Recent studies have elucidated some of the molecular and cellular mechanisms that determine the sensitivity or resistance to ionizing radiation. These findings ultimately may be useful in devising new strategies to improve the

Introduction

Clinical and experimental studies of the acute and late effects of radiation and chemotherapy at the levels of tissues, organs, and gross pathology have enhanced our knowledge of how normal tissues and tumors respond to these agents. These studies have led to the optimization of radiation treatment schedules and to more precise modes of radiation delivery.

Improvements in radiation delivery include hyperfractionated radiotherapy (ie, smaller dose fractions delivered two or three times per day); computerized treatment planning and the use of multiple noncoplanar radiation portals (three-dimensional conformal radiotherapy) to reduce the volume of normal tissue irradiated; intensity-modulated radiation therapy, a technique designed to reduce the radiation dose to surrounding normal tissue for a given dose to the tumor volume; stereotactic radiosurgery and radiotherapy, allowing the delivery of single-dose or fractionated radiotherapy to precisely defined target volumes in the brain; improved combinations of radiotherapy and chemotherapy; and precisely targeted particle-beam therapy (eg, proton-beam treatment).[56-59] Given the state of the art, further refinements of this type are likely to lead to incremental rather than quantum improvements in cancer treatment.

If significant improvements in treatment outcome are to be achieved, they are more likely to result from the clinical application of the relative explosion in knowledge of the molecular mechanisms of the cell response to stress, including genotoxic agents, such as ionizing radiation and many chemotherapeutic drugs. The major sources of this knowledge have included: (1) identification of a series of yeast genes, and their mammalian counterparts, for which mutation or loss result in radiosensitivity; (2) studies of genetic mechanisms and cellular consequences of tumor-suppressor gene mutations (eg, p53, ATM, BRCA1/2, DNA-PK); (3) the discovery of the role of growth factor/cytokine signal transduction and oncogene activation in modulating radiosensitivity; and (4) elucidation of the molecular mechanisms of apoptosis, a form of programmed cell death. The first part of this two-part review, published in last month’s issue, summarized the clinical and tissue kinetic factors that govern the sensitivity of normal tissues to ionizing radiation. This second part will characterize recent insights into the cellular and molecular pathways that determine the sensitivity of normal and tumor cells to ionizing radiation.

Factors Regulating Apoptosis

Mutations of tumor-suppressor genes, some of which are found in the human counterparts of yeast radiation response genes, and oncogene activation occur predominantly in malignant tumors,[60-64] while growth factor/cytokine signal transduction and apoptosis occur in normal development, tissue response to injury, as well as benign and malignant tumors.[61-64] Apoptosis, the major form of programmed cell death in animal cells, is a genetically programmed cascade of events activated in response to cell stress.[66-68] Apoptosis is characterized by nuclear DNA fragmentation, chromatin condensation, and a characteristic cytoplasmic and nuclear morphology, before the cell is eliminated by phagocytosis.

Apoptosis is an important mechanism by which radiation and chemotherapy kill cells.[69-71] As will become evident from the discussion below, tumor-suppressor mutations, oncogene activation, and growth factor signaling all modulate the induction of apoptosis by ionizing radiation. Thus, knowledge of the factors that regulate apoptosis is of paramount importance in understanding the molecular basis of the tumor and normal tissue response to radiation.
A genetic framework for understanding apoptosis in mammalian cells has been established by the study of the cell death (CED) genes of the nematode Caenorhabditis (C) elegans.[67,68] Three of the major CED gene products (and their human homologs) are CED-9 (Bcl-2), CED-4 (Apaf-1), and CED-3 (caspase-3). CED-9/Bcl-2, a pore-forming protein of the outer mitochondrial membrane, is thought to block apoptosis by binding to CED-4/Apaf-1 and/or preventing the release of cytochrome c from mitochondria. The apoptosis-activating factors (Apafs 1-3) are recently identified factors that mediate deoxyadenosine triphosphate (dATP)/cytochrome c-dependent cleavage of procaspases into active enzymes (see below).

The apoptotic cascade (illustrated in Figure 1) results in the activation of an enzyme (flipase) that causes the translocation of phosphatidylserine from the inner to the outer cell membrane. The phosphatidylserine on the cell surface is recognized by annexins on the surface of macrophages, resulting in the elimination of apoptotic cells by phagocytosis, so that they do not generate an inflammatory response.

