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Cancer Treatment with Gene Therapy and Radiation Therapy

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Abstract

Radiation therapy methods have evolved remarkably in recent years which have resulted in more effective local tumor control with negligible toxicity of surrounding normal tissues. However, local recurrence and distant metastasis often occur following radiation therapy mostly due to the development of radioresistance through the deregulation of the cell cycle, apoptosis, and inhibition of DNA damage repair mechanisms. Over the last decade, extensive progress in radiotherapy and gene therapy combinatorial approaches has been achieved to overcome resistance of tumor cells to radiation. In this review, we summarize the results from experimental cancer therapy studies on the combination of radiation therapy and gene therapy.

1. INTRODUCTION

Approximately half of all cancer patients worldwide are treated with radiation therapy alone or in combination with chemotherapy or surgery. Radiation therapy approaches employ ionizing radiation delivered either externally by linear accelerators or X-rays and γ -rays or internally with the use of radioisotopes to destroy cancer cells. Radiation therapy is, in general, localized, noninvasive and does not produce systemic toxicity after treatment in comparison with chemotherapy. However, despite progress in radiation therapy, development of resistance to therapeutic doses of irradiation still remains a major obstacle for anticancer therapy. There are generally two major forms of resistance encountered in clinical practice. One is intrinsic radiation resistance, which is an innate property of the cancer cells. The other is acquired resistance, which develops subsequent to radiotherapy (Shimura, 2011). It is becoming more evident from clinical studies of conventional radiotherapy that multimodality treatment strategies have a higher chance of success, presumably because combined therapies simultaneously utilize diverse target signaling pathways in tumor cells and may have non-overlapping toxicities. In order to overcome mechanisms of radiation resistance, resulting in recurrence of disease, strategies involving a combination of radiation therapy and gene therapy have been extensively investigated. This review focuses on cancer gene therapy strategies that are currently being evaluated in conjunction with radiation therapy, including the employment of radiation-inducible promoters in combination with therapeutic genes that ultimately lead to radiosensitization of tumor cells. Furthermore, in some instances, radiation treatment has resulted in increased proliferation and intratumor distribution and tumor cell killing of replicative viral vectors.

2. MOLECULAR MECHANISMS OF TUMOR RESPONSE TO RADIATION THERAPY

Significant progress has been made in recent years in understanding the molecular mechanisms of cellular responses to radiation therapy (summarized in Fig. 7.1). Exposure of tumor cells to clinically relevant doses of ionizing radiation produces a wide range of DNA damages that include DNA single-strand breaks, DNA double-strand breaks (DSBs), DNA base damage and apyrimidinic/apurinic (AP) sites, as well as DNA–protein cross-links. In addition to direct DNA damage, irradiation triggers a variety of lesions in cellular macromolecules (e.g., lipid peroxidation) in part due to mitochondria-dependent generation of reactive oxygen species (ROS). This complex of DNA and non-DNA lesions can stimulate various signal transduction pathways (such as protein kinase C (PKC), JNK, ceramide, and MAPK activation). Thus, the cellular responses to radiation-mediated DNA damage are characterized by coordinated processes involving DNA repair mechanisms engaged with other events that are occurring in the cell, including cell cycle progression or apoptosis. Initially, the damage to DNA initiates signaling pathways that halt cell cycle progression through G1, intra-S, and G2/M to prevent transfer of DNA damage to progeny cells. Activation of the DNA repair machinery is facilitated by increased accessibility of DNA damage sites. Concurrently with the arrest of cell cycle progression, malignant cells mobilize mechanisms preparing the cell to undergo apoptosis when DNA cannot be successfully repaired. Also, inactivation of phosphatases by mitochondria-dependent generation of ROS induces signaling at the receptor level that consequently causes ligand-independent activation of cell-surface receptors with intrinsic tyrosine kinase activity.

2.1. Molecular mechanisms of DNA damage repair

A growing body of evidence suggests that DNA DSBs are the main cause of cell death induced by ionizing radiation. The two major pathways involved in DSB repair are homologous recombination (HR) and non-homologous end joining (NHEJ). The primary function of HR is to repair DSBs at the replication fork, whereas NHEJ is the key mechanism of DSB repair during G0, G1, and early S phase, and NHEJ continues to repair a minority of breaks during late S and G2 phases of the cell cycle (Fig. 7.1).

The HR pathway employs numerous proteins (RPA, Rad51, Rad52, Rad54, BRCA1, BRCA2, and Mre11–Rad50–Nbs1 (MRN) complex). Ataxia-telangiectasia mutated (ATM), RAD3-related protein kinases (ATR), and RAD3-related protein kinases from the PI3K-related kinase family are central mediators of cellular responses to DNA DSBs in cells. ATM/ATR and Rad3-related protein kinases phosphorylate numerous substrates in the cell that modulate the cell response to DNA damage via activation of receptors/intracellular signaling pathways as well as stimulating cell cycle checkpoints, p53 activity, and DNA repair complex function. ATM is activated when it is recruited to sites of DSB damage by the MRN complex (Bakkenist & Kastan, 2004; Falck, Coates, & Jackson, 2005; Jazayeri et al., 2006; Lee & Paull, 2005). Once activated, ATM and ATR protein kinases phosphorylate numerous substrates in the cell and induce the cell response to DNA damage via facilitating DNA repair and modulation of cell cycle arrest (Gatei et al., 2003). The best characterized among the ATM-activated substrates include p53 (Canman et al., 1998; Khanna & Lavin, 1993), the kinases CHK1 and CHK2 (Zhou et al., 2000), and the histone H2AX and the MRN complex (Burma, Chen, Murphy, Kurimasa, & Chen, 2001). Similarly, ATM and ATR phosphorylate CHK1 and DNA helicase BLM1. Thus, ATM/ATR protein kinases are involved in the modulation of apoptosis via p53 and CHK2 expression, DNA repair (H2AX and MRN), and cell cycle arrest using p53, CHK1, and CHK2 signaling pathways (Andreassen, Ho, & D'Andrea, 2006). Finally, the core DNA repair machinery is activated,

as a result of CHK1/CHK2-mediated cell cycle arrest (mostly at intra-S or G2/M phase) (Zhou & Bartek, 2004).

NHEJ predominantly repairs DSBs that have been generated by ionizing radiation during the cell cycle. The main NHEJ pathway involves binding of the DNA-PK complex composed of Ku70, Ku80/Ku86, and the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) kinase to the ends at a DSB. In this process, the Ku70/Ku80 heterodimers load onto a DNA terminus during the initial “synapsis” phase. The Ku70/Ku80–DNA complex binds to the DNA-PKcs serine/threonine kinase. The activated protein kinase complex is involved in the phosphorylation of the DNA DSB repair/ *V(D)J* recombination protein Artemis. Subsequently, Artemis–DNA-PKcs complex with endonuclease activity processes DNA ends before XRCC4 and DNA ligase IV to facilitate the final ligation step (Burma, Chen, & Chen, 2006; Lieber, Ma, Pannicke, & Schwarz, 2003). In contrast, HR is generally restricted to late S and G2 phase and rejoins DSBs using a sister homologue as a template. An early step in HR involves the generation of a single-stranded region of DNA, followed by invasion of the template strand, which creates a Holliday junction. DNA synthesis using the sister strand as a template is followed by branch migration and subsequent resolution of the heteroduplex. Rad51, a central player in HR, is loaded onto ssDNA and promotes strand invasion, with BRCA2 having a role in delivering Rad51 to the DNA (O’Driscoll & Jeggo, 2006; Pellegrini et al., 2002).

Additionally, clinically relevant low-dose ionizing radiation can generate ROS through radiolysis of water that results in a multitude of oxidized base lesions, DNA strand breaks, and base loss, largely caused by hydroxyl radicals. Inhibition of protein tyrosine phosphatases (PTPases) by ROS and reactive nitrogen species (RNS) leads to activation of receptor and nonreceptor tyrosine kinases and activation of downstream signaling pathways (Chiarugi & Cirri, 2003). Radiation dose-escalation studies revealed that high doses of ionizing irradiation increase the levels of acidic sphingomyelinase and production of ceramide and promote membrane-associated receptor activation by facilitating receptor clustering within the lipid rafts (Galabova-Kovacs et al., 2006). The activation of cell-surface RTK receptors initiates a cascade of proliferative signaling events involving several members of the epidermal growth factor receptor (ERBB1/HER1) family (Goldkorn, Balaban, Shannon, & Matsukuma, 1997; Gulbins & Kolesnick, 2003; Kavanagh, Dent, Schmidt-Ullrich, Chen, & Mikkelsen, 1998; Kavanagh, Lin, Chen, & Schmidt-Ullrich, 1995; Schlessinger & Lemmon, 2006). It was shown that heterodimerization of ERBB1 with other members of the ERBB1 receptor family (ERBB2, ERBB3, and ERBB4) is linked to downstream activation of intracellular signaling pathways, including the RAF-1–MEK1/2–ERK1/2 and the PI3K–phosphoinositide-dependent kinase-1–AKT pathways (Hynes & Lane, 2005; Nyati, Morgan, Feng, & Lawrence, 2006; Schlessinger, 2004). The initial radiation-induced activation of ERK1/2 can temporally delay reactivation of receptors as well as intracellular signaling pathways by facilitating cleavage and release of paracrine ligands in irradiated cells. Several vital signaling pathways are also activated by the receptors including the c-Src tyrosine kinase, the signal transducer and activator of transcription proteins (STATs), and mammalian target of rapamycin (Bjornsti & Houghton, 2004; Ishizawa & Parsons, 2004; Yu & Jove, 2004). Perturbations of such signaling pathways can lead to radiation resistance in cancer cells.

2.2. Downstream signaling networks

Signaling pathway networks activated through both the cell-surface receptors and damaged DNA play an important role in the regulation of the downstream pathways. These signaling pathways are ultimately involved in the activation of a variety of transcription factors (Criswell, Leskov, Miyamoto, Luo, & Boothman, 2003). Radiation-activated transcription factors include tumor suppressor p53 (Lu & Lane, 1993), proinflammatory transcription

factor NF- κ B (Prasad, Ravindran, & Aggarwal, 2010), signal transducer and activator of transcription-3 (STAT3) (Aggarwal et al., 2009), the octamer-binding protein-1 (Oct-1) transcription factor (Jin et al., 2001), the c-Fos/c-Jun AP-1 complex (Hallahan, Virudachalam, et al., 1991; Sherman, Datta, Hallahan, Weichselbaum, & Kufe, 1990), and the early growth response 1 SP1-like transcription factor (Datta et al., 1993), which are important in the regulation of gene expression in major pathways of inflammation, tumor cell proliferation, as well as invasion and metastasis. Radiation-mediated activation of intracellular signaling pathways can modulate levels of transcription of proteins involved in proliferative and inflammatory responses of tumor cells to radiation therapy. Thus, the balance between DNA damage and DNA repair signaling pathways determines the radiosensitivity of tumor cells.

3. GENE TRANSFER SYSTEMS

Radiation treatment is a well-established modality for anticancer therapy. A growing body of evidence suggests that a manipulation of genes involved in tumor progression and radiation resistance represents a promising approach for therapeutic intervention in combination with radiation therapy. Cancer gene therapy is the selective transfer of recombinant DNA/RNA using viral and nonviral gene delivery vectors, into malignant cells as well as tumor-associated cells for therapeutic purposes. Generally, gene therapy approaches are based on the employment of genetically engineered vectors that promote the transfer of the transgene expression unit into the tumor cells and allow transient or stable expression of the therapeutic gene. The search for methods to introduce genes into cancer cells led to many advances in viral and nonviral vector development. Vectors derived from adenoviruses, adeno-associated viruses, retroviruses, herpesviruses, and poxviruses are the most commonly used gene carriers in cancer gene therapy. Nonviral gene transfer systems include DNA transfection using chemical methods such as utilization of a variety of liposomes, cationic polymers, or peptide delivery systems, as well as disruption of the cell membrane by physical methods (such as electroporation or ultrasonography). Many of these gene delivery systems have been tested for cancer therapy in combination with radiation therapy. Each group of vectors demonstrates a number of advantages and limitations. Most commonly used in cancer gene therapy, recombinant viral vectors are biological systems derived from naturally evolved pathogens which as part of the infection process transfer their genomes into the target tumor cells. The common restrictions associated with viral vectors are related to their safety, immunogenicity, preexisting immune response, and limited capacity of transgene insert.

3.1. Adenoviruses

Adenoviruses (Ads) are nonenveloped linear double-stranded DNA viruses. At the present time, human and nonhuman Ad vectors have been modified extensively and are commonly used vectors in clinical trials of cancer gene therapy. Ads can transduce both dividing and quiescent cells. In contrast with parvoviruses and retroviruses, Ads do not integrate into the cell genome and therefore pose no risk of insertional mutagenesis. Helper-dependent high-capacity Ad vectors are completely devoid of all viral coding sequences but retain the viral ITRs and the packaging signal and were demonstrated to be significantly safer in comparison with the first generation of Ad vectors. The Ad vectors can accommodate up to a 30-kb DNA insert and demonstrate a high transduction efficiency and relatively simple production and manipulation. These vectors have been used in gene transfer for basic studies of gene function and for gene therapy of genetic diseases and cancer. Conditionally replicating Ads (CRAds) with *E1A* driven by tumor-specific promoters have often been explored as oncolytic viruses. A substantial problem associated with Ad vectors is high immunogenicity in combination with proinflammatory effects that limit transgene expression and hamper systemic readministration (Bauerschmitz, Barker, & Hemminki,

2002). However, the Ad immunogenicity is applicable to treatment of chronic diseases, and an appropriate immune response induced by Ad vectors may be beneficial in some cancer gene therapy approaches related to the regulation of the host immune system.

3.2. Adeno-associated viruses

Adeno-associated viral (AAV) vectors are replication-defective, single-stranded DNA parvoviruses that require a helper Ad for their replication. Site-specific or random AAV vector integration into the host cell genome, in the absence of a helper virus, results in long-term transgene expression. Also, AAV vectors demonstrate high transduction efficiency in a broad range of quiescent and dividing target cells. In contrast to Ads, AAV vectors do not produce a significant immune response (Zaiss et al., 2002). A major limitation of using AAV vector is a relatively small transgene size (4 kb), a necessity of helper Ad or herpesviruses for successful transduction, and integration into the genome of target tumor cells as well as host cells that could lead to mutagenesis. In the absence of a helper virus, AAV vectors establish latency by preferential integration into a specific site on human chromosome 19. AAV vectors are frequently used for gene therapy for genetic diseases and cancer.

3.3. Retroviruses/lentiviruses

Retroviruses are single-stranded RNA viruses that can integrate into the genome of cells, which results in stable replication and transmission to all the progeny of these cells. Retroviruses continue to be employed as gene delivery vehicles, although recent adverse events following retroviral gene therapy have raised concerns about potential insertional mutagenesis (Hacein-Bey-Abina et al., 2003). Lentiviral vectors can transduce both proliferating and quiescent cells. Advantages of retroviral vectors for gene therapy include potential long-term transgene expression due to integration into the target tumor cells or host genome and low immunogenicity. However, most retroviruses demonstrate low levels of transduction efficiency and only infect dividing cells during mitosis (Adamina, Daetwiler, Rosenthal, & Zajac, 2005).

