [195].

Free radical scavenging is certainly a major antimutagenic pathway, but it seems that amifostine also exploits additional pathways. *p53* is a key tumour suppressor gene, the functional loss of which provides resistance to irradiation. Ionising radiation dramatically accelerates lymphoma development in *p53* heterozygous mice. Upon irradiation, *p53* disruption confers a selective advantage and expansion of *p53*-deficient clones followed by an increased incidence of lymphoma [196]. Amifostine restores the normal *p53* gene function in the presence of specific *p53* gene mutations [197, 198] and also suppresses the activation of the *c-Myc* oncogene [199]. This suggests that amifostine could potentially act as an antileukemogenic agent in cases of genetic predisposition. At low intracellular levels, amifostine exhibits antimutagenic activity against nucleoside reverse transcriptase inhibitors used for the treatment of human immunodeficiency virus 1 infection [200]. Amifostine may, therefore, be classified as a Type D radioprotectant, owing to its genetic interference in addition to its scavenger activity.

The exact nature of the mutagenic event induced by radiation remains elusive. Irradiation of peripheral blood mononuclear cells with 3 Gy results in prolonged up- and downregulation of a variety of genes [201]. The upregulated guanine nucleotide binding protein α 15 and the downregulated chemokine (CX3C) receptor 1 are involved in G-protein-coupled receptor protein signalling pathways. The H4 histone family, member G, replication protein A3 and topoisomerase 2 α all act as DNA binding molecules in DNA metabolism and are downregulated. V-Maf, a musculoaponeurotic fibrosarcoma (avian) oncogene homologue with transcription factor activity, is also upregulated. The identification of a specific phenotype that marks a normal cell as initiated would allow the development of treatment approaches aiming to cleanse the body from such cells.

p53 gene mutations appear to occur late in the process of radiation carcinogenesis [202], and amplification of the murine double minute (*mdm2*; *p53* inactivating) protein has been also documented in some experimental radiation-induced tumours [203]. Anti-*p53* Type II radioprotectants are expected to increase carcinogenesis,

while an attempt to restore *p53* function once radiotherapy is accomplished seems a reasonable approach to eliminate *p53*-linked radiation carcinogenesis. The direct activity of amifostine on *p53* gene function restoration implies that an antimutagenic effect could be achieved even if the compound is administered late after cessation of radiotherapy. Whether *p53* gene therapy (INGN201) [204] or *mdm2* inhibitors such as Nutlin-3 or MI-219 [205, 206] are useful approaches as a cleansing method following irradiation should be tested experimentally. Whether Bcl-2 inhibition (*i.e.* with oblimersen sodium [207]) during radiotherapy may also prove significant in promoting the death of mutated cells is also an interesting speculation. Levels of the anti-apoptotic Bcl-x1 protein have been shown to increase following irradiation promoting the aggressiveness of cancer cells [208, 209].

Non-homologous end joining (NHEJ) of DNA double-stranded breaks, an error-prone repair pathway, is common in mammalian cells compared with the error-free homologous recombination pathway [210]. The D-NHEJ depends on the activities of DNA-dependent protein kinase (DNA-PK) and DNA ligase IV/XRCC4/XLF, while B-NHEJ uses DNA ligase III/XRCC1, PARP-1 and histone H1 [211, 212]. Even partial deficiency of DNA-PKcs leads to increased ionising radiation induced mutagenesis (that is directly related to the level of deficiency) and telomere dysfunction [213]. High mobility group adenine–thymine-hook 2 protein (HMGA2) overexpression interferes with NHEJ processes, promoting genomic instability and tumorigenesis [214]. Vanillin derivatives, on the other hand, bearing important antimutagenic properties, selectively inhibit the activity of DNA-PK so that blockage of the NHEJ is considered to be the cause of its antimutagenic effect, presumably by shifting the balance towards homologous error-prone recombination [215]. Ribonucleoproteins (RNPs) are involved in the packaging, processing and export of pre-RNA molecules in the cytoplasm, and have an important role in gene regulation. A subclass of TNPs, heterogeneous nuclear ribonucleoproteins (hnRNPs), show either mRNA transcript or protein quantity changes following ionising radiation. A role for hnRNPs A1, A18, A2/B1, C1/C2, K and P2 in regulating double-stranded break repair pathways by promoting preferentially homologous recombination or NHEJ repair is implied [216]. This certainly represents a field of research to identify pathways involved in radiation carcinogenesis. The stimulation of homologous error-free recombination repair of double-strand breaks is a field for research for the development of Type D radioprotectants, and hnRNPs may represent a putative target.

