# Molecular Targets for Tumor Radiosensitization

Philip J. Tofilon*,† and Kevin Camphausen‡

*Drug Discovery Department, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, Florida 33612, and the Radiation Oncology Branch, National Cancer Institute, 10 Center Drive Building 10, CRC, Bethesda, Maryland 20892*

*Received October 28, 2008*

## Contents





## 1. Introduction

It is estimated that in 2008 1.4 million patients in North America will be diagnosed with a nonskin form of cancer (www.cancer.org), of which seventy-five percent will receive radiotherapy sometime during the course of their disease. Clearly, radiotherapy continues to serve as a primary cancer treatment modality; consequently, the development of strategies that improve its efficacy is likely to have an impact on a significant number of patients. Toward this end, radiation is typically combined with standard cytotoxic chemotherapeutic agents, an approach that has become the standard of care for a number of solid tumor types including lung, head and neck, gastrointestinal, and brain tumors. The processes through which radio-chemotherapy could provide treatment benefits were initially put forth by Steel and Peckham in 1979[1] and included concepts of spatial cooperation, toxicity independence, and enhanced tumor radiosensitivity. In essence, spatial cooperation involves local tumor control induced by radiation with chemotherapy intended for metastases; toxicity independence refers to additive effects of radiation and drug on tumor control without increased normal tissue toxicity and enhanced tumor response assumes a direct effect of the drug on the tumor cells to enhance their radiosensitivity. However, whether one of these processes, more than one or some other undefined event mediates the effectiveness of a specific cytotoxic drug/radiation combination has for the most part not been determined. Thus, although contributing to successful cancer treatment, this approach typically lacks a mechanistic explanation for any increase in tumor response and on a practical level is often limited by unacceptable levels of normal tissue toxicity.[2,3]

In contrast to the primarily empirical application of radiochemotherapy, advances in defining the molecules mediating cellular radioresponse have generated considerable interest in the development of target-based strategies for enhancing tumor radiosensitivity. As for other forms of targeted therapy now being applied to cancer treatment, this mechanism-based approach to radiosensitization offers the potential for tumor selectivity and the identification of predictive biomarkers. To date, a diverse set of molecules affecting such processes as signal transduction, the DNA damage response, gene expression, and apoptosis have been shown to influence radiosensitivity in one or more experimental models (see Figure 1 for examples), providing a relatively extensive list of potential targets.

However, the translation of such laboratory information into a treatment setting requires more than establishing a causal relationship between a molecule and the radiosensitivity of a tumor cell line. Whereas many of the specifics remain to be defined, it has become increasingly apparent that cell survival after exposure to ionizing radiation reflects the end result of combinatorial and likely redundant processes involving a wide variety of signaling and effector molecules. The consequence of such regulatory complexity is cell-type dependency in the molecular determinants of radiosensitivity. On one hand, this bodes well for the existence of tumor-specific targets for radiosensitization. On the other hand, given the extensive genetic/epigenetic heterogeneity among solid tumors, including those within the same histology, it is unlikely that a single molecule will influence the radiosensitivity of all tumor cells. There are a number of examples showing that targeting a selected radioresponse-associated molecule affects the radiosensitivity of some tumor cell lines but not others (see below). Thus, in addition to outcome-based laboratory studies, the successful application of targeted radiosensitization strategies will require more in-depth, fundamental information, including a delineation of the genetic and epigenetic cellular context under which the regulatory potential of a putative target is operative. Such information, in addition to contributing a mechanistic rationale for proposed tumor selectivity, will provide the foundation for biomarker development and the ability to select appropriate patients.

The goal of this review is to summarize current research efforts in the development of molecular targets for tumor radiosensitization. As illustrated in Figure 1, it is not possible

* To whom correspondence should be addressed. E-mail: philip.tofilon@moffitt.org.
† Moffitt Cancer Center.
‡ National Cancer Institute.