**DNA Damage–Activated Signaling Pathways**

Progress in understanding how DNA damage is signaled to the cellular response machinery has been greatly facilitated by the identification of a group of yeast genes whose mutation results in enhanced radiosensitivity. These genes encode protein kinases and kinase targets, the activation of which by DNA damage ultimately results in cell-cycle arrest and the transcription of DNA repair genes.

**Roles of ATM and Structurally Related Proteins**

In one pathway, activation of yeast phosphatidylinositol (PI)-related kinases MEC1 and TEL1 by damaged DNA results in phosphorylation of the Rad9 protein, its association with the protein kinase Rad53, and the downstream events of cell-cycle arrest and induction of DNA repair genes (Figure 2).[72-74] The human homolog of MEC1 is ATM, the product of the ataxia-telangiectasia susceptibility gene (ataxia-telangiectasia mutated, or ATM), located on human chromosome 11q22-23.[75] This gene encodes a 350-kD protein containing a PI3 kinase-like domain. PI3 kinase is a substrate involved in signal transduction from growth factor receptors; mammalian and yeast proteins that share the PI3K domain are involved in meiotic recombination and DNA damage responses, as well as cell-cycle control.[76]

ATM also appears to relay information from DNA damaged by ionizing radiation to the p53 tumor-suppressor gene (Figure 3).[77] Thus, cells lacking ATM are defective in the induction of p53 by radiation.

Finally, a pathway linking DNA damage to mitotic arrest through the human homolog of the yeast protein CHK1 has recently been elucidated.[78,79] Like the pathway for ATM signaling to p53, the CHK1 pathway involves 14-3-3, a group of proteins that participate in growth factor receptor signaling and in regulating apoptosis.[80] The human 14-3-3 signaling proteins are the homologs of two yeast proteins implicated in regulation of the response to radiation, Rad24 and Rad25.

The mechanisms by which ATM regulates radiosensitivity and chromosome stability are not well established. However, studies using ATM deletion mutants suggest that the C-terminal PI3K domain is required for maintaining S-phase arrest, chromosomal stability, and normal radiosensitivity following ionizing radiation.[81] These functions of ATM also require protein interactions mediated through the leucine zipper region of the protein.

A recent study further suggests that cleavage of ATM by the protease caspase-3, which is activated during apoptosis, blocks its protein kinase activity but not its DNA-binding activity. The kinase-inactivated ATM then acts as a dominant inhibitor of DNA damage signaling and repair.[82] These findings suggest that signal transduction in the nucleus involving the ATM protein may play a major role in limiting damage from ionizing radiation. The mechanism may involve delaying cells with severe chromosomal damage from exiting the S-phase before the damage is repaired.

Most ATM mutations result in inactivation or loss of the protein.[83] It is interesting to speculate whether there exist more subtle alterations of the ATM gene, polymorphisms, or defective ATM-binding factors that account for some of the individual heterogeneity in the response to radiotherapy (see part 1).

Two other proteins structurally related to ATM are the ATM-related (ATR) protein and DNA-dependent protein kinase (DNA-PK). Cells defective in ATM or ATR are hypersensitive to ionizing but not ultraviolet radiation.[84,85] DNA-PK, a nuclear serine/threonine kinase that binds to and is activated by double-strand DNA breaks, is a multiprotein complex consisting of a 470-kD catalytic subunit and a dimeric regulatory subunit (Ku-70 and Ku-80) with DNA-binding activity. DNA-PK has been implicated in genomic surveillance, detection and signaling of DNA damage, and cell-cycle control.
The radiation-sensitive rodent cell line CHO xrs-6 is defective in its ability to repair double-stranded DNA breaks induced by ionizing radiation, and the human Ku-80 (XRCC5) gene product corrects this defect. A mutation in DNA-PK catalytic subunit is responsible for the murine severe combined immunodeficiency defect, characterized by a defect in V(D)J recombination during B- and T-cell development, the inability to repair double-stranded DNA breaks, and increased radiosensitivity. These findings implicate DNA-PK in genetic recombination.

