Radiation therapy is essential for local tumor control for many types of cancer histologies. Technological advancements in recent years have allowed for precise irradiation of target tissues while minimizing the dose to non-target tissues. To enhance radiation damage to cancer cells and further limit the radiation effects on normal tissue, researchers have explored compounds that specifically target cancer cells and make them more sensitive to ionizing radiation. Recent radiosensitization research has focused on promising compounds that alter hypoxia, inhibit topoisomerases, interfere with microtubules, and activate caspases, among other mechanisms. Many such compounds have shown impressive results in pre-clinical trials against a variety of cell types, but their safety, efficacy, and practicality in clinical trials remains to be demonstrated. This review seeks to provide an overview of recent research in radiosensitization, detailing some of the more successful compounds, and illustrating avenues for future research.

Local control is often of critical importance in the treatment of cancer. Radiation therapy is an essential tool in achieving local control for many different types of cancer histology. Modern radiation therapeutic technology allows for the delivery of high doses of radiation to a well-defined, conformal area involved with disease while minimizing the radiation dose to normal, non-target tissue. Despite great technological advancements in radiotherapy, however, the physics of radiation delivery often necessitate some degree of normal tissue irradiation. Compounds that increase cancer cell sensitivity to radiation (‘radiosensitizers’) have been used in an effort to optimize radiation therapy. The purpose of the present review is to summarize recent developments in radiosensitization, and to identify potential avenues for future research.

Hypoxia

Unregulated cell reproduction and growth is the hallmark of many malignancies, and as a result of this unregulated growth, tumors can often outgrow their blood supply and thus enter a relatively hypoxic state. Radioresistance due to hypoxia is a well-established problem in cancer treatment. Radiation therapy damages tumor DNA in a variety of ways, but an important and often predominant mechanism is the interaction of photons with water molecules to create reactive oxygen species. These species then damage tumor DNA, which leads to cell apoptosis, necrosis, or inability to divide if unrepaired. Oxygen in the microenvironment allows for the perpetuation of free radicals and is important in ‘fixing’ damaged DNA by formation of organic peroxides at damaged sites, which are then resistant to cellular repair mechanisms (1). The importance of oxygen in the tumor microenvironment is quantified by the oxygen enhancement ratio (OER) and varies based on the tumor histology and on the type of therapeutic radiation (2).

The hypoxic state also results in stabilization of a transcription factor known as hypoxia-inducible factor 1 (HIF1), which is normally quickly degraded under normoxic conditions (3). Activation of HIF1 is thought to lead to several downstream effects that seem to be associated with higher rates of metastasis and poorer survival, at least in some types of cancer (4).

Because of the adverse radiobiological and clinical consequences of hypoxia, improving tumor oxygenation or otherwise combating the hypoxic tumor microenvironment has been an oncological strategy for over 50 years. Trials of hyperbaric oxygen were some of the earliest to explore hypoxic manipulation, and although this intervention improved locoregional control and disease-specific survival, it has not translated into improvements in overall survival based on a recent meta-analysis of head and neck cancer trials (5). Other researchers have experimented with Carbogen, a mixture of
95% oxygen and 5% carbon dioxide, to increase oxygen concentrations in tumors (6, 7). This strategy is usually used in conjunction with nicotinamide, which is an inhibitor of poly ADP-ribose polymerase 1, an important single-strand break repair enzyme; in models, nicotinamide has been shown to be a radiosensitizer (8, 9). Despite theoretical promise, however, results from trials exploring Carbogen with and without nicotinamide have been disappointing (5). Other strategies have included administration of tirapazamine, which is a benzoazain-class molecule that can be reduced to a cytotoxic free-radical species and can also interact with platinum-based chemotherapy agents in hypoxic cells, resulting in synergistic DNA damage (10). Two phase III trials of tirapazamine with radiation and cisplatin have disappointed: one showed no survival advantage (11) and the other was closed early due to concerns about disproportionate mortality in the tirapazamine arm (12).

Other manipulations of hypoxia-related pathways are in various stages of development. Li and colleagues have recently shown that β-elemene, a molecule occurring in Curcuma wenyujin, a traditional Chinese medicine, improved radiosensitivity of human lung adenocarcinoma cells in mouse models by reducing expression of HIF1α (13). Ning and colleagues have shown that dinitroazetidines, a class of compounds developed in the aerospace sector, trigger apoptosis via free radical cascades and have higher cytotoxicity than cisplatinum with no appreciable systemic toxicity in murine models (14). Although the mechanism of action is not yet understood, these compounds are activated by both ionizing radiation and hypoxic conditions, making them attractive agents for combined-modality oncological therapy.