3.4. Herpesviruses

The herpesviruses, including herpes simplex virus HSV-1, are receiving increasing attention because of their ability to replicate in the nucleus as latent episomes and kill dividing and nondividing tumor cells. Attenuated and replication-competent HSV-1 mutants offer the capability to insert up to almost a 40-kb transgenic sequence. However, low and transient transfection efficiency as well as elicited host immune response and severe symptoms associated with wild-type viruses are serious obstacles for HSV-1-based gene therapy (Marconi et al., 1996).

3.5. Poxviruses

Similar to HSV-1, the vaccinia virus has a large genome (about 186 kb) and can accommodate up to 25 kb foreign DNA. Recombinant vaccinia viral vectors have been used mainly for delivery of cancer-specific antigens and immunoregulatory genes for vaccine-based gene therapy, which is based on the long history of utilization of these viruses for vaccination. However, the strong immune response as a result of preexisting anti-vaccinia immunity following childhood smallpox immunization limits the use of recombinant vaccinia viral vectors for gene therapy (Arlen, Gulley, Madan, Hodge, & Schlom, 2007).

3.6. Nonviral vectors

In an attempt to improve both the efficacy and safety of virus-mediated gene therapy, many vectors have been modified. However, several obstacles including limitation of the size of

the inserted genetic material, difficulty of propagation and purification, and toxic adverse events (in particular immunogenic and oncogenic activity) have encouraged researchers to focus on nonbiological gene transfer systems as an alternative to viral vectors. Progress has been made using a variety of liposomes, cationic polymers, and peptides. In this approach, recombinant DNA is associated with positively charged lipid bilayers, cationic polymers, or peptides and can enter cells either by endocytosis or by fusion with the cell membrane. DNA–protein complexes have been developed employing natural or synthetic peptides that allow the use of these gene delivery vehicles in cell-targeting strategies. In contrast with recombinant viruses, these nonviral gene transfer systems are characterized by low cost and simple production, large gene insert size without toxic effects, and lack of an immune response after readministration. However, nonbiological gene transfer systems produce short-term expression, and their efficiency and specificity of gene delivery is less compared to viral vectors (Glover, Lipps, & Jans, 2005).

4. RADIATION-RESPONSIVE PROMOTERS

Gene therapy strategies offer the potential for specific targeting at several levels including transcriptional targeting. Transcriptional regulatory elements are composed of both *cis*-acting and *trans*-acting elements. The *cis*-acting components include promoters as well as enhancer regions that also regulate expression of a particular gene. The *trans*-acting elements belong to a growing group of transcription factors that bind to specific sites within promoter and/or enhancer regions. Transcriptional control of therapeutic gene expression using promoters that are responsive to ionizing radiation provides an attractive approach for the combination of radiation therapy and gene therapy (summarized in Table 7.1). It has been known for many years that ionizing radiation can modulate gene transcription as well as protein expression of a large number of cellular proteins involved in the early response of tumor cells to ionizing radiation-mediated damage. This includes genes involved in cell cycle checkpoints, cellular stress, DNA repair, and apoptosis. It was shown that expression of radiation-inducible genes such as cytokines and growth factors (e.g., early growth factor-1) (Hallahan, Sukhatme, et al., 1991) as well as immediate-early genes encoding transcription factors (e.g., Fos, Jun, AP-1, NF- κ B) (Weichselbaum et al., 1994) is variable and cell dependent (Hamdi et al., 2005). Activated immediate-early genes interact with the promoter region of so-called late-responding genes, coding for cytokines, involved in the inflammation process (e.g., IL-6) and growth factors, involved in cell survival and tissue recovery (e.g., TGF β 1, TNF- α) (Beetz et al., 2000; Blalock et al., 1999; Martin et al., 1997). Importantly, upregulation of therapeutic genes is achievable at clinically relevant doses of radiation both *in vitro* and *in vivo*. The molecular mechanisms of radiation-inducible gene expression remain unclear but involve putative radiation-responsive elements in gene promoter binding sites for selected transcription factors.

4.1. Early growth response-1 gene

The early growth response-1 (*Egr-1*) promoter, first described more than two decades ago, is one of the most widely investigated radiation-inducible promoters. The *Egr-1* gene encodes a nuclear protein with a Cys2-His2 zinc-finger motif that is partially homologous to the corresponding domain in the Wilms tumor susceptibility gene (Gessler et al., 1990). *Egr-1* is an immediate-early protein that is involved in signal transduction during cellular proliferation and differentiation. It was shown that induction of *Egr-1* expression in the transition of quiescent cells from the G0 to G1 phase of the cell cycle is regulated by efficient *cis*- and *trans*-regulatory mechanisms. The mechanism of regulation of *Egr-1* expression by ionizing radiation is incompletely defined. However, functional analysis of *Egr-1* revealed a number of DNA sequence motifs including GCGGGGGCG consensus sequence identified in the promoters of many regulatory cytokines including *bFGF*, *IL-1*, *PDGF- α* , and *TNF- α* , as well as putative AP-1 sites located in the enhancer region of the

Egr-1 promoter that can modulate the radiation-mediated response (Datta et al., 1992). Ionizing radiation as well as DNA-damaging chemotherapy agents can readily induce the *Egr-1* promoter via a 10-nucleotide motif of consensus sequence in the CArG (CC(A+T rich) 6GG or serum response element) (Datta et al., 1992, 1993; Hsu et al., 2003; Mezhir et al., 2006; Tsai-Morris, Cao, & Sukhatme, 1988). The comparative analysis of a series deletion in the *Egr-1* promoter region using constructs with the chloramphenicol acetyltransferase (*CAT*) reporter gene demonstrated that the three 5' distal CArG boxes were essential to the radiation response. It has been shown that CArG elements are responsive to hydrogen peroxide treatment, and this could play a role in radiation inducibility via ROS produced following irradiation (Datta et al., 1992; Hsu et al., 2003). CArG elements were shown to regulate the expression of other immediate-early genes including several members of the actin family and c-fos via the binding of specific accessory proteins such as Elk-1 and/or SAP-1. The presence of the putative binding site moieties in the *Egr-1* promoter also suggests potential involvement of the Sp-1 transcription factor as well as the Fos-jun heterodimer activator protein (AP)-1. Also, the phosphorylation by mitogen-activated protein (MAP) kinases involves the *Egr-1* promoter response to cellular stress following irradiation of cancer cells (Christy & Nathans, 1989; Datta et al., 1992; Treisman, 1990; Tsai-Morris et al., 1988). A number of gene therapy vectors encoding the therapeutic transgene under the control of the radiation-responsive elements of *Egr-1* promoter were extensively tested in combination with ionizing radiation. The early gene therapy studies of this system utilized an Ad.Egr.TNF adenoviral vector encoding the proapoptotic cytokine TNF- α under the control of CArG elements. It was shown that radiation and chemotherapy increase TNF- α expression (Meyer et al., 2002; Mezhir et al., 2006; Park et al., 2002). Ad.Egr.TNF demonstrated increased antitumor efficacy in combination with radiation therapy in phase I and phase II trials (Bickenbach et al., 2008; Hallahan et al., 1995; McLoughlin et al., 2005; Senzer et al., 2004). Currently, several suicide gene/prodrug systems using radiation-inducible promoters and gene delivery systems are under intense investigation. Recently, *Egr-1*-driven molecular chemotherapy using the herpes simplex virus-thymidine kinase (HSV-TK) with the antiherpetic drug ganciclovir (GCV) (Joki et al., 1995) or cytochrome p450 isozyme (CYP4B1) in combination with 4-ipomeanol (4-IM) (Hsu et al., 2003) systems was tested in combination with radiation *in vitro* and *in vivo*.

4.2. Other radiation-inducible promoters

The promoters from many stress response genes related to cellular fate following DNA damage are potential candidates for cancer gene therapy. In the last two decades, several radiation-inducible promoters including tissue plasminogen activator (*tPA*), *p21/WAF-1*, and *GADD45a* were proposed for gene therapy in combination with radiation therapy strategies.

4.2.1 Tissue plasminogen activator gene—Human tPA is a glycoprotein produced mainly by vascular endothelial cells. tPA activates clot dissolution in the presence of fibrin by conversion of plasminogen to plasmin, thereby cleaving cross-linked fibrin to D-dimer and other degradation products. The *tPA* promoter comprises several putative binding sites for the AP-2, NF κ B-like and AP-2-like regulatory factors. The mutagenesis of the AP-2 and NF κ B binding sequences resulted in the reduction of basal activity and UV-induced tPA expression. Additionally, mutations in the AP-2-like sequence resulted in complete loss of the UV response and produced negligible modification of basal expression. tPA was induced in radioresistant human melanoma cells over 50-fold following X-irradiation at 4.5 Gy (Boothman et al., 1994).

4.2.2 Wild-type p53-activated fragment (p21/WAF1)—WAF1, also known as p21 or CIP1, is a cyclin-dependent kinase inhibitor, suppressing cell growth. It has also been shown to be involved in the immediate-early response to a range of stresses (Ishida, Sasaguri, Kosaka, Nojima, & Ogata, 1997; Steinman et al., 1994). The *p21/WAF1* promoter region has a p53 binding site that has been shown to confer p53-dependent WAF1 expression. These p53 recognition elements comprise four tandem PuPuPuC(A/T) pentamers and located 1.3, 1.7, and 2.2 kb upstream of the first exon of human *p21/WAF1* (Ishida et al., 1997). It was shown *in vitro* that the radiation inducibility of the *p21/WAF1* promoter is p53 dependent (el-Deiry et al., 1993). However, there is also evidence of p53-independent activation pathways and that the *p53* status did not alter significantly the radiation inducibility of *p21/WAF1 in vivo* (Blundell, Kaminski, & Harrison, 2004; Rimner et al., 2001; Sourvinos & Spandidos, 1998). The expression of the inducible nitric oxide synthetase (*iNOS*) gene under the control of the *p21/WAF1* promoter has been employed to improve the efficacy of conventional radiation therapy. It was shown that a *p21/WAF1* promoter-driven *iNOS* gene therapy significantly sensitized both p53 wild-type RIF-1 tumors and p53 mutant HT29 tumors to fractionated radiation therapy (McCarthy et al., 2007; Worthington et al., 2004). Molecular chemotherapy using a recombinant AAV vector expressing the *HSV-TK* gene under the control of the *p21/WAF1* promoter significantly increased killing of MCF7 human breast cancer cells in the presence of the prodrug GCV in combination with fractionated radiation treatment at 1 Gy (Nenoi et al., 2006).

4.2.3 Growth arrest and DNA damage-inducible protein 45 alpha—Growth arrest and DNA damage-inducible protein 45 alpha (*GADD45α*) plays an important role in controlling cell cycle checkpoints, apoptosis, and DNA repair after DNA damage. A number of studies have reported that *GADD45α* expression is modulated by a variety of DNA-damaging agents, including ionizing radiation (Fornace, Alamo, & Alamo, 1988; Fornace et al., 1989; Papathanasiou et al., 1991). Several X-ray-responsive elements were found including the p53 recognition site in *GADD45α*. Detailed analysis of the upstream region (−2244 bp/+89 bp) and the third intron (+1389 bp/+2488 bp) of *GADD45α* revealed binding sites for Oct-1, NF-κB, HNF, NF-AT, and KLF family transcription factors. Radiation treatment stimulated transcription factor binding to *GADD45α* gene promoter after a single dose of 0.5 Gy with expression levels reaching a maximum at 1 h after irradiation (Daino et al., 2003). It was shown that induction of *GADD45α* gene expression following ionizing irradiation is regulated in a p53-dependent manner (Daino et al., 2006; Jin et al., 2001; Takahashi et al., 2001).

4.3. Radiation-inducible hybrid/synthetic enhancer–promoter elements

Synthetic or hybrid enhancer and/or promoters have been designed to improve both the strength and tumor specificity for gene expression. In early experiments using synthetic promoters for radiation-mediated gene therapy, increased levels of expression of *GFP* and *HSV-TK* genes under the control of the CArG elements from the *Egr-1* promoter were detected after single dose and multiple fractions of irradiation using human breast cancer and glioma cell models (Greco et al., 2005). An optimization study of radiation-responsive gene promoters comprised 4 (E4), 6 (E6), 9 (E9) or 12 (E12) copies of the CArG element sequences in tandem was carried out. Radiation treatment of MCF7 human breast cancer cells at 3 Gy increased expression of a reporter gene. The maximal levels of GFP expression were reached using a promoter with nine copies of the CArG element (Scott et al., 2002). Another synthetic promoter containing CArG element (pE9) was made to drive production of *iNOS*, which radiosensitizes hypoxic tumor cells to radiation treatment (Coulter et al., 2008).

5. THERAPEUTIC TRANSGENES

Over the last decades, substantial progress has been made in radiation therapy methods. However, often tumor cells escape irradiation induced cell death either by deregulation of the cell cycle, suppressing apoptosis or by an increased ability of cancer cells to repair the DNA damage. Manipulation of genes involved in radiation resistance represents an important approach for therapeutic intervention in cancer. Combination of radiation therapy with gene therapy provides a promising strategy to modify the radiation response and overcome radioresistance of tumor cells. In the last decade, significant progress in cancer therapy using a combination of gene therapy and radiation treatment has been demonstrated. A broad range of different gene therapy approaches, including cytolytic and corrective gene therapy, have been successfully applied for experimental therapy in combination with radiation therapy in animal models, with translation into early clinical trials (summarized in Table 7.2).

5.1. DNA repair pathways

Exposure to ionizing radiation initiates a cascade of multiple signaling pathways including a number of DNA repair pathways which are essential for the intrinsic resistance of the tumor cell to radiotherapy (Chen, Sin, Leung, Ng, & Poon, 2005; Fischer & Meese, 2007; Kauffmann et al., 2008; Munshi et al., 2005; Rass & Reichrath, 2008). It is well known that the HR and NHEJ pathways are involved in radiation-induced repair. Extensive efforts have been made to develop gene therapy strategies to interfere with the main DSB repair pathways. In the last decade, gene therapy approaches were tested for downregulation of protein expression involved in HR or NHEJ by using short-interfering RNA (siRNA) against BRCA2 and Rad51 (Vernole et al., 2009; Yu et al., 2008) or Ku70 and Ku80 (Li et al., 2003; Nimura et al., 2007), respectively. Recently, a strategy for sensitizing tumors to radiotherapy employing short modified double-stranded DNA molecules (siDNA) has been explored. Use of this signal-interfering DNA, mimicking DSB damage, recruits the Ku70/Ku80/DNA-PKcs DNA damage repair complex. Activation of the DNA-PK kinase phosphorylates a large number of proteins in the DNA damage response cascade including H2AX, Rpa32, Chk1, Chk2, Nbs1, and p53 that results in the inhibition of DNA repair (Berthault et al., 2011; Quanz et al., 2009). Gene correction therapies in combination with radiation have also been evaluated for inhibition of the base excision repair (BER) pathways that are responsible for the repair of oxidative and alkylation DNA damage. Combination of intratumoral (i.t.) injection of Ad5/F35 vector expressing fiber from Ad serotype 35 and siRNA against the human apurinic/aprimidinic endonuclease (APE1), the important enzyme in the BER pathway, with irradiation at 6 Gy produced delay in LOVO colon tumor growth in comparison with radiation therapy alone (Xiang et al., 2008). It was shown that inhibition of the poly(ADP-ribose) polymerase-1 enzyme, which plays a vital role in DNA damage signaling and regulation of the BER pathways, significantly improved survival of patients with BRCA1 or BRCA2 mutation in the tumor cells (Ratnam & Low, 2007). The efficacy of cancer gene therapy by targeting the poly (ADP-ribose) polymerase dominant negative gene in combination with single dose radiation at 5 Gy was evaluated using a prostate tumor xenograft model (Trofimova et al., 2002).