**p53 Mediation of Cellular Response to DNA Damage**

Exposure to x-rays results in single-and double-stranded breaks of the sugar-phosphate backbone of DNA. The mechanisms by which cells sense this damage are not well understood. Although the proximate sensor molecule has not been identified, later events within this process include the molecular association of p53 protein, several protein kinases (c-Abl and ATM), and the broken ends of the DNA. As a result, p53 protein is activated, its half-life increases, and a nuclear signal is generated that leads to various biochemical responses. These biochemical responses determine whether the cell enters into apoptosis or a survival/repair pathway: the cell fate decision (Figure 3). The factors that determine this decision may include the type and extent of damage, genetic factors, and cellular context (ie, environmental factors).

The p53 protein is a 393-amino acid phosphoprotein containing distinct domains that mediate transcription activation, sequence-specific DNA binding, recognition of DNA damage, activation of the p53 molecule, and protein-protein interactions (eg, oligomerization and binding to transcription factors or DNA repair proteins). Activation of p53 following irradiation leads to its: (1) accumulation due, mainly, to an increase in nuclear half-life; and (2) binding to specific DNA sequences within the regulatory regions of certain genes, resulting in transcriptional activation or silencing.[reviewed in reference 60]

Transcription activation mediated by p53 involves the binding of p53 to a consensus DNA sequence (PuPuPuC(A/G) repeated four times) in target genes. These target genes encode proteins that: (1) push cells into apoptosis [Bax (the inhibitory binding partner of Bcl-2) and IGF-BP3 (which binds insulin-like growth factor 1 [IGF-1] and prevents it from activating an antiapoptotic signaling pathway, see below)]; (2) promote cell-cycle arrest and DNA repair (p21WAF1/CIP1 and Gadd45); and (3) bind to and inhibit the transactivational activity of p53 (Mdm2).[88-92]

**Cell-Cycle Checkpoints**

Ionizing radiation can induce cell-cycle arrest or delay in G1, S, and G2.[93] The mechanisms that allow cells to respond to DNA damage by arresting in a compartment in which the damage can be repaired or limited are called DNA-damage cell-cycle checkpoints.[reviewed in references 61 and 62] Checkpoint mechanisms are major determinants of the cellular response to DNA-damaging agents, including radiation and chemotherapy.

For example, p53 gene mutations in cultured Burkitt’s lymphoma cells resulted in loss of the ability of these cells to arrest in late G1 in a manner normally mediated by the p53-dependent G1 cyclin-dependent kinase inhibitor p21WAF1/CIP1.[90] These cells became two- to threefold more resistant to radiation, most likely because of loss of the ability of mutant p53 to induce apoptosis.[94]

On the other hand, loss of the G2 DNA damage checkpoint renders lymphoma and carcinoma cells more sensitive to the cytotoxic actions of radiation and chemotherapeutic agents. Thus, treatment of cells with agents that inhibit the G2 checkpoint (eg, pentoxifylline [Trental] or UCN-01 [7-hydroxy-staurosporine]) resulted in a 1.8- to 2.8-fold increase in sensitivity to gamma radiation.[95,96] The rapid entry of cells with DNA damage into mitosis resulted in further chromosomal damage, chromosome loss, and cell death.

MCF-7 human breast cancer cells with wild-type p53 were resistant to UCN-01-mediated abrogation of the G2 checkpoint. These cells were less sensitive to DNA damage than cells with a dominant negative mutant p53, probably reflecting a contribution of p53 to the G2 checkpoint in this cell type.[95]

Conversely, transient overexpression of the Bcr-Abl kinase conferred resistance to apoptosis and reduced radio-sensitivity on lymphoma cells.[97] Bcr-Abl is an oncogenic form of c-Abl, formed by the 9:22 chromosomal translocation in Philadelphia chromosome 1-positive chronic myelogenous leukemia. The c-Abl gene product, a nuclear tyrosine kinase, has been implicated in the activation of p53 following DNA damage.[98] The radioprotective action of Bcr-Abl appeared to be mediated by a prolonged G2 cell-cycle block.[97]
The DNA damage-responsive G₂ checkpoint can be activated even at very low doses of radiation (<100 cGy).[93] Arrest of the cell cycle at G₂ is mediated by inhibition of cyclin B1 expression and/or enhanced tyrosine phosphorylation of p34cdc2.[93,97] Since mammalian cells arrest in G₂ even if they lack functional p53, it is clear that there is another G₂ checkpoint protein(s) capable of recognizing radiation-induced DNA damage and initiating the cellular responses. In yeast, the Rad9 and CHK1/Rad27 proteins mediate these functions.[see reference 61 and references contained therein] Thus, lack of functional Rad9 in Saccharomyces cerevisiae results in a defect in the ability to arrest in G₂ and in increased sensitivity to x-rays.[99] The mammalian counterpart of one of these proteins (CHK1) was identified recently (see above).