Nitroimidazoles have long been known to select for hypoxic cells and increase DNA damage caused by ionizing radiation (15). After reduction, these compounds act similarly to oxygen, stabilizing damaged DNA so that it is difficult to repair; because oxygen reverses the initial reduction reaction, these compounds are selectively activated in hypoxic environments (15, 16). This concept was refined to conceive and then synthesize molecules with two reduction-oxidation loci with different potentials, so-called 'bi-bioreductive' molecules (17). Although the utilization of two nitroimidazoles for this purpose was abandoned due to in vivo practicability (17), other researchers have used a combination of a nitroimidazole and a quinone for bi-bioreductives (bypassing some of the practical issues with dinitroimidazoles, such as solubility) with good effect, although these studies are in the early stages (16).

**Topoisomerase Inhibition**

Topoisomerase enzymes are essential for human DNA replication and transcription. Topoisomerases allow for the unwinding of supercoiled and coiled DNA during replication by catalyzing transient single-strand breaks via formation of a phosphotyrosine bond (18). The first topoisomerase inhibitor studied was camptothecin, derived from a closely related but inactive compound from the Chinese tree *Camptotheca acuminata* (19). Camptothecin and its related compounds are thought to cause cytotoxic damage by: (a) stabilizing the topoisomerase/DNA interaction, leading to a collision with the DNA replication fork and a subsequent double-strand break (20); (b) terminating RNA synthesis prematurely (21); and (c) interacting with cell-cycle checkpoints to induce apoptosis (22). Camptothecin and its derivatives also seem to induce a finite period of radiosensitivity distinct from their directly cytotoxic effects, although details about the nature and mechanism of this radiosensitization remain elusive (19).

Despite its potent antitumor effects, camptothecin was found to be too toxic for practical use (23). Other camptothecin derivatives have been studied, most notably irinotecan and topotecan. These compounds have antitumor activity but also have significant toxicity at clinically effective doses and are currently approved only for recurrent or metastatic malignancies, although trials are ongoing (25, 26). As some researchers have pointed out, the toxicity of these two camptothecin analogs is due to the instability of the lactone ring within the molecule (18). Subsequent stabilization of the lactone ring in a compound known as CPT417 revealed topoisomerase inhibition with better tumor inhibition than topotecan and negligible toxicity in studies of mammary adenocarcinoma in mice; this compound has the advantages of being inexpensively and simply synthesized, and orally bioavailable (18). Clinical trials of CPT417 are currently underway.

Other camptothecin-related molecules are in preclinical development. Substitution of hydrophilic moieties at the 7-position of the molecule, the so-called 7-oxyliminomethyl derivatives, have had antitumoric effects against several human xenografts (26). Other researchers have substituted a difluoromethylene focus in the camptothecin core (BMS-286309), increasing stability and bioavailability; in vitro and in vivo experiments comparing this molecule to irinotecan have shown similar antitumoric effects but lower toxicity with BMS-286309 (27).

**Microtubule Manipulation**

Interfering with microtubule function has been a longstanding oncological treatment strategy: stabilizing microtubule formation leads to cell-cycle arrest and eventual cell death. Microtubule stabilizers are additionally viewed as radiosensitizers as they often arrest cells in the G2-M stage, which is the most radiosensitive phase of the cell cycle (28). Taxanes are the most developed and widely-used class of microtubule stabilizers, but clinical utility is often limited by
neurotoxicity, as well as the existence of multidrug-resistance genes, enabling tumors to efflux drug, among other resistance mechanisms (29). Researchers have explored non-taxane microtubule stabilizers in an attempt to overcome resistance and achieve a more-favorable toxicity profile. One of the most promising of such agents is patupilone (EPO906, epothilone B) (28). Patupilone has been shown to overcome resistance with no neurotoxicity in human colonic adenocarcinoma xenografts (28) and has also been shown to be synergistic with radiation therapy in human medulloblastoma xenografts (30). Another microtubule disrupter that is promising in its pre-clinical results is pyrrolo-1,5-benzoxazepine-15 (PBOX-15) (31). Rather than stabilizing polymerized microtubules, the PBOX family of compounds affect the depolymerization of microtubules and initiate apoptotic pathways. Pre-clinical studies on prostate, glioma, and lung cancer cells show that PBOX-15 remains cytotoxic in tumors with multidrug-resistance genes, overcomes hypoxic effects, and works synergistically with ionizing radiation therapy (31).