5.2. Radiotargeted gene therapy

DNA damage mediated by radiotargeted gene therapy provides an additional approach to sensitize tumor cells to irradiation. The utilization of tumor-targeted gene transfer for the expression of high-affinity membrane receptors can enhance the specificity as well as extent of localization of radiolabeled ligand within the tumor mass and decrease normal tissue toxicity. Using a genetic radioisotope targeting strategy, induction of high levels of human somatostatin receptor subtype 2 (hSSTR2) or gastrin-releasing peptide receptor (GRPR)

expression resulted in selective tumor uptake of radiolabeled peptides and significant inhibition of tumor growth of human tumor xenografts was achieved (Cotugno et al., 2011; Rogers et al., 2002; Stackhouse et al., 1999). A second strategy involves transduction of the sodium iodide symporter (*NIS*) or the noradrenaline transporter (*NAT*) genes in combination with free radionuclide therapy. *NIS* and *NAT* are responsible for the active uptake of ^{131}I -iodine (Na^{131}I) and ^{131}I -meta-iodobenzylguanidine (^{131}I -MIBG), respectively. Therapy studies have been performed on several tumor xenograft models using *NIS/NAT* gene transfer followed by the administration of targeted radionuclides Na^{131}I for *NIS* and ^{131}I -MIBG. There is considerable clinical experience in the use of ^{131}I -MIBG and Na^{131}I in the treatment of medullary thyroid and hepatocellular carcinoma (Jia et al., 2011; Spitzweg et al., 2007). The use of gene transfer technology to induce expression of high-affinity membrane receptors or transporters can enhance the specificity and extent of radioligand or radionuclide localization in tumors, and the use of radionuclides with appropriate emissions can deliver radiation-absorbed cytotoxic doses across several cell diameters to compensate for limited transduction efficiency.

5.3. Modulation of the apoptosis pathways

It is now well known that exposure of tumor cells to clinically relevant doses of radiation generates DNA damage in the nucleus as well as triggers a large network of intracellular signaling pathways that collectively produce a multilayered biological response. Apoptosis is recognized as a fundamental mechanism of cellular response to ionizing radiation. Suppressed apoptosis is an important distinctive feature that contributes to the malignant phenotype of cancer. Also, the ability of a cancer cell to respond to radiotherapy is believed to be due, in part, to its apoptotic capacity. Thus, combination of radiation and gene correction strategies is a promising approach to increase apoptosis in tumor cells. Apoptosis is controlled by multiple genes, many of which are mutated in human cancers. *p53* is one of the genes most widely used for gene therapy in combination with radiation. The *p53* tumor suppressor protein exerts its growth inhibitory activity by interacting with diverse signaling pathways which leads to many outcomes in cells, including cell cycle arrest and apoptosis. The mutation/deletion of *p53* impairs cellular response to DNA damage, prevents apoptosis, and remains a strong predictor of radioresistance (Lowe et al., 1994). Restoration of *p53* activity by i.t. injection of Ad vectors encoding *p53* has been shown to significantly increase tumor cell sensitivity to irradiation. Combination of radiation with wild-type *p53* gene therapy has a synergistic suppressive effect on tumor growth via increasing apoptosis in a wide variety of tumor xenograft models including colorectal, ovarian, nasopharyngeal, head and neck, and squamous cell carcinomas (Gallardo et al., 1996; Higuchi et al., 2003; Li et al., 1999; Spitz et al., 1996; Wakasa et al., 2002; Xu et al., 1999). As a result, advanced clinical studies are now underway which demonstrate promising results in patients with SCCHN, thyroid tumors, non-small cell lung cancer (NSCLC), prostate cancer, or pancreatic cancer with Ad-*p53* i.t. injections (bronchoalveolar lavage for NSCLC) combined with fractionated radiation therapy. Ad-mediated overexpression of the *p53* family of genes, *p73* and *p51/p63*, produces cell cycle arrest and apoptosis in colorectal cancer cells resistant to *p53*-mediated apoptosis (Sasaki et al., 2001). A number of gene therapy approaches were evaluated for the modulation of apoptosis response to radiation through overexpression of proapoptotic genes, including *bax* (Kaliberov et al., 2002); family of cysteine proteases—caspases 1, 3, 8, and 9 (Shariat et al., 2001; Uchida et al., 2003; Winter et al., 2004; Yanamandra et al., 2004); the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) (Kaliberov et al., 2004); Fas ligand (CD95L/Apo1L) (Shinoura et al., 2000); melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL-24) (Su et al., 2006); and/or downregulation of antiapoptotic genes by siRNA, antisense oligos, or catalytic RNA (ribozymes), including *Bcl-2* (Hara et al., 2005), *Bcl-XL* (Mercatante et al., 2002), and *XIAP* and *survivin* (Cao et al., 2004).

5.4. Targeting of tumor microenvironment

Data from experimental and clinical studies indicate that the tumor–microenvironment interaction have an essential role in tumor growth. Development of radiation resistance is accompanied by remarkable changes in the gene expression profile of surrounding normal cells. Cross talk between malignant cells and other tumor-associated cells represents a new target for anticancer therapy, which has been explored through different forms of intervention. Strategies to inhibit tumor–microenvironment interaction include inhibition of expression of growth factors and their receptors, inhibition of growth factor signaling pathways, abrogation of the binding between ligands and their receptors, and prevention of intracellular transduction of the growth factor signal. The formation of new blood vessels is an important step in cancer progression. Tumor angiogenesis is a complex of coordinated interactions between numerous proteins, involved in different signaling pathways. Each step provides an opportunity for therapeutic intervention. Angiogenesis mediated by the vascular endothelial growth factor (VEGF) constitutes a new target for anticancer therapy, which has been explored through different forms of intervention aimed at blocking of tumor neovascularization. Strategies to inhibit tumor angiogenesis include inhibition of angiogenic factor production and their receptors, inhibition of the VEGF signaling pathway, inhibition of the binding between VEGF and its receptors, and inhibition of intracellular transduction of the VEGF signal (Kaliberov et al., 2005; Shi et al., 2003; Yang, Elliott, & Head, 2002). The importance of signal transduction through the VEGF receptor 2 is illustrated by use of soluble KDR. Treatment with AdVEGF-sKDR, encoding *sKDR* under the control of the human *VEGF* promoter, significantly inhibited the proliferation of human vascular endothelial cells and cancer cells. Ionizing radiation upregulated VEGF promoter activity in prostate carcinoma and endothelial cells. *In vivo* tumor therapy studies demonstrated significant inhibition of DU145 tumor growth in mice that received combined AdVEGF-sKDR infection and ionizing radiation versus AdVEGF-sKDR alone or radiation therapy alone (Kaliberov et al., 2005).

TNF- α has a broad range of potent anticancer properties including activation and recruitment of immune cells, and induction of hemorrhagic necrosis by disruption of the tumor vasculature. The oxidative damage produced by TNF- α might enhance cellular damage produced by ionizing radiation (Zimmerman, Marafino, Chan, Landre, & Winkelhake, 1989). Combination of *TNF- α* gene therapy with radiation produced an additive or a supra-additive increase in antitumor efficacy of a range of tumor cells in comparison with these modalities alone (Azria et al., 2003; Gridley et al., 1994; Hallahan et al., 1990). Furthermore, the combination of radiation with *TNF- α* gene delivery using Ad vectors encoding *TNF- α* under the control of the radiation-inducible CArG elements from the *Egr-1* gene increased radiation targeting specificity of cancer gene therapy (Weichselbaum & Kufe, 2009; Weichselbaum et al., 1994).

Cell growth is controlled by a balance of growth-promoting and growth-inhibiting factors. It is well known that a disturbed balance of growth factors in tumor cells results in uncontrolled cellular proliferation. Recent evidence suggests that radioresistance is due to, in part, tumor cell repopulation and involves several signaling pathways, including of the epidermal growth factor receptor (EGFR) pathway. Members of the EGFR family are overexpressed in many epithelial tumors. Cancer cells have been shown to upregulate the tyrosine kinase activity of EGFR in response to radiation therapy via ROS-mediated inhibition of protein tyrosine phosphatases, the enzymes responsible for dephosphorylating activated tyrosine kinases. Signaling by EGFR can also be upregulated in a delayed manner through the induction of paracrine growth factors, such as EGFR ligand transforming growth factor- α . EGFR overexpression has been linked to poor clinical outcomes with radiation therapy (Barrett et al., 2005; Harari & Huang, 2001; Sartor, 2000). Based on these findings, a variety of EGFR signaling-targeted therapeutic strategies have been developed including

monoclonal antibodies (cetuximab), tyrosine kinase inhibitors (erlotinib) as well as antisense oligonucleotides to EGFR or c-raf-1, serine/threonine protein kinase (Dritschilo et al., 2006; Gokhale et al., 1999; Lai et al., 2009; Lammering et al., 2003).

5.5. Immunomodulation

Immunotherapy is based on activation of a host immune response against the tumor cells using cytokines and activated immune cells. Immuno-modulation represents an approach to cancer therapy, in which induction of an antitumor immune response leads to tumor cell killing. Cancer cells express several unique tissue-specific proteins that can potentially function as target antigens for genetic immunotherapy. Although a large number of tumor-associated antigens that can be recognized by the immune system are expressed on the cell surface, tumor cells demonstrate relatively low immunogenicity. A large body of evidence demonstrates the ability of radiation to induce inflammation and expression of host-derived proinflammatory cytokines, such as IL-1 β and TNF- α via modification of the tumor microenvironment (Apetoh et al., 2007; Hallahan, Spriggs, Beckett, & Kufe, 1989; Ishihara, Tsuneoka, Dimchev, & Shikita, 1993). Also, it was shown that different immunotherapeutic strategies can be combined with radiation therapy to enhance antitumor effects as well as employing radiation therapy to promote tumor antigen presentation by dendritic cells (Demaria, Bhardwaj, McBride, & Formenti, 2005). Recently developed approaches in this field are strategies based on dendritic cells (Chakravarty et al., 1999; Demaria et al., 2004; Nikitina & Gabrilovich, 2001; Teitz-Tennenbaum et al., 2003), targeting of toll-like transmembrane receptors with CpG oligodeoxynucleotides (Jahrsdorfer & Weiner, 2008; Krieg, 2004; Mason et al., 2005; Yuan, Qiao, & Chen, 2011), vaccination with modified autologous tumor cells (Chakraborty et al., 2004; Lumniczky et al., 2002), and cytokines. Cytokines are proteins produced following activation of lymphocytes, macrophages, or other cells and are employed in immunotherapy to enhance immune mechanisms directed against tumors. Cytokines can induce a cascade of activation, proliferation, and differentiation of immune cells and increase an antitumor immune response. Interleukin 2 (IL-2), produced by activated T lymphocytes and critical for immune responsiveness, is one of the first cytokines/lymphokines that have been extensively explored in combination with radiation (Bray et al., 2003; Formenti, 2010; Hillman et al., 2004). Promising results have also emerged from studies using a wide range of immunomodulators in combination with radiation, including IL-3 (Chiang et al., 2000; Oh et al., 2004), IL-4 (Iwadate et al., 2003), IL-12 (Lohr et al., 2000; Nishitani et al., 2002; Yang et al., 2004), mda-7/IL-24 (Emdad et al., 2006; Yacoub et al., 2008; Yang et al., 2009), and TNF- α (Gridley et al., 2004; Weichselbaum et al., 1994, 2002).

5.6. Oncolytic virotherapy

At the present time, several strategies of cytoreductive gene therapy such as oncolytic virotherapy have been used for cancer treatment in combination with radiation. Conditional oncolytic virotherapy has employed replication-competent viral vectors that specifically kill tumor cells and represents a promising tool for cancer treatment. A major advantage of replicative oncolytic viral vectors is that their number should increase via viral replication within infected tumor cells, and the resulting viral progeny can infect and kill neighboring cancer cells within the tumor mass. G207 is an attenuated/replication-competent HSV-1 mutant that lacks both copies of the *ICP34.5 (RL1)* gene and contains an insertion of *lacZ* in the *ICP6 (UL39)* gene. These gene modifications offer a new modality in cancer therapy through the ability of G207 to selectively replicate within and kill malignant cells with minimal toxicity to normal tissues. G207 induced a significant inhibition of tumor growth alone and in combination with radiation in human head and neck cancer and prostate tumor xenograft models (Kim et al., 2005; Walker et al., 1999). Significant tumor growth delays were noted in *p53* mutant tumors receiving virotherapy using ONYX-015 (*d11520*) CRAd

vector containing a deletion in E1B-55kD protein in conjunction with irradiation at 5 Gy in comparison with CRAd or radiation alone (Geoerger et al., 2003; Rogulski et al., 2000). An EBV-dependent transcriptional targeting strategy was used to construct a novel conditionally replicating Ad, ad.oriP.E1A. The experimental group treated with ad.oriP.E1A in combination with ionizing radiation showed complete regressions of established tumors for at least 2 weeks in two distinct EBV-positive nasopharyngeal carcinoma xenograft models (Chia et al., 2004). CV706 is an oncolytic CRAd vector, in which the essential *E1A* viral replication gene is under the control of an exogenous minimal enhancer/promoter construct derived from the 5' flank of the human prostate-specific antigen (*PSA*) gene promoter that is preferentially active in prostate cancer cells (Rodriguez et al., 1997). CV706 has been shown to selectively kill human prostate cancer xenografts in preclinical models (DeWeese et al., 2001). KD1 and KD3 are replication-competent infectivity-enhanced Ads that contain mutations in the *E1A* gene that knock out binding of the E1A protein to cellular p300/CBP and pRB; these mutations facilitate KD1 and KD3 proliferation selectively in tumor cells, but not in normal cells. It was shown that the combination of irradiation with CRAd increased suppression of the growth of human tumor xenografts in nude mice in comparison with these treatments alone (Toth et al., 2003). Enhanced antitumor efficacy was also observed in radiation combination studies with Ad5-Delta24RGD (Lamfers et al., 2002) and the probasin promoter-driven CRAd expressing the *NIS* gene (Trujillo, Oneal, McDonough, Qin, & Morris, 2010). In an attempt to improve both the efficacy and safety of oncolytic virotherapy, a replication-competent Ad vector encoding a *CD/HSV-1 TK* fusion gene was constructed. Phase I studies demonstrated the safety of intraprostatic administration of this Ad in combination with conventional-dose three-dimensional conformal radiation therapy in patients with intermediate to high-risk prostate cancer (Freytag et al., 2003).