Resistance to Apoptosis

Resistance to apoptosis appears to play a significant role in the chemoresistance and radioresistance of tumors[reviewed in references 69-71] and may explain, in part, why the two forms of treatment resistance often occur together. The p53 gene induces apoptosis by a mechanism involving the regulation of the transcription of genes that encode apoptosis-regulatory proteins and by a mechanism that does not require gene transcription.[100] Probably the most important of these mechanisms is the upregulation of the proapoptotic protein Bax and the downregulation of its antiapoptotic binding partner Bcl-2.[70,88,101]

Most p53 mutations are point mutations in the DNA-binding domain, resulting in a mutant protein defective in sequence-specific DNA binding.[102] Such mutant p53 proteins invariably fail to activate the Bax promoter, although they often exhibit transactivation activity for other p53-regulated genes.[103]

The loss of the ability to transactivate the Bax gene may explain why so many human cancers (approximately 50%) contain p53 mutations. It may also account for the low levels of Bax found in many human breast cancers and breast carcinoma cell lines,[104] and it may help to understand how certain colon cancers of the microsatellite mutator phenotype (MMP) develop, despite the presence of wild-type p53. These tumors frequently contain frameshift mutations in the Bax gene.[105] Thus, the induction of wild-type p53 would lead to production of a defective Bax protein. It is not known whether the MMP phenotype is associated with clinical radioresistance in tumor or normal tissue cells.

p53 and DNA repair

The p53 gene was indirectly implicated in DNA repair based on its ability to induce Gadd 45 and p21/WAF1/CIP1 expression. Recent studies suggest a direct role for p53 in nucleotide excision repair and also in base excision repair.[106,107] Wild-type (but not mutant) p53 modulated nucleotide excision repair associated with the RNA polymerase II basal transcription factor TFIIH, via protein interactions with DNA helicases ERCC2 and ERCC3. These proteins are encoded by two xeroderma pigmentosum (XP) complementation genes, XP-B and XP-D, respectively. Cells defective for the XP-B and XP-D helicases were also defective in p53-mediated apoptosis. Xeroderma pigmentosum is an autosomal-recessive disorder characterized by extreme sensitivity to sunlight, premature aging of the skin, a high risk of skin cancer, and a defect in nucleotide excision repair. This phenotype may be due to mutations in various genes involved in repair of ultraviolet radiation induced DNA damage.

Although the XP genes have not been convincingly linked to clinical radiosensitivity,[108,109] cells with a defect in ERCC1/ERCC4 (XP-F) complex-mediated 5’-endonuclease activity are unusually sensitive to gamma rays under hypoxic conditions. This increased hypoxic radiosensitivity involves a mechanism distinct from nucleotide excision repair, possibly the formation of radiation-induced DNA-protein cross-links.[110] The x-ray repair cross-complementing gene 1 (XRCC-1) product was linked to the repair of ionizing radiation-induced single-strand DNA breaks and to maintenance of the kinetics of sister chromatid exchanges.[111]

The exact function of XRCC-1 is unknown, but some studies suggest that XRCC-1 may interact with DNA polymerase-beta, poly (adenosine diphosphate-ribose) polymerase (PARP), and DNA ligase III to form a protein complex that functions as a DNA [n]ick sensor.[112] Interestingly, PARP is a target of caspases during apoptosis (see below). The role of XRCC-1 in clinical radiosensitivity is not established. Polymorphisms of the XRCC-1 gene and levels of XRCC-1 messenger RNA do not correlate with the radiosensitivity of head and neck tumor cell lines.[113]