Centrosome aberrations rapidly appear following irradiation of normal cells [217]. These are linked to prolonged cell-cycle arrest and are considered as an important mechanism involved in radiation-induced genomic instability, predisposing cells for the subsequent development of tumours. Exposure of human mammary epithelial cells to 2 Gy irradiation leads to an increase of centrosomal aberrations that are reduced when cells are treated for 8 post-irradiation days with TGF β [218]. This effect of TGF β is not related to a reduction of induced aberrations but to elimination of cells with established centrosomal aberrations. This

observation is interesting because a growth factor, namely TGF β , appears to be an important agent for the cleansing of cells with genomic instability. This effect was proved to be mediated by p53 activation, highlighting again the potential for eliminating mutated cells via this approach. Because TGF β is also a factor involved in Type II and III radiation sequelae, it is possible that anti-TGF β agents aiming to mitigate such radiation toxicities may instead enhance genomic instability, and that TGF β administration aiming to decrease genomic instability may enhance Type II/III toxicities. However, short-term administration of TGF β in the form of, for example, avotermin [219] for a short period after the cessation of Type II radiation sequelae may be feasible and could be tested at experimental level. Such an approach may also be significant in reducing the load of mutated cells after exposure to lower, Type II/III toxicity-free radiation doses such as those used in diagnostic radiology or following exposure during space missions.

Telomerases also appear to have an important role in radiation-induced genomic instability. Telomeric repeat binding factor (TRF-2) is a telomere-binding protein with roles in telomere length regulation, and is upregulated in certain tumours. In the absence of telomerase activity, TRF-2 becomes a potent oncogene accelerating epithelial carcinogenesis [220]. There is increasing evidence that many DNA double-strand break repair proteins, including Ku, DNA-PKcs, RAD51D and MRN, BRCA1, hRad9 and PARP1, are involved in telomere maintenance. TRF-2 is not confined to its telomeric environment but it may migrate to the sites of DNA breakage following radiation [221]. X-ray induces phosphorylation of TRF-2 and plays a functional role in DNA double-strand break repair [222]. There is, therefore, increasing evidence that telomere maintenance mechanisms constitute an integral part of DNA damage response machinery. NF- κ B, a transcription factor that becomes functionally activated upon radiation exposure, upregulates the telomerase activity by binding to the κ B-binding region in the promoter region of the *TERT* gene [223]. Enhancing NF- κ B, therefore, may protect genomic integrity in addition to protecting normal tissues against Type I toxicities.

Type E radioprotectants: protecting bystander cells

The exact molecular events defining the non-targeted effects of radiation remain poorly understood. This is an important field of research, given that understanding the pathways may help in the reduction of radiotherapy sequelae, enhance its antitumour efficacy and, most importantly, reduce the risk of secondary carcinomas.

Gap junctions allow the flow between cells of small molecules (1000–1500 Da) such as calcium ions, nucleotides and peptides, and this route is considered a major pathway of bystander effect manifestation within an organ [224, 225]. Targeting gap junctions and their constituent proteins, namely connexins, may prove important in blocking the bystander effects within a partially irradiated organ (*e.g.* the lung or liver). Gamma-hexachlorocyclohexane (lindane) induces gap junction endocytosis, a process that is activated by the ERK pathway [226]. TGF β -3 treatment downregulates connexin 43

and induces the ERK pathway [227]. Phorbol ester 12-O-tetradecanoylphorbol-13-acetate and chlorohydroxyfurones, by-products of drinking water chlorination, also appear to inhibit gap junction [228].

The long-range abscopal effects are postulated to be mediated through large molecules (1000–10 000 kDa) such as lipid peroxide products and cytokines (IL-1, IL-6, TGF β and TNF α). Such cytokines induce nitric oxide synthase 2 and increased nitric oxide content in target cells. Injection of Cu/Zn-SOD or nitric oxide synthase inhibitors such as L-NAME lead to reduced expression of bystander effects in experimental animals [229]. Macrophages appear to be the source of the long- and short-range bystander signals through cytokine production [230]. Whether abrogation of macrophage activation may have a role in protection against Type D radiation toxicities is an emerging hypothesis. Macrophage migration inhibitory factor (MIF) is a macrophage-produced cytokine that induces TNF α secretion and nitric oxide production, also contributing to the recruitment of leukocytes. Its activity can be blocked with monoclonal antibodies or by targeting MIF tautomerase activity using small molecules such as (*S,R*)-3-(4-hydroxyphenyl)-4, 5-dihydro-5-isoxazole acetic acid methyl ester [231]. Inhibitors of protein kinase-C, such as 1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride, also display important inhibitory activity on macrophage activation [232].

Because hypomethylation of DNA is a bystander effect of radiation [22] that may be important in genomic instability and carcinogenesis, agents that can restore this effect may also be useful Type V radioprotectants. CpG dinucleotides are major sites of DNA methylation in mammals. During somatic cell differentiation, DNA methylation occurs and represses germline-specific genes, a process catalysed by DNA methyltransferases (DNMTs). Demethylation is an important subsequent step in permitting expression of tissue-specific genes and occurs either by inhibition of DNMTs or as an active process involving the DNA repair-related demethylase [233]. The latter process is largely obscure, although it seems to be related to DNA repair machinery, including DNA glycosylases. Growth arrest and DNA-damage-inducible protein GADD45 α is a non-enzymatic factor involved in base excision repair that actively promotes DNA demethylation. Cytidine deaminase also appears to be involved, converting 5-methylcytosine into a thymine; this is followed by excision and replacement of methylated nucleotides. DNA demethylation correlates with extensive histone modification and exchange that is facilitated by histone chaperone proteins such as histone cell cycle regulation defective homolog A and nucleosome assembly protein. It is expected that explanation of the process of demethylation will help in the development of strategies to block radiation-induced demethylation in bystander cells.