6. MOLECULAR CHEMOTHERAPY AND RADIATION THERAPY

One of the most widely investigated radiation therapy and gene therapy combination approaches utilizes molecular chemotherapy or suicide gene-directed enzyme/prodrug therapy (GDEPT). This strategy involves delivery of a specific enzyme that can produce cell death through the conversion of an inactive nontoxic prodrug into a cytotoxic drug metabolite. Specifically, targeted expression of the prodrug-activating enzyme avoids systemic toxicity and results in a high drug concentration in the tumor mass and an improved therapeutic index compared to systemic drug administration. The key element of a molecular chemotherapy system is a gene that encodes an enzyme, which converts a prodrug to an active cytotoxic drug. Importantly, prodrug-activating enzymes are normally absent or poorly expressed in mammalian cells. This means that tumor targeting of gene therapy, using specific gene delivery vehicles, can restrict enzyme expression to the transduced tumor cells and adjacent surrounding tumor cells through diffusion of the drug metabolite to generate a bystander effect.

6.1. Cytosine deaminase/5-fluorocytosine

One of the most promising molecular chemotherapy systems is cytosine deaminase/5-fluorocytosine (CD/5-FC) that has been studied intensively during the last decade. CD is a bacterial or yeast enzyme that can convert the antifungal agent 5-FC into the chemotherapy agent 5-fluorouracil (5-FU). Importantly, CD is present in prokaryotes and fungi but not in higher eukaryotes, where it is an important member of the pyrimidine salvage pathway. The antifungal agent 5-FC has been extensively used in the past as an antifungal agent for therapy of *Cryptococcus* and *Candida* infection and requires activation by the fungal CD. Therefore, its toxicological properties in humans are well established. 5-FU is a pyrimidine antagonist in which the hydrogen atom at the C-5 position of uracil is substituted by fluorine. Since the structure of 5-FU is similar to that of uracil, 5-FU is converted into

biologically active metabolites, 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) or 5-fluorouridine-5'-triphosphate as well as being metabolized to an inactive form, 5-fluoro- β -alanine, ammonia, and CO₂, by the same anabolic and catabolic pathways as that of uracil. 5-Fluorouracil triphosphate is incorporated into RNA and interferes with RNA processing, while FdUMP with the coenzyme N⁵,N¹⁰-methylene tetrahydrofolate forms a covalent ternary complex with thymidylate synthase (TS), an essential DNA *de novo* synthetic enzyme that catalyzes the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). This irreversibly inhibits the conversion of dUMP to dTMP and hence the disruption of nucleotide pools of dTMP, a thymidine precursor in DNA synthesis. The *codA* gene encoding bacterial CD (bCD) has been cloned from *Escherichia coli* into eukaryotic expression vectors and was first used for GDEPT of mouse fibroblasts *in vitro* and in murine tumor models almost two decades ago (Mullen, Coale, Lowe, & Blaese, 1994).

One of the major advantages of the CD/5-FC-mediated molecular chemotherapy system is a strong bystander effect that does not require gap junctions in contrast to other suicide gene therapy systems such as the HSV-TK/GCV, since 5-FU is a small, uncharged molecule capable of nonfacilitated diffusion through cellular membranes (Hoganson, Batra, Olsen, & Boucher, 1996; Nishihara et al., 1998; Trinh, Austin, Murray, Knick, & Huber, 1995). The bystander effect can be defined as an enlargement of cancer cell cytotoxicity in which nontransfected neighboring cells are killed through the transfer of metabolites of 5-FU from CD-expressing cells in close proximity. This means that even if only a small number of cancer cells express the *CD* gene, tumor eradication may still be achieved by using CD/5-FC molecular chemotherapy. Thus, the bystander activity is important for successful GDEPT due to the low transfection efficiency of gene delivery vectors currently employed for cancer gene therapy. Generally, molecular mechanisms of therapeutic agent-mediated cytotoxicity can be divided into two groups that involve development of a local immune-mediated effect and distant bystander activity. The bystander effect may be produced via the diffusion of a soluble chemotherapy drug such as 5-FU to adjacent surrounding cells (Huber, Austin, Richards, Davis, & Good, 1994; Kuriyama et al., 1998), via transfer of toxic products through gap junctions (Dilber et al., 1997; Elshami et al., 1996), or via apoptotic vesicles (Freeman et al., 1993). It was shown that gap junctions produce restricted transfer due to the requirement of cell-to-cell contact, and the levels of intercellular gap junction communication are often decreased between tumor cells (Holder, Elmore, & Barrett, 1993; Mesnil, Piccoli, Tiraby, Willecke, & Yamasaki, 1996).

Another advantage of the CD/5-FC system is based on the extensive pre-clinical and clinical experience with 5-FU as both a chemotherapeutic agent and a strong radiation sensitizer. Several groups have reported *in vivo* results with CD to have a significant bystander effect at clinically relevant 5-FC doses and radiation regimens (Kambara et al., 2002; Kievit et al., 2000; Kim et al., 1998). Intrinsic differences in sensitivity to 5-FU and ionizing radiation may contribute to overall efficacy of molecular chemotherapy in combination with radiation therapy. The precise mechanisms underlying the interaction of radiation and 5-FU are not clear. However, there are a number of potential pathways by which 5-FU could increase radiation sensitivity at the cellular level. Although the disruption of either RNA or DNA synthesis can produce cytotoxicity, a substantial body of evidence suggests that radiosensitization is a result of TS inhibition (Lawrence, Tepper, & Blackstock, 1997; Miller & Kinsella, 1992). It was shown that 5-FU produces tumor cell killing particularly during S phase, when cells are relatively radioresistant (McGinn & Lawrence, 2001). Also, the combination of 5-FU and radiation sensitization probably results in cells that are progressing inappropriately through S phase due to misrepair of DNA damage imposed by ionizing radiation. This loss of the S-phase checkpoint in cancer cells may provide the molecular basis for selective killing of tumors compared with normal tissues (Davis, Tang, Maybaum,

& Lawrence, 1995; Lawrence, Davis, & Loney, 1996). The hydrolytic deamination of cytosine that produces a miscoding uracil residue is a frequent DNA lesion induced by oxidative damage, through exposure to ROS-producing agents such as ionizing radiation. Cytostatic agents interfering with pyrimidine metabolism may enhance uracil incorporation by increasing the dUTP:dTTP ratio. Thus, cytostatic drugs inhibiting TS, for example, 5-FU may enhance uracil incorporation and thus contribute to the cytotoxicity of combined CD/5-FC GDEPT with radiation therapy. Analysis of the linear quadratic or single-hit-multi-target parameters of survival curves indicated a significant reduction in cell survival at both low and high doses of radiation therapy (Pederson et al., 1997; Stackhouse et al., 2000). The combination of CD/5-FC and ionizing radiation produced a measurable antitumor effect using 2 or 5 Gy per fraction over 1 week which is a clinically relevant dose regimen of radiation therapy (Stackhouse et al., 2000). Additional preclinical studies have been carried out using yCD (Hamstra et al., 1999), and clinical trials have been conducted in patients with prostate cancer administered a replicative Ad expressing *CD* in combination with radiotherapy with promising results (Freytag et al., 2002, 2007).

A major problem associated with this GDEPT approach is the low affinity displayed by the *CD* gene product toward 5-FC in comparison with cytosine. Recent studies have demonstrated that substitution of an alanine (A) for the aspartic acid (D) at position 314 of bCD increased relative specificity of the mutant bCD-D314A enzyme to 5-FC in comparison with bCDwt and may be a superior suicide gene (Mahan, Ireton, Knoeber, Stoddard, & Black, 2004; Mahan, Ireton, Stoddard, & Black, 2004). It was shown that infection of AdbCD-D314A vector encoding a mutant *bCD* gene resulted in increased 5-FC-mediated cell killing, compared with AdbCDwt. Furthermore, a significantly greater inhibition of growth of human pancreatic tumor xenografts was produced by the combination of AdbCD-D314A/5-FC with radiation as compared with molecular chemotherapy or radiation therapy alone (Kaliberova et al., 2008). Also, animal studies showed significant inhibition of subcutaneous or intracranial tumor growth of human glioma xenografts by the combination of AdbCD-D314A/5-FC with ionizing radiation as compared with either agent alone or AdbCDwt/5-FC plus radiation. The results suggest that the combination of CD/5-FC with radiation produces markedly increased cytotoxic effects in cancer cells *in vitro* and *in vivo* (Kaliberov et al., 2007).

6.2. Herpes simplex virus-thymidine kinase/ganciclovir

Another well-studied suicide gene/prodrug system for cancer therapy employs a combination of HSV-TK with antiherpetic drugs such as GCV or its analogues (acyclovir and valacyclovir) (Eastham et al., 1996). The HSV-TK/GCV system is characterized by the effective phosphorylation of GCV into a toxic compound that is incorporated into DNA during its replication. This incorporation into guanine sites in newly synthesized DNA chains causes termination of synthesis and the selective killing of dividing cells by activation of apoptosis pathways. It was shown that HSV-TK/GCV molecular chemotherapy sensitized cancer cells to ionizing radiation (Atkinson & Hall, 1999; Kim et al., 1997). A number of Ad vectors encoding the *HSV-TK* gene were employed in combination with ionizing radiation and have shown therapeutic effects in clinical trials without substantial toxic effects when used alone or in combination with radiotherapy (Blackburn et al., 1998; Cheon et al., 2000; Eastham et al., 1996). In an extensive series of preclinical studies, a combination of radiation therapy and molecular chemotherapy using Ad vectors encoding *HSV-TK* or *CD:HSV-TK* fusion gene demonstrated potent tumor cell radiosensitization activity (Chen et al., 2001; Teh et al., 2001; Tetzlaff et al., 2006).

Currently, a number of alternative suicide GDEPT systems using various gene delivery systems are under intense investigation. A series of clinical trials using a replication-defective Ad vector expressing *E. coli* nitroreductase (*NTR*) gene were initiated in patients

with primary or secondary liver cancer, head and neck cancer, or prostate cancer (Searle et al., 2004). It was shown that the prodrug CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide) is activated by NTR to a potent DNA-cross-linking agent. Adenoviral delivery of *NTR* gene in combination with CB1954 administration in nude mice enhanced the effect of fractionated radiotherapy using four fractions at 3 Gy in SW480 xenograft tumors (White et al., 2008). Another example of a novel molecular chemotherapy system is the combination of gene delivery of horseradish peroxidase (HRP) with indole-3-acetic (IAA) as a prodrug was developed and its antitumor effect was evaluated *in vitro* and *in vivo* (Xu et al., 2011). Metabolism of IAA by HRP produces a wide range of cytotoxic species, including superoxide and peroxy radicals (de Melo, de Lima, Pithon-Curi, & Curi, 2004) and increased cytotoxicity in combination with irradiation (Greco et al., 2002).

7. CONCLUSION

Although in recent years significant progress in radiation therapy methods has been achieved, development of resistance to irradiation remains a major problem for successful control of cancer in patients. The combination of gene therapy and radiation therapy could improve the outcome of cancer treatment. Important advances in the combination of irradiation and gene therapy over the last decade are a result of improved understanding of the molecular mechanisms that mediate cancer progression and resistance to radiation treatment. Ionizing radiation produces DNA damage and activates multiple intracellular signaling pathways. The ability of radiation to activate pathways depends on the levels of DNA damage, generation of ROS/RNS, activation of a variety of transcription factors, as well as alterations in the expression of growth factor receptors and their cognate ligands. A growing body of evidence suggests that interaction between tumor cells and surrounding normal cells controls local tumor development and involves the development of radiation resistance. Intervention against the interaction between tumor cells and their microenvironment is a promising area for the development of combination of gene therapy and radiation therapy modalities. This would provide the possibility to utilize multiple targeting approaches for combined gene therapy and radiotherapy.

Modifications of gene delivery systems, such as using tumor-specific promoters for selective expression of therapeutic genes, or development of new oncolytic virotherapy vectors that are designed to selectively replicate in tumor cells or tumor-associated cells, should significantly increase the efficacy and safety of cancer gene therapy. These efforts for development of cancer gene therapy vectors in combination with radiation can be illustrated by using transductional and transcriptional approaches for targeting of Ad vectors. Importantly, irradiation at therapeutic doses can increase the level of viral uptake, replication, and gene expression. Manipulation of the Ad capsid can modify viral tropism by binding of Ad to receptors other than the coxsackie and adenovirus receptor (CAR)—the primary receptor for Ad5. This can be achieved through genetic modification of Ad5 fiber knob, employment of adaptor molecules (i.e., fusion protein comprising extracellular domain of CAR and tumor-specific single-chain variable fragment), or chemical modification such as PEGylation. The modification of the fiber or fiber knob in Ad serotype 5 with an alternative fiber knob can increase CAR-independent binding of Ad5-based vectors. Additionally, the use of specific promoters allows for Ad vector-mediated tumor targeting of therapeutic gene expression. These modifications increase specificity and reduce the risk of toxic side effects with the combination of gene therapy and radiation therapy. Challenges regarding current approaches and future directions include uptake of vectors in metastatic tumor sites after systemic administration, and adequate expression of the transgene to produce radiosensitization. Importantly, the combination of such targeted cancer therapies can contribute to the growing field of personalized cancer medicine that is based on the unique genetic characteristics of the tumor as well as the patient.