BRCA Genes and the DNA Damage Response

Most hereditary breast and ovary cancers (approximately 80%) are linked to mutations of the BRCA1
Both BRCA1 and BRCA2 participate in cell-cycle regulation, apoptosis, and DNA repair and recombination pathways. The BRCA1 gene contains a C-terminal module (BRCT domain) homologous to those of many DNA repair and checkpoint proteins. BRCA1 and BRCA2 associate and colocalize with the DNA recombinase Rad51. Immediately after exposure to DNA-damaging agents, BRCA1 becomes hyperphosphorylated and translocates with BRCA2 and Rad51 to sites of damaged DNA containing proliferating cell nuclear antigen (PCNA), suggesting roles for these proteins in DNA damage signaling and repair.

BRCA1 (-/-) murine fibroblasts are defective in transcription-coupled repair of oxidative DNA damage and are hypersensitive to ionizing radiation. BRCA1 expression was increased in ovarian cancer cells selected for resistance to the alkylating agent, cisplatin. However, unregulated expression of wild-type BRCA1 inhibited the repair of DNA breaks induced by doxorubicin and rendered human prostate cancer cells hypersensitive to doxorubicin and ionizing radiation. Treatment of radioresistant H-ras transformed rat embryo fibroblasts with one such compound resulted in radiosensitization of these cells and increased apoptosis following irradiation.

Functional activation of the H-Ras oncogene and human papilloma virus 16-E7 gene cooperated with mutant p53 in the establishment of a radioresistant cell phenotype. The radioresistance was associated with tumorigenicity, but there was no correlation between radioresistance and spontaneous metastases in tumorigenic rat embryo fibroblast cell clones. In a prior study, it was possible to separate transformation by Ras from induction of radioresistance, suggesting that the acquisition of radioresistance is distinct from that of other phenotypic characteristics of tumor progression.

In studies of rat embryo fibroblasts, the activated H-Ras oncogene binds directly to Rad51, via 8 conserved BRC motifs encoded by exon 11. Interestingly, transgenic mice homozygous for a mutant BRCA2 truncated within exon 11 survived to adulthood. These mice exhibited abnormalities of growth and development, a high incidence of T-cell lymphomas, increased repair of double-stranded DNA breaks, and radioresensitivity. Defective repair of DNA strand breaks induced by radiation or chemotherapy drugs (eg, etoposide) was also documented in human cells with BRCA2 mutations.

Breast cancers from patients with mutations of BRCA1 or BRCA2 had a two- to threefold increase in chromosomal loss compared to sporadic cancers, suggesting a role for these genes in the maintenance of genomic integrity. These observations suggest that like p53 mutations, BRCA1/2 mutations may lead to tolerance to certain types of DNA damage, permitting the survival of cells with defective genomes and, thus, promoting carcinogenesis and tumor progression. In a mouse model, conditional inactivation of BRCA1 resulted in genetic instability and mammary tumorigenesis that was accelerated in a p53 null genetic background. Mutations of BRCA1/2 may also confer abnormal sensitivity to therapeutic x-rays and DNA-damaging drugs, but it is not known whether the normal tissues of individuals with these mutations are abnormally sensitive to x-rays or chemotherapy.

**Oncogene Activation and Radiosensitivity**

Although oncogene activation is usually thought of as a mechanism of cell transformation, it may also result in significant alterations in radiosensitivity. For example, expression of activated p21Ras, a cytoplasmic guanosine triphosphatase (GTPase) involved in growth factor signal transduction, renders cells more resistant to ionizing radiation. This finding may be related to activation of an anti-apoptotic pathway downstream of p21Ras. In studies of rat embryo fibroblasts, the activated H-Ras oncogene and human papilloma virus 16-E7 gene cooperated with mutant p53 in the establishment of a radioresistant cell phenotype. The radioresistance was associated with tumorigenicity, but there was no correlation between radioresistance and spontaneous metastases in tumorigenic rat embryo fibroblast cell clones. In a prior study, it was possible to separate transformation by Ras from induction of radioresistance, suggesting that the acquisition of radioresistance is distinct from that of other phenotypic characteristics of tumor progression.