### Second Mitochondria-derived Activator of Caspase (SMAC) Mimetics

There are two distinct apoptotic pathways based on internal or external cellular stimulations, but both pathways result in the activation of a family of proteases known as caspases. Damage from chemotherapeutics and ionizing radiation primarily effect the intrinsic pathway (32). In non-apoptotic states, caspases are regulated by inhibitor of apoptosis proteins (IAPs) (32, 33). When internal cellular signals initiate the intrinsic apoptotic pathway, however, the mitochondria release several molecules, including SMAC. SMAC antagonizes the IAPs, removing caspase inhibition and allowing the cell to proceed with apoptosis (33).

SMAC-mimetics have been studied as monotherapeutic agents against a variety of malignant histological types, but the resistance of some cell types to SMAC mimetics has led to their use in combination with ionizing radiation as radiosensitizers, such that caspase activation would theoretically be enhanced by the additional pro-apoptotic signaling caused by ionizing radiation (33-36). Tat-SMACN7, one such SMAC mimic, was equally potent at cell killing *in vitro* and *in vivo* in both SMAC-resistant and SMAC-sensitive esophageal and non-small cell lung cancer cells (33). Similar studies of SMAC-resistant and SMAC-sensitive breast cancer cell lines were found to be equally susceptible to the combination of SM-164 (another SMAC mimic) and radiation therapy (34); other studies with SM-164 showed it to be an effective radiosensitizer in head and neck squamous carcinoma cell lines in xenografts (35). Studies using LCL161, also a SMAC mimic, in combination with radiation found not only that this compound worked synergistically with irradiation to induce apoptosis in esophageal squamous cell cancer lines, but also seemed to promote the extrinsic apoptotic pathway, based on increased levels of tumor necrosis factor-α (36).

### Targeted-Agents

Many molecularly targeted-agents have shown great promise as radiosensitizers, through a variety of pathways. Sorafenib is an inhibitor of various cell-surface and intracellular kinases involved in cellular and tumoral growth, including tyrosine kinases of vascular endothelial growth factor (VEGF) receptor and platelet-derived growth factor receptor. Rapidly Accelerated Fibrosarcoma (RAF) kinases, and serine/threonine kinases (37). Sorafenib has also been shown to sensitize hepatocellular carcinoma (HCC) cells to radiation: in a study of four radioresistant HCC cell lines, sorafenib combined with radiation led to kinase-independent enhancement in radiation-induced apoptosis through down-regulation of phospho-Signal transducer and activator of transcription 3 (STAT3), a protein thought to be critical in HCC progression; radiosensitizing effects were shown *in vitro* and *in vivo* with HCC xenografts in nude mice (38). The clinical application of sorafenib with radiation in humans has been demonstrated in a study of 31 patients with advanced HCC treated with sorafenib and radiotherapy, with a primary tumor response rate of 100%, with acceptable toxicity (39). A phase II trial of radiation therapy and sorafenib in 40 patients with unresectable HCC showed a less robust but still promising tumor response rate in 55% of patients, although there were three treatment-related fatalities (40). Further studies are needed to assess the translational benefits of sorafenib as radiation sensitizer.

Erlotinib is a small-molecule reversible inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinases and has been used against a variety of malignancies, including non-small cell lung cancer, pancreatic cancer, and even some non-malignant processes such as myeloproliferative disorders (41). Erlotinib also has a breadth of radiosensitizing mechanisms, including reduction in fraction of cells in the S-phase of mitosis, inhibiting expression of radiation response genes such as *Rad51*, and the enhancement of radiation-induced apoptosis by blocking EGFR signaling (42). The clinical applicability of erlotinib as a radiosensitizer has been shown to be very promising in phase II trials of patients with locally advanced cervical cancer (43) and brain metastases from non-small cell lung cancer (44), both of which demonstrated excellent response rates with tolerable toxicity. Cetuximab, a recombinant monoclonal antibody inhibitor of EGFR tyrosine kinase, is thought to lead to radiosensitivity through mechanisms similar to those of erlotinib and has been studied with radiation in patients with head and neck cancers (45), locally advanced rectal cancer (46), and non-small cell lung cancer.
Although cetuximab with radiation was shown to improve locoregional control of head and neck cancer and reduce mortality compared to radiation-alone (45), no survival advantage was found in patients with lung cancer when cetuximab was added to concurrent chemoradiation with carboplatin (47). Further studies are needed to optimize the specific contexts and treatment regimens in which cetuximab has a role.

Other monoclonal antibodies have been used as radiosensitizers. Bevacizumab, a recombinant antibody against the angiogenesis stimulator VEGFA, has been used without radiation for a variety of malignancies, including colorectal, lung, breast, renal, and central nervous system tumors. Recently, bevacizumab has shown great promise as a radiosensitizing agent in trials of patients with locally advanced cervical cancer (48) and locally advanced head and neck cancer (49). Although the mechanism of radiosensitization is incompletely understood, it has been suggested that bevacizumab leads to a somewhat counterintuitive normalization of chaotic tumor vasculature, allowing for increased perfusion and improved oxygenation in the tumor microenvironment, which in turn enhances radiosensitivity (50).