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References

- Adamina M, Daetwiler S, Rosenthal R, Zajac P. Clinical applications of recombinant virus-based cancer immunotherapy. *Expert Opinion on Biological Therapy*. 2005; 5:1211–1224. [PubMed: 16120051]
- Aggarwal BB, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, Koca C, et al. Signal transducer and activator of transcription-3, inflammation, and cancer: How intimate is the relationship? *Annals of the New York Academy of Sciences*. 2009; 1171:59–76. [PubMed: 19723038]
- Andreassen PR, Ho GP, D'Andrea AD. DNA damage responses and their many interactions with the replication fork. *Carcinogenesis*. 2006; 27:883–892. [PubMed: 16490739]
- Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Medicine*. 2007; 13:1050–1059.
- Arlen PM, Gulley JL, Madan RA, Hodge JW, Schlom J. Preclinical and clinical studies of recombinant poxvirus vaccines for carcinoma therapy. *Critical Reviews in Immunology*. 2007; 27:451–462. [PubMed: 18197807]
- Atkinson G, Hall SJ. Prodrug activation gene therapy and external beam irradiation in the treatment of prostate cancer. *Urology*. 1999; 54:1098–1104. [PubMed: 10604717]
- Azria D, Dorvillius M, Gourgou S, Martineau P, Robert B, Pigniere M, et al. Enhancement of radiation therapy by tumor necrosis factor alpha in human colon cancer using a bispecific antibody. *International Journal of Radiation Oncology, Biology, Physics*. 2003; 55:1363–1373.
- Bakkenist CJ, Kastan MB. Phosphatases join kinases in DNA-damage response pathways. *Trends in Cell Biology*. 2004; 14:339–341. [PubMed: 15246426]
- Barrett DM, Black SM, Todor H, Schmidt-Ullrich RK, Dawson KS, Mikkelsen RB. Inhibition of protein-tyrosine phosphatases by mild oxidative stresses is dependent on S-nitrosylation. *The Journal of Biological Chemistry*. 2005; 280:14453–14461. [PubMed: 15684422]
- Bauerschmitz GJ, Barker SD, Hemminki A. Adenoviral gene therapy for cancer: From vectors to targeted and replication competent agents (review). *International Journal of Oncology*. 2002; 21:1161–1174. [PubMed: 12429964]
- Beetz A, Peter RU, Oppel T, Kaffenberger W, Rupec RA, Meyer M, et al. NF-kappaB and AP-1 are responsible for inducibility of the IL-6 promoter by ionizing radiation in HeLa cells. *International Journal of Radiation Biology*. 2000; 76:1443–1453. [PubMed: 11098847]
- Berthault N, Maury B, Agrario C, Herbette A, Sun JS, Peyrieras N, et al. Comparison of distribution and activity of nanoparticles with short interfering DNA (Dbait) in various living systems. *Cancer Gene Therapy*. 2011; 18:695–706. [PubMed: 21799529]
- Bickenbach KA, Veerapong J, Shao MY, Mauceri HJ, Posner MC, Kron SJ, et al. Resveratrol is an effective inducer of CAR-driven TNF-alpha gene therapy. *Cancer Gene Therapy*. 2008; 15:133–139. [PubMed: 18157146]
- Bjornsti MA, Houghton PJ. The TOR pathway: A target for cancer therapy. *Nature Reviews. Cancer*. 2004; 4:335–348.
- Blackburn RV, Galoforo SS, Corry PM, Lee YJ. Adenoviral-mediated transfer of a heat-inducible double suicide gene into prostate carcinoma cells. *Cancer Research*. 1998; 58:1358–1362. [PubMed: 9537229]
- Blalock WL, Weinstein-Oppenheimer C, Chang F, Hoyle PE, Wang XY, Algate PA, et al. Signal transduction, cell cycle regulatory, and anti-apoptotic pathways regulated by IL-3 in hematopoietic cells: Possible sites for intervention with anti-neoplastic drugs. *Leukemia*. 1999; 13:1109–1166. [PubMed: 10450743]

- Blundell R, Kaminski N, Harrison D. Increase in p21 expression independent of the p53 pathway in bleomycin-induced lung fibrosis. *Experimental and Molecular Pathology*. 2004; 77:231–237. [PubMed: 15507241]
- Boothman DA, Lee IW, Sahijdak WM. Isolation of an X-ray-responsive element in the promoter region of tissue-type plasminogen activator: Potential uses of X-ray-responsive elements for gene therapy. *Radiation Research*. 1994; 138:S68–S71. [PubMed: 8146331]
- Bray D, Yu SZ, Koprowski H 2nd, Rhee J, Kumar S, Pericle F, et al. Combination nonviral interleukin 2 gene therapy and external-beam radiation therapy for head and neck cancer. *Archives of Otolaryngology—Head & Neck Surgery*. 2003; 129:618–622. [PubMed: 12810463]
- Burma S, Chen BP, Chen DJ. Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. *DNA Repair*. 2006; 5:1042–1048. [PubMed: 16822724]
- Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *The Journal of Biological Chemistry*. 2001; 276:42462–42467. [PubMed: 11571274]
- Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science*. 1998; 281:1677–1679. [PubMed: 9733515]
- Cao C, Mu Y, Hallahan DE, Lu B. XIAP and survivin as therapeutic targets for radiation sensitization in preclinical models of lung cancer. *Oncogene*. 2004; 23:7047–7052. [PubMed: 15258565]
- Chakraborty M, Abrams SI, Coleman CN, Camphausen K, Schlom J, Hodge JW. External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. *Cancer Research*. 2004; 64:4328–4337. [PubMed: 15205348]
- Chakravarty PK, Alfieri A, Thomas EK, Beri V, Tanaka KE, Vikram B, et al. Flt3-ligand administration after radiation therapy prolongs survival in a murine model of metastatic lung cancer. *Cancer Research*. 1999; 59:6028–6032. [PubMed: 10626784]
- Chen Y, DeWeese T, Dilley J, Zhang Y, Li Y, Ramesh N, et al. CV706, a prostate cancer-specific adenovirus variant, in combination with radiotherapy produces synergistic antitumor efficacy without increasing toxicity. *Cancer Research*. 2001; 61:5453–5460. [PubMed: 11454691]
- Chen GG, Sin FL, Leung BC, Ng HK, Poon WS. Glioblastoma cells deficient in DNA-dependent protein kinase are resistant to cell death. *Journal of Cellular Physiology*. 2005; 203:127–132. [PubMed: 15493013]
- Cheon J, Kim HK, Moon DG, Yoon DK, Cho JH, Koh SK. Adenovirus-mediated suicide-gene therapy using the herpes simplex virus thymidine kinase gene in cell and animal models of human prostate cancer: Changes in tumour cell proliferative activity. *BJU International*. 2000; 85:759–766. [PubMed: 10759680]
- Chia MC, Shi W, Li JH, Sanchez O, Strathdee CA, Huang D, et al. A conditionally replicating adenovirus for nasopharyngeal carcinoma gene therapy. *Molecular Therapy*. 2004; 9:804–817. [PubMed: 15194047]
- Chiang CS, Hong JH, Wu YC, McBride WH, Dougherty GJ. Combining radiation therapy with interleukin-3 gene immunotherapy. *Cancer Gene Therapy*. 2000; 7:1172–1178. [PubMed: 10975678]
- Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends in Biochemical Sciences*. 2003; 28:509–514. [PubMed: 13678963]
- Christy B, Nathans D. Functional serum response elements upstream of the growth factor-inducible gene zif268. *Molecular and Cellular Biology*. 1989; 9:4889–4895. [PubMed: 2513479]
- Cotugno G, Aurilio M, Annunziata P, Capalbo A, Faella A, Rinaldi V, et al. Noninvasive repetitive imaging of somatostatin receptor 2 gene transfer with positron emission tomography. *Human Gene Therapy*. 2011; 22:189–196. [PubMed: 20825281]
- Coulter JA, McCarthy HO, Worthington J, Robson T, Scott S, Hirst DG. The radiation-inducible pE9 promoter driving inducible nitric oxide synthase radiosensitizes hypoxic tumour cells to radiation. *Gene Therapy*. 2008; 15:495–503. [PubMed: 18256696]
- Criswell T, Leskov K, Miyamoto S, Luo G, Boothman DA. Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. *Oncogene*. 2003; 22:5813–5827. [PubMed: 12947388]

- Daino K, Ichimura S, Neno M. Comprehensive search for X-ray-responsive elements and binding factors in the regulatory region of the GADD45a gene. *Journal of Radiation Research (Tokyo)*. 2003; 44:311–318.
- Daino K, Ichimura S, Neno M. Both the basal transcriptional activity of the GADD45A gene and its enhancement after ionizing irradiation are mediated by AP-1 element. *Biochimica et Biophysica Acta*. 2006; 1759:458–469. [PubMed: 17084916]
- Datta R, Rubin E, Sukhatme V, Qureshi S, Hallahan D, Weichselbaum RR, et al. Ionizing radiation activates transcription of the EGR1 gene via CArG elements. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; 89:10149–10153. [PubMed: 1332031]
- Datta R, Taneja N, Sukhatme VP, Qureshi SA, Weichselbaum R, Kufe DW. Reactive oxygen intermediates target CC(A/T)6GG sequences to mediate activation of the early growth response 1 transcription factor gene by ionizing radiation. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90:2419–2422. [PubMed: 8384722]
- Davis MA, Tang HY, Maybaum J, Lawrence TS. Dependence of fluorodeoxyuridine-mediated radiosensitization on S phase progression. *International Journal of Radiation Biology*. 1995; 67:509–517. [PubMed: 7775826]
- de Melo MP, de Lima TM, Pithon-Curi TC, Curi R. The mechanism of indole acetic acid cytotoxicity. *Toxicology Letters*. 2004; 148:103–111. [PubMed: 15019094]
- Demaria S, Bhardwaj N, McBride WH, Formenti SC. Combining radiotherapy and immunotherapy: A revived partnership. *International Journal of Radiation Oncology, Biology, Physics*. 2005; 63:655–666.
- Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *International Journal of Radiation Oncology, Biology, Physics*. 2004; 58:862–870.
- DeWeese TL, van der Poel H, Li S, Mikhak B, Drew R, Goemann M, et al. A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Research*. 2001; 61:7464–7472. [PubMed: 11606381]
- Dilber MS, Abedi MR, Christensson B, Bjorkstrand B, Kidder GM, Naus CC, et al. Gap junctions promote the bystander effect of herpes simplex virus thymidine kinase *in vivo*. *Cancer Research*. 1997; 57:1523–1528. [PubMed: 9108455]
- Dritschilo A, Huang CH, Rudin CM, Marshall J, Collins B, Dul JL, et al. Phase I study of liposome-encapsulated c-ras antisense oligodeoxyribonucleotide infusion in combination with radiation therapy in patients with advanced malignancies. *Clinical Cancer Research*. 2006; 12:1251–1259. [PubMed: 16489081]
- Eastham JA, Chen SH, Sehgal I, Yang G, Timme TL, Hall SJ, et al. Prostate cancer gene therapy: Herpes simplex virus thymidine kinase gene transduction followed by ganciclovir in mouse and human prostate cancer models. *Human Gene Therapy*. 1996; 7:515–523. [PubMed: 8800746]
- el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, et al. WAF1, a potential mediator of p53 tumor suppression. *Cell*. 1993; 75:817–825. [PubMed: 8242752]
- Elshami AA, Saavedra A, Zhang H, Kucharczuk JC, Spray DC, Fishman GI, et al. Gap junctions play a role in the ‘bystander effect’ of the herpes simplex virus thymidine kinase/ganciclovir system *in vitro*. *Gene Therapy*. 1996; 3:85–92. [PubMed: 8929915]
- Emdad L, Sarkar D, Lebedeva IV, Su ZZ, Gupta P, Mahasreshti PJ, et al. Ionizing radiation enhances adenoviral vector expressing mda-7/IL-24-mediated apoptosis in human ovarian cancer. *Journal of Cellular Physiology*. 2006; 208:298–306. [PubMed: 16646087]
- Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*. 2005; 434:605–611. [PubMed: 15758953]
- Fischer U, Meese E. Glioblastoma multiforme: The role of DSB repair between genotype and phenotype. *Oncogene*. 2007; 26:7809–7815. [PubMed: 18066094]
- Formenti SC. Immunological aspects of local radiotherapy: Clinical relevance. *Discovery Medicine*. 2010; 9:119–124. [PubMed: 20193637]

- Fornace AJ Jr, Alamo I Jr, Hollander MC. DNA damage-inducible transcripts in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:8800–8804. [PubMed: 3194391]
- Fornace AJ Jr, Nebert DW, Hollander MC, Luethy JD, Papathanasiou M, Fargnoli J, et al. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Molecular and Cellular Biology*. 1989; 9:4196–4203. [PubMed: 2573827]
- Freeman SM, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, et al. The “bystander effect”: Tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Research*. 1993; 53:5274–5283. [PubMed: 8221662]
- Freytag SO, Khil M, Stricker H, Peabody J, Menon M, DePeralta-Venturina M, et al. Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. *Cancer Research*. 2002; 62:4968–4976. [PubMed: 12208748]
- Freytag SO, Stricker H, Peabody J, Pegg J, Paielli D, Movsas B, et al. Five-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy for treatment of prostate cancer. *Molecular Therapy*. 2007; 15:636–642. [PubMed: 17228316]
- Freytag SO, Stricker H, Pegg J, Paielli D, Pradhan DG, Peabody J, et al. Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer. *Cancer Research*. 2003; 63:7497–7506. [PubMed: 14612551]
- Galabova-Kovacs G, Kolbus A, Matzen D, Meissl K, Piazzolla D, Rubiolo C, et al. ERK and beyond: Insights from B-Raf and Raf-1 conditional knockouts. *Cell Cycle*. 2006; 5:1514–1518. [PubMed: 16861903]
- Gallardo D, Drazan KE, McBride WH. Adenovirus-based transfer of wild-type *p53* gene increases ovarian tumor radiosensitivity. *Cancer Research*. 1996; 56:4891–4893. [PubMed: 8895740]
- Gatei M, Sloper K, Sorensen C, Syljuasen R, Falck J, Hobson K, et al. Ataxia-telangiectasia-mutated (ATM) and NBS1-dependent phosphorylation of Chk1 on Ser-317 in response to ionizing radiation. *The Journal of Biological Chemistry*. 2003; 278:14806–14811. [PubMed: 12588868]
- Georger B, Grill J, Opolon P, Morizet J, Aubert G, Lecluse Y, et al. Potentiation of radiation therapy by the oncolytic adenovirus dl1520 (ONYX-015) in human malignant glioma xenografts. *British Journal of Cancer*. 2003; 89:577–584. [PubMed: 12888833]
- Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GA. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. *Nature*. 1990; 343:774–778. [PubMed: 2154702]
- Glover DJ, Lipps HJ, Jans DA. Towards safe, non-viral therapeutic gene expression in humans. *Nature Reviews. Genetics*. 2005; 6:299–310.
- Gokhale PC, McRae D, Monia BP, Bagg A, Rahman A, Dritschilo A, et al. Antisense raf oligodeoxynucleotide is a radiosensitizer in vivo. *Antisense & Nucleic Acid Drug Development*. 1999; 9:191–201. [PubMed: 10355825]
- Goldkorn T, Balaban N, Shannon M, Matsukuma K. EGF receptor phosphorylation is affected by ionizing radiation. *Biochimica et Biophysica Acta*. 1997; 1358:289–299. [PubMed: 9366260]
- Greco O, Powell TM, Marples B, Joiner MC, Scott SD. Gene therapy vectors containing CARG elements from the *Egr1* gene are activated by neutron irradiation, cisplatin and doxorubicin. *Cancer Gene Therapy*. 2005; 12:655–662. [PubMed: 15818381]
- Greco O, Tozer GM, Dachs GU. Oxidative and anoxic enhancement of radiation-mediated toxicity by horseradish peroxidase/indole-3-acetic acid gene therapy. *International Journal of Radiation Biology*. 2002; 78:173–181. [PubMed: 11869472]
- Gridley DS, Glisson WC, Uhm JR. Interaction of tumour necrosis factor- α and radiation against human colon tumour cells. *Therapeutic Immunology*. 1994; 1:25–31. [PubMed: 7584476]
- Gridley DS, Miller GM, Luo X, Cao JD, Timiryasova TM, Fodor I, et al. Proton radiation and TNF- α /Bax gene therapy for orthotopic C6 brain tumor in Wistar rats. *Technology in Cancer Research & Treatment*. 2004; 3:217–227. [PubMed: 15059028]
- Gulbins E, Kolesnick R. Raft ceramide in molecular medicine. *Oncogene*. 2003; 22:7070–7077. [PubMed: 14557812]

- Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *The New England Journal of Medicine*. 2003; 348:255–256. [PubMed: 12529469]
- Hallahan DE, Beckett MA, Kufe D, Weichselbaum RR. The interaction between recombinant human tumor necrosis factor and radiation in 13 human tumor cell lines. *International Journal of Radiation Oncology, Biology, Physics*. 1990; 19:69–74.
- Hallahan DE, Mauceri HJ, Seung LP, Dunphy EJ, Wayne JD, Hanna NN, et al. Spatial and temporal control of gene therapy using ionizing radiation. *Nature Medicine*. 1995; 1:786–791.
- Hallahan DE, Spriggs DR, Beckett MA, Kufe DW. Increased tumor necrosis factor alpha mRNA after cellular exposure to ionizing radiation. *Proceedings of the National Academy of Sciences of the United States of America*. 1989; 86:10104–10107. [PubMed: 2602359]
- Hallahan DE, Sukhatme VP, Sherman ML, Virudachalam S, Kufe D, Weichselbaum RR. Protein kinase C mediates x-ray inducibility of nuclear signal transducers EGR1 and JUN. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88:2156–2160. [PubMed: 1900938]
- Hallahan DE, Virudachalam S, Beckett M, Sherman ML, Kufe D, Weichselbaum RR. Mechanisms of X-ray-mediated protooncogene c-jun expression in radiation-induced human sarcoma cell lines. *International Journal of Radiation Oncology, Biology, Physics*. 1991; 21:1677–1681.
- Hamdi M, Kool J, Cornelissen-Steijger P, Carlotti F, Popeijus HE, van der Burgt C, et al. DNA damage in transcribed genes induces apoptosis via the JNK pathway and the JNK-phosphatase MKP-1. *Oncogene*. 2005; 24:7135–7144. [PubMed: 16044158]
- Hamstra DA, Rice DJ, Pu A, Oyedijo D, Ross BD, Rehemtulla A. Combined radiation and enzyme/prodrug treatment for head and neck cancer in an orthotopic animal model. *Radiation Research*. 1999; 152:499–507. [PubMed: 10521927]
- Hara T, Omura-Minamisawa M, Chao C, Nakagami Y, Ito M, Inoue T. Bcl-2 inhibitors potentiate the cytotoxic effects of radiation in Bcl-2 overexpressing radioresistant tumor cells. *International Journal of Radiation Oncology, Biology, Physics*. 2005; 61:517–528.
- Harari PM, Huang SM. Radiation response modification following molecular inhibition of epidermal growth factor receptor signaling. *Seminars in Radiation Oncology*. 2001; 11:281–289. [PubMed: 11677653]
- Higuchi Y, Asaumi J, Murakami J, Matsuzaki H, Wakasa T, Inoue T, et al. Thermoradiotherapy combined with adenoviral p53 gene therapy in head and neck squamous cell carcinoma cell lines. *Oncology Reports*. 2003; 10:415–420. [PubMed: 12579282]
- Hillman GG, Slos P, Wang Y, Wright JL, Layer A, De Meyer M, et al. Tumor irradiation followed by intratumoral cytokine gene therapy for murine renal adenocarcinoma. *Cancer Gene Therapy*. 2004; 11:61–72. [PubMed: 14681727]
- Hoganson DK, Batra RK, Olsen JC, Boucher RC. Comparison of the effects of three different toxin genes and their levels of expression on cell growth and bystander effect in lung adenocarcinoma. *Cancer Research*. 1996; 56:1315–1323. [PubMed: 8640820]
- Holder JW, Elmore E, Barrett JC. Gap junction function and cancer. *Cancer Research*. 1993; 53:3475–3485. [PubMed: 8393376]
- Hsu H, Rainov NG, Quinones A, Eling DJ, Sakamoto KM, Spear MA. Combined radiation and cytochrome CYP4B1/4-ipomeanol gene therapy using the EGR1 promoter. *Anticancer Research*. 2003; 23:2723–2728. [PubMed: 12894565]
- Huber BE, Austin EA, Richards CA, Davis ST, Good SS. Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with cytosine deaminase gene: Significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:8302–8306. [PubMed: 8058798]
- Hynes NE, Lane HA. ERBB receptors and cancer: The complexity of targeted inhibitors. *Nature Reviews. Cancer*. 2005; 5:341–354.
- Ishida A, Sasaguri T, Kosaka C, Nojima H, Ogata J. Induction of the cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1) by nitric oxide-generating vasodilator in vascular smooth muscle cells. *The Journal of Biological Chemistry*. 1997; 272:10050–10057. [PubMed: 9092548]

- Ishihara H, Tsuneoka K, Dimchev AB, Shikita M. Induction of the expression of the interleukin-1 beta gene in mouse spleen by ionizing radiation. *Radiation Research*. 1993; 133:321–326. [PubMed: 8383862]
- Ishizawar R, Parsons SJ. c-Src and cooperating partners in human cancer. *Cancer Cell*. 2004; 6:209–214. [PubMed: 15380511]
- Iwadate Y, Yamaura A, Sakiyama S, Sato Y, Tagawa M. Glioma-specific cytotoxic T cells can be effectively induced by subcutaneous vaccination of irradiated wild-type tumor cells without artificial cytokine production. *International Journal of Oncology*. 2003; 23:483–488. [PubMed: 12851699]
- Jahrsdorfer B, Weiner GJ. CpG oligodeoxynucleotides as immunotherapy in cancer. *Update on Cancer Therapeutics*. 2008; 3:27–32. [PubMed: 19255607]
- Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, et al. ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nature Cell Biology*. 2006; 8:37–45.
- Jia ZY, Deng HF, Huang R, Yang YY, Yang XC, Qi ZZ, et al. In vitro and in vivo studies of adenovirus-mediated human norepinephrine transporter gene transduction to hepatocellular carcinoma. *Cancer Gene Therapy*. 2011; 18:196–205. [PubMed: 21072068]
- Jin S, Fan F, Fan W, Zhao H, Tong T, Blanck P, et al. Transcription factors Oct-1 and NF-YA regulate the p53-independent induction of the GADD45 following DNA damage. *Oncogene*. 2001; 20:2683–2690. [PubMed: 11420680]
- Joki T, Nakamura M, Ohno T. Activation of the radiosensitive EGR-1 promoter induces expression of the herpes simplex virus thymidine kinase gene and sensitivity of human glioma cells to ganciclovir. *Human Gene Therapy*. 1995; 6:1507–1513. [PubMed: 8664375]
- Kaliberov S, Buchsbaum DJ, Gillespie GY, Curiel DT, Carpenter M, Arafat WO, et al. Adenovirus-mediated transfer of Bax driven by the vascular endothelial growth factor promoter induces apoptosis in lung cancer cells. *Molecular Therapy*. 2002; 6:190–198. [PubMed: 12161185]
- Kaliberov SA, Kaliberova LN, Buchsbaum DJ. Combined ionizing radiation and *sKDR* gene delivery for treatment of prostate carcinomas. *Gene Therapy*. 2005; 12:407–417. [PubMed: 15616600]
- Kaliberov SA, Kaliberova L, Stockard CR, Grizzle WE, Buchsbaum DJ. Adenovirus-mediated flt-1 targeted proapoptotic gene therapy of human prostate cancer. *Molecular Therapy*. 2004; 10:1059–1070. [PubMed: 15564138]
- Kaliberov SA, Markert JM, Gillespie GY, Krendelchtchikova V, Della Manna D, Sellers JC, et al. Mutation of *Escherichia coli* cytosine deaminase significantly enhances molecular chemotherapy of human glioma. *Gene Therapy*. 2007; 14:1111–1119. [PubMed: 17495948]
- Kaliberova LN, Della Manna DL, Krendelchtchikova V, Black ME, Buchsbaum DJ, Kaliberov SA. Molecular chemotherapy of pancreatic cancer using novel mutant bacterial cytosine deaminase gene. *Molecular Cancer Therapeutics*. 2008; 7:2845–2854. [PubMed: 18790765]
- Kambara H, Tamiya T, Ono Y, Ohtsuka S, Terada K, Adachi Y, et al. Combined radiation and gene therapy for brain tumors with adenovirus-mediated transfer of cytosine deaminase and uracil phosphoribosyltransferase genes. *Cancer Gene Therapy*. 2002; 9:840–845. [PubMed: 12224025]
- Kauffmann A, Rosselli F, Lazar V, Winnepenninckx V, Mansuet-Lupo A, Dessen P, et al. High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene*. 2008; 27:565–573. [PubMed: 17891185]
- Kavanagh BD, Dent P, Schmidt-Ullrich RK, Chen P, Mikkelsen RB. Calcium-dependent stimulation of mitogen-activated protein kinase activity in A431 cells by low doses of ionizing radiation. *Radiation Research*. 1998; 149:579–587. [PubMed: 9611096]
- Kavanagh BD, Lin PS, Chen P, Schmidt-Ullrich RK. Radiation-induced enhanced proliferation of human squamous cancer cells *in vitro*: A release from inhibition by epidermal growth factor. *Clinical Cancer Research*. 1995; 1:1557–1562. [PubMed: 9815956]
- Khanna KK, Lavin MF. Ionizing radiation and UV induction of p53 protein by different pathways in ataxia-telangiectasia cells. *Oncogene*. 1993; 8:3307–3312. [PubMed: 8247533]
- Kievit E, Nyati MK, Ng E, Stegman LD, Parsels J, Ross BD, et al. Yeast cytosine deaminase improves radiosensitization and bystander effect by 5-fluorocytosine of human colorectal cancer xenografts. *Cancer Research*. 2000; 60:6649–6655. [PubMed: 11118048]

- Kim SH, Kim JH, Kolozsvary A, Brown SL, Freytag SO. Preferential radiosensitization of 9L glioma cells transduced with HSV-tk gene by acyclovir. *Journal of Neuro-Oncology*. 1997; 33:189–194. [PubMed: 9195489]
- Kim JH, Kolozsvary A, Rogulski K, Khil MS, Brown SL, Freytag SO. Selective radiosensitization of 9L glioma in the brain transduced with double suicide fusion gene. *The Cancer Journal from Scientific American*. 1998; 4:364–369. [PubMed: 9853135]
- Kim SH, Wong RJ, Kooby DA, Carew JF, Adusumilli PS, Patel SG, et al. Combination of mutated herpes simplex virus type 1 (G207 virus) with radiation for the treatment of squamous cell carcinoma of the head and neck. *European Journal of Cancer*. 2005; 41:313–322. [PubMed: 15661558]
- Krieg AM. Antitumor applications of stimulating toll-like receptor 9 with CpG oligodeoxynucleotides. *Current Oncology Reports*. 2004; 6:88–95. [PubMed: 14751085]
- Kuriyama S, Masui K, Sakamoto T, Nakatani T, Kikukawa M, Tsujinoue H, et al. Bystander effect caused by cytosine deaminase gene and 5-fluorocytosine in vitro is substantially mediated by generated 5-fluorouracil. *Anticancer Research*. 1998; 18:3399–3406. [PubMed: 9858915]
- Lai SY, Koppikar P, Thomas SM, Childs EE, Egloff AM, Seethala RR, et al. Intratumoral epidermal growth factor receptor antisense DNA therapy in head and neck cancer: First human application and potential antitumor mechanisms. *Journal of Clinical Oncology*. 2009; 27:1235–1242. [PubMed: 19204206]
- Lamfers ML, Grill J, Dirven CM, Van Beusechem VW, Geoerger B, Van Den Berg J, et al. Potential of the conditionally replicative adenovirus Ad5-Delta24RGD in the treatment of malignant gliomas and its enhanced effect with radiotherapy. *Cancer Research*. 2002; 62:5736–5742. [PubMed: 12384532]
- Lammering G, Hewit TH, Valerie K, Lin PS, Contessa JN, Schmidt-Ullrich RK. Anti-erbB receptor strategy as a gene therapeutic intervention to improve radiotherapy in malignant human tumours. *International Journal of Radiation Biology*. 2003; 79:561–568. [PubMed: 14530165]
- Lawrence TS, Davis MA, Loney TL. Fluoropyrimidine-mediated radio-sensitization depends on cyclin E-dependent kinase activation. *Cancer Research*. 1996; 56:3203–3206. [PubMed: 8764107]
- Lawrence TS, Tepper JE, Blackstock AW. Fluoropyrimidine-radiation Interactions in cells and tumors. *Seminars in Radiation Oncology*. 1997; 7:260–266. [PubMed: 10717223]
- Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science*. 2005; 308:551–554. [PubMed: 15790808]
- Li GC, He F, Shao X, Urano M, Shen L, Kim D, et al. Adenovirus-mediated heat-activated antisense Ku70 expression radiosensitizes tumor cells in vitro and in vivo. *Cancer Research*. 2003; 63:3268–3274. [PubMed: 12810658]
- Li JH, Lax SA, Kim J, Klamut H, Liu FF. The effects of combining ionizing radiation and adenoviral p53 therapy in nasopharyngeal carcinoma. *International Journal of Radiation Oncology, Biology, Physics*. 1999; 43:607–616.
- Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. *Nature Reviews. Molecular Cell Biology*. 2003; 4:712–720.
- Lohr F, Hu K, Haroon Z, Samulski TV, Huang Q, Beaty J, et al. Combination treatment of murine tumors by adenovirus-mediated local B7/IL12 immunotherapy and radiotherapy. *Molecular Therapy*. 2000; 2:195–203. [PubMed: 10985949]
- Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, et al. *p53* status and the efficacy of cancer therapy in vivo. *Science*. 1994; 266:807–810. [PubMed: 7973635]
- Lu X, Lane DP. Differential induction of transcriptionally active p53 following UV or ionizing radiation: Defects in chromosome instability syndromes? *Cell*. 1993; 75:765–778. [PubMed: 8242748]
- Lumniczky K, Desaknai S, Mangel L, Szende B, Hamada H, Hidvegi EJ, et al. Local tumor irradiation augments the antitumor effect of cytokine-producing autologous cancer cell vaccines in a murine glioma model. *Cancer Gene Therapy*. 2002; 9:44–52. [PubMed: 11916244]
- Mahan SD, Ireton GC, Knoeber C, Stoddard BL, Black ME. Random mutagenesis and selection of *Escherichia coli* cytosine deaminase for cancer gene therapy. *Protein Engineering, Design & Selection*. 2004; 17:625–633.