Functional activation of the H-Ras protein requires three steps: farnesylation, proteolysis, and methylation. Farnesyl transferase inhibitors have been developed to target Ras activation in tumors. Treatment of radioresistant H-ras transformed rat embryo fibroblasts with one such compound resulted in radiosensitization of these cells and increased apoptosis following irradiation. However, FTI-277 did not affect the radiosensitivity of untransformed control cells. Thus, targeting of Ras activation might be a clinically useful method to achieve differential
radiosensitization of ras-dependent cancers relative to the surrounding normal tissues.

Myc

The c-Myc proto-oncogene encodes a transcription factor involved in the regulation of cell proliferation, differentiation, and transformation. Under certain conditions (e.g., growth factor deprivation), expression of c-Myc can induce apoptosis, both by p53-dependent and p53-independent mechanisms.[140,141] In a study of rat embryo fibroblasts, transfection with the H-ras oncogene conferred radioresistance, characterized by a more shallow slope (i.e., an increased D0) of the radiation dose-response curve at higher doses.[136] On the other hand, transfection with the v-Myc oncogene did not alter radiosensitivity. However, a combination of H-Ras plus v-Myc yielded a synergistic effect on radioresistance, characterized by a broader shoulder and increased D0. Thus, Myc is another potential genetic modifier of cellular radio-sensitivity.[136,142]

Other Oncogenes

Other oncogenes implicated in the regulation of the response to ionizing radiation include: (1) Raf-1, a serine-threonine kinase that mediates Ras signal transduction, leading to the activation of the extracellular receptor-activated (ERK) family of mitogen-activated proteins (MAPKs; see below); (2) Bcr-Abl (see above); and (3) v-Mos, the oncogenic counterpart of c-Mos, another serine-threonine kinase involved in signaling pathways leading to MAPK activation. Although dominant oncogenes usually enhance radioresistance, some reports suggest that the same oncogenes may also confer increased sensitivity to radiation and anticancer drugs.[see references 134 and 143] The differences could be due to modulation of the effects of oncogenes by the expression of apoptosis-regulatory proteins, such as p53 and Bcl-2.[143]

Growth Factor/Cytokine Signaling Pathways

Various growth factors and cyto-kines present in the cell microenvironment can inhibit apoptosis, resulting in enhanced cell survival and decreased radiosensitivity.

Insulin-Like Growth Factor-1

The best studied survival factor is IGF-1.[144,145] The activated IGF receptor transduces an antiapoptotic signal via a set of domains on the intracellular portion of the receptor that are distinct from, but overlap with, those involved in cell proliferation and differentiation.[146] This pathway involves activation of c-Akt (protein kinase B), a signaling protein downstream of PI3 kinase.[147] Inhibition of c-Myc-induced apoptosis by constitutive activation of Ras signaling also occurs by a mechanism involving PI3K and c-Akt, suggesting that this is a general antiapoptotic signaling pathway.[137]

c-Akt Survival Pathway

Because of the involvement of c-Akt in antiapoptotic signaling, it was of interest to identify targets of the Akt serine/threonine kinase linked to cell survival. Several potential target proteins were identified,[148-151] furthering our understanding of the pathways activated by IGF-1 and other growth factors relevant to cell survival and to cancer chemoresistance and radioresistance. For example, c-Akt phosphorylates the proapoptotic protein Bad, a member of the Bcl-2 family of apoptosis regulators.[148] The Bad protein functions as a death ligand, by binding to Bcl-XL, a Bcl-2-like antiapoptotic protein located in the outer mitochondrial membrane.[152] The ligation of Bcl-XL by Bad in the mitochondrial membrane results in loss of cytochrome c from the mitochondria and consequent activation of caspase-9 in the cytosol by cytochrome c and Apaf-1. Phosphorylation of Bad by c-Akt inactivates its ability to bind to Bcl-XL and effectuate cell death.