Monoclonal antibodies against human epidermal growth factor receptor 2 (HER2), such as trastuzumab, have significantly improved outcomes for patients with breast cancer with an overexpression of this cell membrane receptor (51). These agents may also have a role in the treatment of esophageal, gastric, and other malignancies (52). Investigators have recently shown trastuzumab to have radiosensitizing activity both alone and synergistically with concomitant gefitinib (a small molecule inhibitor of HER2 and other EGFRs), seemingly through reduction in radiation-induced phosphorylation of EGFR, Methyl ethyl ketone 1/2 (MEK1/2) and protein kinase B (Akt), although these pathways are incompletely understood (53). The activity of trastuzumab as a radiation sensitizer has been also tested in humans, as a prospective trial of 308 patients with non-metastatic breast cancer treated with surgery with or without neoadjuvant chemotherapy, followed by adjuvant radiation and trastuzumab (54). In that study, the 4-year locoregional control and overall survival rates were 95% and 98%, respectively, with acceptable toxicities. More studies of this and other HER-2/neu agents are needed to elucidate their radiosensitization mechanism(s) and evaluate their clinical risks and benefits.

Photosensitizers

Radiosensitizing compounds have also been used with non-ionizing radiation, such as light in the 600-800 nm spectrum of wavelengths, to treat cancer as a modality known as photodynamic therapy (PDT). These ‘photosensitizers’ absorb photons of a certain wavelength and enter an excited state that can interact with O₂ to produce singlet oxygen, leading to free radical cascade and oxidative cytotoxicity (55). The most widely-used photosensitizers have been porphyrin-related compounds and derivatives, which are heterocyclic tetraters of pyrrrole molecules (56). Because of some disadvantages to first-generation porphyrins that included suboptimal efficacy as well as inconvenient toxicity profiles, second-generation porphyrins were developed, including reduced porphyrins called chlorins as well as porphyrins with metal ions within the heterocyclic ring known as phthalocyanines (55, 57). Additional porphyrin-related compounds in pre-clinical or clinical stages of development include synthetic porphyrins called porphycenes (58), and pro-porphyrins such as 5-aminolevulinic acids and esters, which are converted into porphyrins by endogenous cytosolic enzymes (59).

Although photosensitizers in the context of PDT have been studied in esophageal (60), lung (60, 61), and other malignancies, PDT is currently reserved as a complementary therapy or for patients who are not candidates for surgery, chemotherapy, and radiation.

Other Directions

There are myriad other mechanisms being explored for radiosensitization. Curcumin, an important constituent of the spice, known as turmeric, has long been known as a chemopreventative, but researchers have recently shown that it is also a potent radiosensitizer of cervical cancer cell lines. Although the precise mechanism is unclear, curcumin in combination with ionizing radiation leads to an increase in reactive oxygen species, which in turn leads to non-apoptotic cell death via sustained activation of extracellular signal-related kinase-1/-2, at least in some cell types (62). Hydroxychalcones are another type of naturally occurring compound present in many types of plant matter; these compounds have been shown to have antitumoric as well as radiosensitization effects on colonic and pancreatic tumor lines (63). The mechanism in this case is ‘proteotoxicity’ via the hydroxychalcone activation of heat-shock factor 1, resulting in heat-shock cascade, and apoptosis (63). Boronic chalcones also have radiosensitizing effects, but their mechanism is distinct from that of hydroxychalcones. A study of one boronic chalcone (AM114) in colonic cancer cells showed cytotoxicity and radiosensitization that resulted from interference with the 20S subunit of the proteasome, leading to cell death due to the accumulation of intracellular p53 and p21 (64).

Conclusion

Radiation therapy is essential in local control of many, if not most, cancer histologies. Technological advances such as computed tomography-based planning, intensity-modulated radiation therapy, image-guided radiation therapy, and
stereotactic radiosurgery have allowed for precise irradiation of target volumes while minimizing the dose to normal, non-target tissue. The physics of radiation delivery, however, do not allow for the complete avoidance of normal tissue, at least with current technology. The exploitation of tumor cell cycle, metabolic, and microenvironmental properties using radiosensitizing compounds can selectively enhance damage to cancerous cells. This review has explored some of the recent research into and application of these compounds, that continue to be a promising avenue through which we can maximize the benefits of radiation therapy.

References