Conclusion

It is evident that during the past decade a large number of experimental and clinical data providing new concepts have been accumulated, in addition to a large pool of chemical and molecular agents that can be effective in the protection and treatment of early and late

Table 2. Known agents with radioprotective activity

Radioprotectant type	Agent	Reference	Radioprotectant type	Agent	Reference			
Type A	A1	Hydroxytryptamine	Type C	C1	Tenovil (rhIL-10)			
		Amifostine			Dekavil (IL-10/F8 fusion)			
		Cobalt chloride			SeV- mediated transfer of IL-10 gene			
	Deferoxamine	IL-10 inducers (Y-40128, WY14643, reboxetine, edelfosine)						
	A2	Clioquinol			Infliximab (anti-TNF α)			
		Isofluran			Tolcumab (anti-IL6 Ab)			
		Okadaic acid			IL-6 blockers (A-285222, Baicalein)			
		Vanadate			Anakinra (IL-1 receptor antagonist)			
		Tilorone			Anti-CD54 Ab			
		Baicalein			Pravastatin			
	A3	FG-4497			VEGF blockers	C2		[124–126]
		Superoxide dismutases			TNP-470			[130]
		Glutathione			HIF blockers (PX-478, YC-1)			[131]
		N-acetyl-cysteine			TGF β blockers (naringenin, halogunginon, relaxin, SB-525334, SB203580, pirferidone, pentoxifylline)			[132, 133]
		Amifostine (WR-1065)			PDGFR inhibitors (imatinib, SU9518)			[140–148]
Fullerenols		Infliximab (anti-TNF α Ab)	[150, 151]					
Type B	B1	Cerium oxide	HGF gene transfer	[158]				
		Tempol	Retinoic acid	[170–172]				
		Amifostine (WR-33278)	Anti-bFGF	[173]				
	B2	Resveratrol	ACE inhibitors	[177]				
		Butin	COX inhibitors	[178]				
		Vectors with repair enzymes	Amifostine	[179]				
		Sodium orthovanadate	INGN201 (p53 gene therapy)	[191–194]				
		Antisense-PUMA	Mdm2 inhibitors (untl-1, MI-219)	[199]				
		Inhibitors of GSK-3 β	Oblimersen sodium (anti-bcl-2)	[200, 201]				
		HPV16 E5 viral protein	Vanillin derivatives	[202]				
Angiotensin receptor blockers	Avotermin (TGF β)	[209]						
Flagellin analogues	NF- κ B inducers	[213]						
Type C	A1	RTA401	Macrophage activation suppressors	[217]				
		Autophagy modulators	Amifostine and Type A agents	[218]				
		Haemopoietin growth factors	Type D agents					
		Keratinocyte growth factor	Gap junction inhibitors (lindane, TPA)	[226–228]				
		Becaplermin (rhPDGF-BB)	NOS-inhibitors (L-NAME)	[229]				
		Telbermin (rhVEGF)	Macrophage activation inhibitors	[231, 232]				
		FGF-P peptide	Demethylation targeting agents	[233]				
		FGF1:FGF2 chimeric GF						
		Velafermin (rhFGF-20)						
Type D	B1		Type D					
		Type E			B2		Type E	

ACE, angiotensin-converting-enzyme; COX, cyclooxygenase; FGF, fibroblast growth factor; GSK-3 β , glycogen synthase kinase 3-beta; HGF, hepatocyte growth factor; HIF, hypoxia inducible factor; HPV, human papillomavirus; IL, interleukin; NOS, nitric oxide synthase; PDGFR, platelet derived growth factor receptor; PUMA, p53 upregulated modulator of apoptosis; TGF, transforming growth factor; TNF, tumour necrosis factor; TNP, methionine aminopeptidase-2 (MetAP-2) inhibitor; VEGF, vascular endothelial growth factor.

radiation damage, as well as in the reduction of carcinogenesis following exposure to therapeutic or diagnostic ionising radiation. Where we stand in the clinical setting is rather disappointing. Amifostine, an important multitype radioprotective agent, is the only agent tested and officially approved for a very narrow spectrum of radiation toxicity; namely, radiation-induced xerostomia, regardless of the large amount of clinical data supporting its activity in the prevention of oesophagitis, pneumonitis and intestinal toxicity. Such indications, however, have been included in the clinical recommendation guidelines of certain organisations [234, 235].

Table 2 summarises the types of radiation damage and the corresponding agents expected to have a radioprotective role. It is obvious that many of the agents may be valuable for protection against multiple types of radiation damage, given that the underlying biological pathways overlap to a certain extent. It is also stressed that some agents, although protective against one type of radiation, can aggravate another type of damage; therefore, the time points of their usage may be a critical issue for clinical trials. Establishing central committees, recruiting experts in the field and absorbing significant funds from governmental and charitable agencies active in the field of cancer and radiation protection is required to sustain large-scale clinical experimentation with an aim to uncover the value of the plethora of agents with putative radioprotectant activity.

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