Interleukin-1

Interleukin-1 (IL-1) is a multifunctional mediator of biological responses related to tissue injury, infection, and immunity.[153] Interleukin-1, interleukin-6 (IL-6), and tumor necrosis factor (TNF) are major mediators of acute phase reactions and inflammation. Interleukin-1 can block or enhance apoptosis of various normal and tumor cell types via signaling through the activated type I IL-1 receptor.[154,155] These responses depend on both the cell type and environmental conditions, particularly hypoxia.[154,156] In studies of cytokine-mediated radioprotection, systemic administration of IL-1, TNF, or stem-cell factor (SCF) protected mice against lethal doses of whole-body irradiation.[157-159] At doses of 1,200 to 1,300 cGy, death in untreated animals was due mainly to bone marrow failure. The radioprotective action of IL-1 appeared to be mediated by upregulation of the expression of c-Kit, the SCF receptor, on hematopoietic stem and progenitor cells.[157] Interleukin-1 and SCF acted synergistically to increase the number of c-Kit-positive bone marrow cells. The ability of IL-1 and TNF
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Scatter Factor/Hepatocyte Growth Factor
Scatter factor (SF; also known as hepatocyte growth factor) is an invasogenic and angiogenic cytokine involved in tumorigenesis and cancer progression.[160,161] Its receptor is a transmembrane tyrosine kinase encoded by a proto-oncogene (c-Met).[162] Scatter factor protects epithelial cells against apoptosis induced by detachment from the substrate (anoikis) or by the protein kinase inhibitor staurosporine[163,164]; scatter factor also protects breast cancer cells from cytotoxicity and apoptosis induced by DNA-damaging agents (eg, x-rays and doxorubicin).[165] Protection against apoptosis resulted in increased clonogenic survival. Scatter factor rendered bone marrow stem cells more resistant to ionizing radiation, as shown by a larger shoulder (n) and a shallower slope (increased Do) of the dose-response curve.[166] The protective activity of SF in breast cancer cells may be due to its ability to block downregulation of the antiapoptotic protein Bcl-XL that is induced by cytotoxins, such as doxorubicin. The radioprotective activities of the cytokines SF and IL-1 may be relevant to cancer therapy. Both of these cyto-kines accumulate in human breast cancers and other malignancies. The highest titers of SF or IL-1-beta were found in the most biologically aggressive tumors,[167-171] and high SF content in invasive breast cancers was a powerful, independent indicator of a poor prognosis.[172]

Tumor Necrosis Factor-Alpha
In addition to protecting against whole-body irradiation, tumor necrosis factor-alpha (TNF-alpha) can modulate the radiosensitivity of tumor cells in vitro. Tumor necrosis factor-alpha accumulates in the culture medium of irradiated sarcoma cells,[173,174] and sublethal concentrations of TNF-alpha can synergistically enhance radiosensitivity.[134,174] Ligation of three type I TNF receptors by a trimeric TNF-alpha molecule induces apoptosis through a series of protein-protein interactions involving death domains and death effector domains.[167,175] These result in the activation of cysteine aspartyl proteases (caspases), that function as the executioners of the cell death program by cleavage of various death substrates (eg, nuclear lamins, PARP, ATM, fodrin, and other caspases).[reviewed in references 67 and 68]

Mitogen-Activated Protein Kinases
Mitogen-activated protein kinases (MAPKs) are protein kinases phosphorylated and activated in response to extracellular stimuli that regulate a variety of cellular processes, including proliferation, differentiation, and apoptosis. MAPKs are classified into three main pathways: extracellular signal-regulated kinases (ERKs), Jun N-terminal kinases (JNKs), and p38 kinases. Each pathway is activated by a specific set of stimuli and plays a role in the regulation of cell proliferation, survival, and death.

Other Growth Factors
Various other growth factors may protect specific cell populations against apoptosis. Of special interest for cancer therapy are vascular endothelial cells. Several angiogenic growth factors (eg, vascular endothelial growth factor [VEGF], basic fibroblast growth factor [bFGF], SF) protect endothelial cells against apoptosis.[179,180] For example, bFGF inhibits radiation-induced apoptosis in bovine aortic endothelia by a receptor-mediated mechanism involving the activation of protein kinase C.[179] Thus, endothelial mitogens that act as survival factors are potential targets for angiogenesis inhibition.