- Mahan SD, Ireton GC, Stoddard BL, Black ME. Alanine-scanning mutagenesis reveals a cytosine deaminase mutant with altered substrate preference. *Biochemistry*. 2004; 43:8957–8964. [PubMed: 15248753]
- Marconi P, Krisky D, Oligino T, Poliani PL, Ramakrishnan R, Goins WF, et al. Replication-defective herpes simplex virus vectors for gene transfer in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:11319–11320. [PubMed: 8876133]
- Martin M, Vozenin MC, Gault N, Crechet F, Pfarr CM, Lefaix JL. Coactivation of AP-1 activity and TGF-beta1 gene expression in the stress response of normal skin cells to ionizing radiation. *Oncogene*. 1997; 15:981–989. [PubMed: 9285693]
- Mason KA, Ariga H, Neal R, Valdecanas D, Hunter N, Krieg AM, et al. Targeting toll-like receptor 9 with CpG oligodeoxynucleotides enhances tumor response to fractionated radiotherapy. *Clinical Cancer Research*. 2005; 11:361–369. [PubMed: 15671567]
- McCarthy HO, Worthington J, Barrett E, Cosimo E, Boyd M, Mairs RJ, et al. p21(WAF1)-mediated transcriptional targeting of inducible nitric oxide synthase gene therapy sensitizes tumours to fractionated radiotherapy. *Gene Therapy*. 2007; 14:246–255. [PubMed: 17006546]
- McGinn CJ, Lawrence TS. Recent advances in the use of radiosensitizing nucleosides. *Seminars in Radiation Oncology*. 2001; 11:270–280. [PubMed: 11677652]
- McLoughlin JM, McCarty TM, Cunningham C, Clark V, Senzer N, Nemunaitis J, et al. TNFerade, an adenovector carrying the transgene for human tumor necrosis factor alpha, for patients with advanced solid tumors: Surgical experience and long-term follow-up. *Annals of Surgical Oncology*. 2005; 12:825–830. [PubMed: 16132372]
- Mercatante DR, Mohler JL, Kole R. Cellular response to an antisense-mediated shift of Bcl-x pre-mRNA splicing and antineoplastic agents. *The Journal of Biological Chemistry*. 2002; 277:49374–49382. [PubMed: 12381725]
- Mesnil M, Piccoli C, Tiraby G, Willecke K, Yamasaki H. Bystander killing of cancer cells by herpes simplex virus thymidine kinase gene is mediated by connexins. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:1831–1835. [PubMed: 8700844]
- Meyer RG, Kupper JH, Kandolf R, Rodemann HP. Early growth response-1 gene (Egr-1) promoter induction by ionizing radiation in U87 malignant glioma cells in vitro. *European Journal of Biochemistry*. 2002; 269:337–346. [PubMed: 11784328]
- Mezhir JJ, Smith KD, Posner MC, Senzer N, Yamini B, Kufe DW, et al. Ionizing radiation: A genetic switch for cancer therapy. *Cancer Gene Therapy*. 2006; 13:1–6. [PubMed: 16082378]
- Miller EM, Kinsella TJ. Radiosensitization by fluorodeoxyuridine: Effects of thymidylate synthase inhibition and cell synchronization. *Cancer Research*. 1992; 52:1687–1694. [PubMed: 1532343]
- Mullen CA, Coale M, Lowe R, Blaese RM. Tumors expressing the cytosine deaminase suicide gene can be eliminated in vivo with 5-fluorocytosine and induce protective immunity to wild type tumor. *Cancer Research*. 1994; 54:1503–1506. [PubMed: 8137255]
- Munshi A, Kurland JF, Nishikawa T, Tanaka T, Hobbs ML, Tucker SL, et al. Histone deacetylase inhibitors radiosensitize human melanoma cells by suppressing DNA repair activity. *Clinical Cancer Research*. 2005; 11:4912–4922. [PubMed: 16000590]
- Nenoi M, Daino K, Ichimura S, Takahash S, Akuta T. Low-dose radiation response of the p21WAF1/CIP1 gene promoter transduced by adeno-associated virus vector. *Experimental & Molecular Medicine*. 2006; 38:553–564. [PubMed: 17079872]
- Nikitina EY, Gabrilovich DI. Combination of gamma-irradiation and den-dritic cell administration induces a potent antitumor response in tumor-bearing mice: Approach to treatment of advanced stage cancer. *International Journal of Cancer*. 2001; 94:825–833.
- Nimura Y, Kawata T, Uzawa K, Okamura J, Liu C, Saito M, et al. Silencing Ku80 using small interfering RNA enhanced radiation sensitivity in vitro and in vivo. *International Journal of Oncology*. 2007; 30:1477–1484. [PubMed: 17487369]
- Nishihara E, Nagayama Y, Narimatsu M, Namba H, Watanabe M, Niwa M, et al. Treatment of thyroid carcinoma cells with four different suicide gene/prodrug combinations in vitro. *Anticancer Research*. 1998; 18:1521–1525. [PubMed: 9673364]

- Nishikawa T, Ramesh R, Munshi A, Chada S, Meyn RE. Adenovirus-mediated mda-7 (IL24) gene therapy suppresses angiogenesis and sensitizes NSCLC xenograft tumors to radiation. *Molecular Therapy*. 2004; 9:818–828. [PubMed: 15194048]
- Nishitani MA, Sakai T, Ishii K, Zhang M, Nakano Y, Nitta Y, et al. A convenient cancer vaccine therapy with in vivo transfer of interleukin 12 expression plasmid using gene gun technology after priming with irradiated carcinoma cells. *Cancer Gene Therapy*. 2002; 9:156–163. [PubMed: 11857033]
- Nyati MK, Morgan MA, Feng FY, Lawrence TS. Integration of EGFR inhibitors with radiochemotherapy. *Nature Reviews. Cancer*. 2006; 6:876–885.
- O'Driscoll M, Jeggo PA. The role of double-strand break repair—Insights from human genetics. *Nature Reviews. Genetics*. 2006; 7:45–54.
- Oh YT, Chen DW, Dougherty GJ, McBride WH. Adenoviral interleukin-3 gene-radiation therapy for prostate cancer in mouse model. *International Journal of Radiation Oncology, Biology, Physics*. 2004; 59:579–583.
- Papathanasiou MA, Kerr NC, Robbins JH, McBride OW, Alamo I Jr, Barrett SF, et al. Induction by ionizing radiation of the gadd45 gene in cultured human cells: Lack of mediation by protein kinase C. *Molecular and Cellular Biology*. 1991; 11:1009–1016. [PubMed: 1990262]
- Park JO, Lopez CA, Gupta VK, Brown CK, Mauceri HJ, Darga TE, et al. Transcriptional control of viral gene therapy by cisplatin. *The Journal of Clinical Investigation*. 2002; 110:403–410. [PubMed: 12163460]
- Pederson LC, Buchsbaum DJ, Vickers SM, Kancharla SR, Mayo MS, Curiel DT, et al. Molecular chemotherapy combined with radiation therapy enhances killing of cholangiocarcinoma cells *in vitro* and *in vivo*. *Cancer Research*. 1997; 57:4325–4332. [PubMed: 9331094]
- Pellegrini L, Yu DS, Lo T, Anand S, Lee M, Blundell TL, et al. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. *Nature*. 2002; 420:287–293. [PubMed: 12442171]
- Prasad S, Ravindran J, Aggarwal BB. NF-kappaB and cancer: How intimate is this relationship. *Molecular and Cellular Biochemistry*. 2010; 336:25–37. [PubMed: 19823771]
- Quanz M, Berthault N, Roulin C, Roy M, Herbette A, Agrario C, et al. Small-molecule drugs mimicking DNA damage: A new strategy for sensitizing tumors to radiotherapy. *Clinical Cancer Research*. 2009; 15:1308–1316. [PubMed: 19190126]
- Rass K, Reichrath J. UV damage and DNA repair in malignant melanoma and nonmelanoma skin cancer. *Advances in Experimental Medicine and Biology*. 2008; 624:162–178. [PubMed: 18348455]
- Ratnam K, Low JA. Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. *Clinical Cancer Research*. 2007; 13:1383–1388. [PubMed: 17332279]
- Rimner A, Wischhusen J, Naumann U, Gleichmann M, Steinbach JP, Weller M. Identification by suppression subtractive hybridization of p21 as a radio-inducible gene in human glioma cells. *Anticancer Research*. 2001; 21:3505–3508. [PubMed: 11848516]
- Rodriguez R, Schuur ER, Lim HY, Henderson GA, Simons JW, Henderson DR. Prostate attenuated replication competent adenovirus (ARCA) CN706: A selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Research*. 1997; 57:2559–2563. [PubMed: 9205053]
- Rogers BE, Zinn KR, Lin CY, Chaudhuri TR, Buchsbaum DJ. Targeted radiotherapy with [⁹⁰Y]-SMT 487 in mice bearing human nonsmall cell lung tumor xenografts induced to express human somatostatin receptor subtype 2 with an adenoviral vector. *Cancer*. 2002; 94:1298–1305. [PubMed: 11877760]
- Rogulski KR, Freytag SO, Zhang K, Gilbert JD, Paielli DL, Kim JH, et al. In vivo antitumor activity of ONYX-015 is influenced by p53 status and is augmented by radiotherapy. *Cancer Research*. 2000; 60:1193–1196. [PubMed: 10728673]
- Sartor CI. Biological modifiers as potential radiosensitizers: Targeting the epidermal growth factor receptor family. *Seminars in Oncology*. 2000; 27:15–20. discussion 92–100. [PubMed: 11236022]
- Sasaki Y, Morimoto I, Ishida S, Yamashita T, Imai K, Tokino T. Adeno-virus-mediated transfer of the p53 family genes, p73 and p51/p63 induces cell cycle arrest and apoptosis in colorectal cancer

- cell lines: Potential application to gene therapy of colorectal cancer. *Gene Therapy*. 2001; 8:1401–1408. [PubMed: 11571580]
- Schlessinger J. Common and distinct elements in cellular signaling via EGF and FGF receptors. *Science*. 2004; 306:1506–1507. [PubMed: 15567848]
- Schlessinger J, Lemmon MA. Nuclear signaling by receptor tyrosine kinases: The first robin of spring. *Cell*. 2006; 127:45–48. [PubMed: 17018275]
- Scott SD, Joiner MC, Marples B. Optimizing radiation-responsive gene promoters for radiogenetic cancer therapy. *Gene Therapy*. 2002; 9:1396–1402. [PubMed: 12365005]
- Searle PF, Chen MJ, Hu L, Race PR, Lovering AL, Grove JJ, et al. Nitroreductase: A prodrug-activating enzyme for cancer gene therapy. *Clinical and Experimental Pharmacology & Physiology*. 2004; 31:811–816. [PubMed: 15566399]
- Senzer N, Mani S, Rosemurgy A, Nemunaitis J, Cunningham C, Guha C, et al. TNFerade biologic, an adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor alpha gene: A phase I study in patients with solid tumors. *Journal of Clinical Oncology*. 2004; 22:592–601. [PubMed: 14726502]
- Shariat SF, Desai S, Song W, Khan T, Zhao J, Nguyen C, et al. Adenovirus-mediated transfer of inducible caspases: A novel “death switch” gene therapeutic approach to prostate cancer. *Cancer Research*. 2001; 61:2562–2571. [PubMed: 11289132]
- Sherman ML, Datta R, Hallahan DE, Weichselbaum RR, Kufe DW. Ionizing radiation regulates expression of the *c-jun* protooncogene. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87:5663–5666. [PubMed: 2116003]
- Shi W, Teschendorf C, Muzyczka N, Siemann DW. Gene therapy delivery of endostatin enhances the treatment efficacy of radiation. *Radiotherapy and Oncology*. 2003; 66:1–9. [PubMed: 12559515]
- Shimura T. Acquired radioresistance of cancer and the AKT/GSK3beta/cyclin D1 overexpression cycle. *Journal of Radiation Research (Tokyo)*. 2011; 52:539–544.
- Shinoura N, Yamamoto N, Asai A, Kirino T, Hamada H. Adenovirus-mediated transfer of Fas ligand gene augments radiation-induced apoptosis in U-373MG glioma cells. *Japanese Journal of Cancer Research*. 2000; 91:1044–1050. [PubMed: 11050476]
- Sourvinos G, Spandidos DA. Decreased BRCA1 expression levels may arrest the cell cycle through activation of p53 checkpoint in human sporadic breast tumors. *Biochemical and Biophysical Research Communications*. 1998; 245:75–80. [PubMed: 9535786]
- Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, Roth JA. Adenoviral-mediated wild-type *p53* gene expression sensitizes colorectal cancer cells to ionizing radiation. *Clinical Cancer Research*. 1996; 2:1665–1671. [PubMed: 9816114]
- Spitzweg C, Baker CH, Bergert ER, O'Connor MK, Morris JC. Image-guided radioiodide therapy of medullary thyroid cancer after carcinoembryonic antigen promoter-targeted sodium iodide symporter gene expression. *Human Gene Therapy*. 2007; 18:916–924. [PubMed: 17931047]
- Stackhouse MA, Buchsbaum DJ, Kancharla SR, Grizzle WE, Grimes C, Laffoon K, et al. Specific membrane receptor gene expression targeted with radiolabeled peptide employing the erbB-2 and DF3 promoter elements in adenoviral vectors. *Cancer Gene Therapy*. 1999; 6:209–219. [PubMed: 10359206]
- Stackhouse MA, Pederson LC, Grizzle WE, Curiel DT, Gebert J, Haack K, et al. Fractionated radiation therapy in combination with adenoviral delivery of the cytosine deaminase gene and 5-fluorocytosine enhances cytotoxic and antitumor effects in human colorectal and cholangiocarcinoma models. *Gene Therapy*. 2000; 7:1019–1026. [PubMed: 10871750]
- Steinman RA, Hoffman B, Iro A, Guillouf C, Liebermann DA, el-Houseini ME. Induction of p21 (WAF-1/CIP1) during differentiation. *Oncogene*. 1994; 9:3389–3396. [PubMed: 7936667]
- Su ZZ, Lebedeva IV, Sarkar D, Emdad L, Gupta P, Kitada S, et al. Ionizing radiation enhances therapeutic activity of mda-7/IL-24: Overcoming radiation- and mda-7/IL-24-resistance in prostate cancer cells overexpressing the antiapoptotic proteins bcl-xL or bcl-2. *Oncogene*. 2006; 25:2339–2348. [PubMed: 16331261]
- Takahashi S, Saito S, Ohtani N, Sakai T. Involvement of the Oct-1 regulatory element of the gadd45 promoter in the p53-independent response to ultraviolet irradiation. *Cancer Research*. 2001; 61:1187–1195. [PubMed: 11221850]

- Teh BS, Aguilar-Cordova E, Kernen K, Chou CC, Shalev M, Vlachaki MT, et al. Phase I/II trial evaluating combined radiotherapy and in situ gene therapy with or without hormonal therapy in the treatment of prostate cancer—A preliminary report. *International Journal of Radiation Oncology, Biology, Physics*. 2001; 51:605–613.
- Teitz-Tennenbaum S, Li Q, Rynkiewicz S, Ito F, Davis MA, McGinn CJ, et al. Radiotherapy potentiates the therapeutic efficacy of intratumoral dendritic cell administration. *Cancer Research*. 2003; 63:8466–8475. [PubMed: 14679011]
- Tetzlaff MT, Teh BS, Timme TL, Fujita T, Satoh T, Tabata K, et al. Expanding the therapeutic index of radiation therapy by combining in situ gene therapy in the treatment of prostate cancer. *Technology in Cancer Research & Treatment*. 2006; 5:23–36. [PubMed: 16417399]
- Toth K, Tarakanova V, Doronin K, Ward P, Kuppuswamy M, Locke JE, et al. Radiation increases the activity of oncolytic adenovirus cancer gene therapy vectors that overexpress the ADP (E3-11.6K) protein. *Cancer Gene Therapy*. 2003; 10:193–200. [PubMed: 12637940]
- Treisman R. The SRE: A growth factor responsive transcriptional regulator. *Seminars in Cancer Biology*. 1990; 1:47–58. [PubMed: 2133110]
- Trinh QT, Austin EA, Murray DM, Knick VC, Huber BE. Enzyme/prodrug gene therapy: Comparison of cytosine deaminase/5-fluorocytosine versus thymidine kinase/ganciclovir enzyme/prodrug systems in a human colorectal carcinoma cell line. *Cancer Research*. 1995; 55:4808–4812. [PubMed: 7585511]
- Trofimova I, Dimtchev A, Jung M, Rosenthal D, Smulson M, Dritschilo A, et al. Gene therapy for prostate cancer by targeting poly(ADP-ribose) polymerase. *Cancer Research*. 2002; 62:6879–6883. [PubMed: 12460902]
- Trujillo MA, Oneal MJ, McDonough S, Qin R, Morris JC. A probasin promoter, conditionally replicating adenovirus that expresses the sodium iodide symporter (NIS) for radiovirotherapy of prostate cancer. *Gene Therapy*. 2010; 17:1325–1332. [PubMed: 20428214]
- Tsai-Morris CH, Cao XM, Sukhatme VP. 5' flanking sequence and genomic structure of Egr-1, a murine mitogen inducible zinc finger encoding gene. *Nucleic Acids Research*. 1988; 16:8835–8846. [PubMed: 3140220]
- Uchida H, Shinoura N, Kitayama J, Watanabe T, Nagawa H, Hamada H. 5-Fluorouracil efficiently enhanced apoptosis induced by adenovirus-mediated transfer of caspase-8 in DLD-1 colon cancer cells. *The Journal of Gene Medicine*. 2003; 5:287–299. [PubMed: 12692863]
- Vernole P, Muzi A, Volpi A, Dorio AS, Terrinoni A, Shah GM, et al. Inhibition of homologous recombination by treatment with BVDU (brivudin) or by RAD51 silencing increases chromosomal damage induced by bleomycin in mismatch repair-deficient tumour cells. *Mutation Research*. 2009; 664:39–47. [PubMed: 19428379]
- Wakasa T, Inoue T, Kawai N, Murakami J, Kishi K, Fukui K. The combination of ionizing radiation and expression of a wild type p53 gene via recombinant adenovirus induced a prominent tumour suppressing effect in human oral squamous cell carcinoma. *The British Journal of Radiology*. 2002; 75:657–662. [PubMed: 12153939]
- Walker JR, McGeagh KG, Sundaresan P, Jorgensen TJ, Rabkin SD, Martuza RL. Local and systemic therapy of human prostate adenocarcinoma with the conditionally replicating herpes simplex virus vector G207. *Human Gene Therapy*. 1999; 10:2237–2243. [PubMed: 10498254]
- Weichselbaum RR, Hallahan DE, Beckett MA, Mauceri HJ, Lee H, Sukhatme VP, et al. Gene therapy targeted by radiation preferentially radiosensitizes tumor cells. *Cancer Research*. 1994; 54:4266–4269. [PubMed: 8044769]
- Weichselbaum RR, Kufe D. Translation of the radio- and chemo-inducible TNFerade vector to the treatment of human cancers. *Cancer Gene Therapy*. 2009; 16:609–619. [PubMed: 19444302]
- Weichselbaum RR, Kufe DW, Hellman S, Rasmussen HS, King CR, Fischer PH, et al. Radiation-induced tumour necrosis factor- α expression: Clinical application of transcriptional and physical targeting of gene therapy. *The Lancet Oncology*. 2002; 3:665–671. [PubMed: 12424068]
- White CL, Menghistu T, Twigger KR, Searle PF, Bhide SA, Vile RG, et al. *Escherichia coli* nitroreductase plus CB1954 enhances the effect of radiotherapy in vitro and in vivo. *Gene Therapy*. 2008; 15:424–433. [PubMed: 18079753]

- Winter RN, Rhee JG, Kyprianou N. Caspase-1 enhances the apoptotic response of prostate cancer cells to ionizing radiation. *Anticancer Research*. 2004; 24:1377–1386. [PubMed: 15274298]
- Worthington J, McCarthy HO, Barrett E, Adams C, Robson T, Hirst DG. Use of the radiation-inducible WAF1 promoter to drive iNOS gene therapy as a novel anti-cancer treatment. *The Journal of Gene Medicine*. 2004; 6:673–680. [PubMed: 15170738]
- Xiang DB, Chen ZT, Wang D, Li MX, Xie JY, Zhang YS, et al. Chimeric adenoviral vector Ad5/F35-mediated APE1 siRNA enhances sensitivity of human colorectal cancer cells to radiotherapy in vitro and in vivo. *Cancer Gene Therapy*. 2008; 15:625–635. [PubMed: 18535621]
- Xu Y, Liu Z, Kong H, Sun W, Liao Z, Zhou F, et al. Co-expression of in-terleukin 12 enhances antitumor effects of a novel chimeric promoter-mediated suicide gene therapy in an immunocompetent mouse model. *Biochemical and Biophysical Research Communications*. 2011; 412:763–768. [PubMed: 21875574]
- Xu L, Pirollo KF, Tang WH, Rait A, Chang EH. Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results in regression of human head and neck xenografts. *Human Gene Therapy*. 1999; 10:2941–2952. [PubMed: 10609655]
- Yacoub A, Hamed H, Emdad L, Dos Santos W, Gupta P, Broaddus WC, et al. MDA-7/IL-24 plus radiation enhance survival in animals with intracranial primary human GBM tumors. *Cancer Biology & Therapy*. 2008; 7:917–933. [PubMed: 18376144]
- Yanamandra N, Kondraganti S, Srinivasula SM, Gujrati M, Olivero WC, Dinh DH, et al. Activation of caspase-9 with irradiation inhibits invasion and angiogenesis in SNB19 human glioma cells. *Oncogene*. 2004; 23:2339–2346. [PubMed: 14767475]
- Yang YJ, Chen DZ, Li LX, Sheng QS, Jin ZK, Zhao DF. Targeted IL-24 gene therapy inhibits cancer recurrence after liver tumor resection by inducing tumor cell apoptosis in nude mice. *Hepatobiliary & Pancreatic Diseases International*. 2009; 8:174–178. [PubMed: 19357032]
- Yang DC, Elliott RL, Head JF. Gene targets of antisense therapies in breast cancer. *Expert Opinion on Therapeutic Targets*. 2002; 6:375–385. [PubMed: 12223074]
- Yang Y, Liu SZ, Fu SB. Anti-tumor effects of pNEgr-mIL-12 recombinant plasmid induced by X-irradiation and its mechanisms. *Biomedical and Environmental Sciences*. 2004; 17:135–143. [PubMed: 15386939]
- Yu H, Jove R. The STATs of cancer—New molecular targets come of age. *Nature Reviews. Cancer*. 2004; 4:97–105.
- Yu D, Sekine E, Fujimori A, Ochiya T, Okayasu R. Down regulation of BRCA2 causes radiosensitization of human tumor cells in vitro and in vivo. *Cancer Science*. 2008; 99:810–815. [PubMed: 18377429]
- Yuan S, Qiao T, Chen W. CpG oligodeoxynucleotide 1826 enhances the Lewis lung cancer response to radiotherapy in murine tumor. *Cancer Biotherapy & Radio-pharmaceuticals*. 2011; 26:203–208.
- Zaiss AK, Liu Q, Bowen GP, Wong NC, Bartlett JS, Muruve DA. Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. *Journal of Virology*. 2002; 76:4580–4590. [PubMed: 11932423]
- Zhou BB, Bartek J. Targeting the checkpoint kinases: Chemosensitization versus chemoprotection. *Nature Reviews. Cancer*. 2004; 4:216–225.
- Zhou BB, Chaturvedi P, Spring K, Scott SP, Johanson RA, Mishra R, et al. Caffeine abolishes the mammalian G(2)/M DNA damage checkpoint by inhibiting ataxia-telangiectasia-mutated kinase activity. *The Journal of Biological Chemistry*. 2000; 275:10342–10348. [PubMed: 10744722]
- Zimmerman RJ, Marafino BJ Jr, Chan A, Landre P, Winkelhake JL. The role of oxidant injury in tumor cell sensitivity to recombinant human tumor necrosis factor in vivo. Implications for mechanisms of action. *Journal of Immunology*. 1989; 142:1405–1409.

Figure 7.1.
Molecular mechanisms of cell response to radiotherapy.

Table 7.1

Summary of radiation-responsive promoters employed in combination with irradiation

Promoter (putative radiation response elements)	Gene/assay	References
Egr-1 (CArG, Sp-1, MAP)	<i>CAT</i>	Datta et al. (1992)
	<i>TNF-α</i>	Mezhrir et al. (2006), Meyer, Kupper, Kandolf, and Rodemann (2002), Park et al. (2002), Hallahan et al. (1995), Senzer et al. (2004), McLoughlin et al. (2005), Bickenbach et al. (2008)
	<i>CYP4B1</i>	Hsu et al. (2003)
	<i>HSV-TK</i>	Joki, Nakamura, and Ohno (1995)
tPA (AP-2, AP-2-like, NFκB)	<i>tPA</i>	Boothman, Lee, and Sahjidak (1994)
p21/WAF1 (p53)	<i>iNOS</i>	Worthington et al. (2004), McCarthy et al. (2007)
	<i>HSV-TK</i>	Nenoi, Daino, Ichimura, Takahashi, and Akuta (2006)
GADD45α (AP-1, p53, Oct-1, NF-κB, HNF, NF-YA, KLF)	Electrophoretic mobility shift analysis (EMSA), chromatin immunoprecipitation (ChIP)	Daino, Ichimura, and Nenoi (2003), Takahashi, Saito, Ohtani, and Sakai (2001), Daino, Ichimura, and Nenoi (2006)
Synthetic promoter containing isolated CArG elements	<i>GFP, HSV-TK</i>	Greco, Powell, Marples, Joiner, and Scott (2005)
	<i>GFP</i>	Scott, Joiner, and Marples (2002)
	<i>iNOS</i>	Coulter et al. (2008)

Table 7.2

Summary of gene therapy strategies to enhance radiation therapy efficacy

Target	Signaling pathways/mechanisms	References
<i>Suppression of radiation-induced DNA damage repair</i>		
BRCA2	HR	Yu, Sekine, Fujimori, Ochiya, and Okayasu (2008)
Rad51		Vernole et al. (2009)
Ku70	NHEJ	Li et al. (2003)
Ku80/Ku86		Nimura et al. (2007)
APE1	BER	Xiang et al. (2008)
Poly(ADP-ribose)		Ratnam and Low (2007), Trofimova et al. (2002)
<i>Radiosensitization by tumor targeting</i>		
hSSTR2	Radiolabeled ligand	Rogers, Zinn, Lin, Chaudhuri, and Buchsbaum (2002), Cotugno et al. (2011)
GRPR		Stackhouse et al. (1999)
NIS	Radionuclide uptake	Spitzweg, Baker, Bergert, O'Connor, and Morris (2007)
NAT		Jia et al. (2011)
<i>Facilitation of apoptosis</i>		
p53	Apoptosis induction	Gallardo, Drazan, and McBride (1996), Spitz et al. (1996), Li, Lax, Kim, Klamut, and Liu (1999), Xu, Pirollo, Tang, Rait, and Chang (1999), Wakasa et al. (2002), Higuchi et al. (2003)
p73, p51/p63		Sasaki et al. (2001)
Bax		Kaliberov et al. (2002)
Caspases	Apoptosis induction/execution	Shariat et al. (2001), Uchida et al. (2003), Winter, Rhee, and Kyprianou (2004), Yanamandra et al. (2004)
TRAIL	Multiple pathways	Kaliberov, Kaliberova, Stockard, Grizzle, and Buchsbaum (2004)
Fas ligand		Shinoura, Yamamoto, Asai, Kirino, and Hamada (2000)
IL-24/mda-7		Nishikawa, Ramesh, Munshi, Chada, and Meyn (2004)
Bcl-2	Inhibition of antiapoptotic pathways	Hara et al. (2005)
Bcl-XL		Mercatante, Mohler, and Kole (2002)
XIAP, survivin		Cao, Mu, Hallahan, and Lu (2004)
<i>Modulation of tumor-associated microenvironment</i>		
Endostatin	Inhibition of angiogenesis	Shi, Teschendorf, Muzyczka, and Siemann (2003)
Soluble VEGF receptor 2		Kaliberov, Kaliberova, and Buchsbaum (2005)
TNF- α	Multiple pathways	Hallahan, Beckett, Kufe, and Weichselbaum (1990), Gridley, Glisson, and Uhm (1994), Azria et al. (2003), Weichselbaum and Kufe (2009), Sartor (2000)
EGFR	Inhibition of proliferation	Gokhale et al. (1999), Dritschilo et al. (2006)
c-raf-1		Lammering et al. (2003), Lai et al. (2009)
<i>Immunomodulation</i>		
IL-2	Facilitating the antitumor immune response	Bray et al. (2003), Hillman et al. (2004), Formenti (2010)
IL-3		Chiang, Hong, Wu, McBride, and Dougherty (2000), Oh, Chen, Dougherty, and McBride (2004)

Target	Signaling pathways/mechanisms	References
IL-4		Iwadate, Yamaura, Sakiyama, Sato, and Tagawa (2003)
IL-12		Lohr et al. (2000), Nishitani et al. (2002), Yang, Liu, and Fu (2004)
IL-24/mda-7		Emdad et al. (2006), Yacoub et al. (2008), Yang et al. (2009)
TNF- α		Weichselbaum et al. (1994, 2002), Gridley et al. (2004)
<i>Radiosensitization by molecular chemotherapy</i>		
CD	Disruption of DNA synthesis	Pederson et al. (1997), Stackhouse et al. (2000), Kaliberova et al. (2008), Kaliberov et al. (2007)
HSV-TK		Kim, Kim, Kolozsvary, Brown, and Freytag (1997), Atkinson and Hall (1999), Blackburn, Galoforo, Corry, and Lee (1998), Cheon et al. (2000), Chen et al. (2001), Teh et al. (2001), Tetzlaff et al. (2006)
CD:HSV-TK fusion		Freytag et al. (2002, 2007)
NTR		White et al. (2008)
HRP		Greco, Tozer, and Dachs (2002)