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response to mitogenic stimuli, environmental stresses, and apoptogenic agents, such as ionizing radiation.[181] Three families of MAPKs have been identified: (1) extracellular receptor-activated kinases (ERKs); (2) c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK); and (3) p38. Activation of MAPKs occurs by a specific sequence of phosphorylations, resulting in the phosphorylation and activation of transcription factors (TFs) and consequent responses: MAPKKK ® MAPKK ® MAPK ® TFs (eg, c-Jun, c-Fos, Elk-1) → proliferation, apoptosis, differentiation. Interest in MAPK pathways increased following the discovery that MAPK is overexpressed 5- to 20-fold in human breast cancer cells in vivo, and that overexpression is linked to the metastatic potential of the tumor cells.[182]

Overexpression of the activated JNK/SAPK was linked to induction of apoptosis after exposure to gamma rays and other genotoxins.[183] Overexpression of the Mos/MAPK pathway induced apoptosis, chromosomal damage, and/or the formation of binucleated cells in Swiss 3T3 mouse fibroblasts.[184] This response was p53-dependent, suggesting that constitutive MAPK activation induces DNA damage that is potentially lethal if sensed by p53. The linkage between MAPKs and sublethal DNA damage has potential therapeutic implications for cancers in which MAPKs are overexpressed (eg, breast cancer).

**Apoptosis vs Other Modes of Cell Death**

As described above, radioresistant and chemoresistant cell phenotypes have been linked to cellular resistance to apoptosis. The mechanisms are complex, and may involve tumor-suppressor gene mutations (eg, p53), accumulation of antiapoptotic cytokines (eg, IGF-1 and SF), or other mechanisms. For example, ceramide is a lipid second messenger that has been linked to the induction of apoptosis by ionizing radiation, TNF-alpha, and Fas ligand. Loss of ceramide production rendered cells resistant to radiation-induced apoptosis.[185]

Although apoptosis is a major mode of death induced by radiation and DNA-damaging drugs, it is not always the dominant mode of cell death. Non-apoptotic cell death may proceed via nonprogrammed cell death (necrosis) or by genetically programmed mechanisms distinct from classic apoptosis. The absence of specific molecular markers makes nonapoptotic death difficult to quantitate and characterize.

Several reports suggest a contribution of nonapoptotic cell death to radiation-induced cell killing in some cell types.[186,187] For example, transfection of rat 208F fibroblasts with the H-ras oncogene inhibited radiation-induced apoptosis but did not alter the clonogenic survival response.[186] These studies indicate that the radiosensitivity of a cell population cannot be predicted solely from the apoptotic component of cell death.

An example of programmed cell death distinct from classic apoptosis is mitotic death in cells that have not completed cytokinesis and reentered interphase. Thus, Chang liver cells treated with vanadyl[4] to generate hydroxyl free radicals demonstrated autophagocytosis of mitotic chromosomes (M-phase chromatin)[188]; this contrasts with classic apoptotic pathways, in which apoptotic cells are recognized and destroyed by macrophages.

These studies agree with classic radiobiology studies examining the kinetics of cell death after irradiation.[189] In most cell types, radiation-sterilized cells do not die immediately, or even soon, after irradiation. Instead, cells that will ultimately die generally are indistinguishable from cells that will survive, for one or several cell divisions after irradiation. The progeny of the former cells then begin to die, often during abnormal and prolonged mitoses, leading to the death of the entire abortive clone.[189] This delayed death, which requires that cells enter into mitosis and divide in order to die, is characteristic of radiation and radiomimetic drugs that produce similar DNA damage.

**Conclusions**

Recent studies have elucidated the molecular and cellular mechanisms that determine the sensitivity or resistance to ionizing radiation. Much of this knowledge was obtained by studying tumor and nontumor cell types that underexpress or overexpress proteins involved in the regulation of the DNA damage response, cell-cycle progression, growth factor signal transduction, and apoptosis. These findings may ultimately be useful in devising new strategies to improve the therapeutic ratio in cancer treatment.

Despite the rapid advances in knowledge of cellular functions that affect radiosensitivity, we still cannot account for most of the clinically observed heterogeneity of normal tissue and tumor responses to radiotherapy, nor can we accurately predict which individual tumors will be controlled locally and which patients will develop more severe normal tissue damage after radiotherapy.
However, several candidate genes for which deletion or loss of function mutations may be associated with altered cellular radiosensitivity (eg, ATM, *p53*, BRCA1, BRCA2, DNA-PK) have been identified. Some of the differences in normal tissue sensitivity to radiation may stem from mutations with milder effects, heterozygosity, or polymorphisms of these genes. Finally, molecular mechanisms linking genetic instability, radiosensitivity, and predisposition to cancer are being unraveled.

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