10.1021/cr800504x CCC: $71.50 
Published on Web 04/02/2009

**Philip J. Tofilon** received his undergraduate degree in physiology from the University of Illinois (Urbana) in 1977. He began his graduate training at the University of California, San Francisco (UCSF), in the pharmacology department and then moved with his thesis advisor to the University of Nebraska Medical Center in Omaha where he received his Ph.D. in 1981. In the fall of 1981 he returned to UCSF accepting a postdoctoral fellowship with Dr. Dennis Deen in the Brain Tumor Research Center where he was introduced to radiobiology, which has continued to be his primary area of research. In 1984, he joined the faculty of the Department of Experimental Radiation Oncology at the University of Texas M.D. Anderson Cancer Center in Houston as an assistant professor. His research at M.D. Anderson focused on drug−radiation interactions. He was eventually promoted to professor in 1996 with a joint appointment as professor in the Department of Neurosurgery. In June of 2001, he joined the National Cancer Institute as Chief of the newly created Molecular Radiation Therapeutics Branch. He relocated to the H. Lee Moffitt Cancer Center in 2006 as Professor/Member in the Drug Discovery Department. His current research is focused on the identification of the molecular determinants of radiosensitivity and the development of molecularly targeted radiosensitizers.

**Kevin Camphausen** received his undergraduate degree from Purdue University in 1988. He then completed his MD (1996) and medical internship (1997) at Georgetown University Medical Center. He next completed his residency in radiation oncology at the Joint Center of Radiation Therapy at Harvard Medical School (2001). While there, he worked in the laboratory of Dr. Judah Folkman studying the interactions of antiangiogenic therapy and radiotherapy. He joined the Radiation Oncology Branch in 2001 as a tenure track investigator. He has recently received tenure at the NIH (2007) and was promoted to be the Chief of the Radiation Oncology Branch (ROB), Center for Cancer Research (CCR), NCI (2007). He is a physician/scientist who has a laboratory program focusing on the development and characterization of agents that alter the tumoral response to radiation. He has been successful in translating his work from the laboratory to the clinic. Dr. Camphausen is an internationally recognized leader in his field and an expert in the field of drug-induced tumor radiosensitization including the use of antiangiogenic agents in combination with radiotherapy.

to address all molecules that have been reported to influence radiosensitivity. Therefore, toward defining current issues/

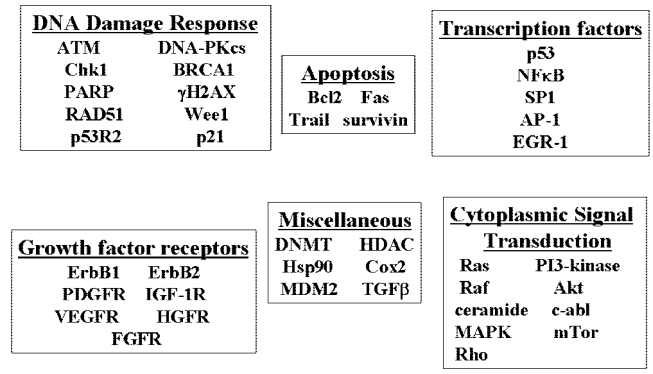


**Figure 1.** Examples of molecules suggested as potential targets for radiosensitization and their associated cellular processes.

advances, we have focused on molecular targets for which there is a strong rationale supporting tumor selectivity over normal cells and on those that are or are likely to be the subject of clinical trials. Targets have been divided into those that affect intrinsic cellular radiosensitivity, which can be evaluated using in vitro models, and those that operate via the tumor microenvironment. It should be noted that a number of terms have been used to the describe drug/radiation interactions resulting in increased cellular radiosensitivity (e.g., greater than additive, supra-additive, enhancement, radiosensitization). In essence, these terms are based on the mathematical analysis of in vitro cell survival curves and whether the "drug" in question has any toxicity when delivered alone. However, although serving as descriptors for the observed effect, the individual terms impart no unique mechanistic insight. For the purposes of this review, an increase in cellular susceptibility to radiation-induced death is referred to as radiosensitization.

## 2. Targets for Intrinsic Tumor Cell Radiosensitization

Cell survival (defined as the ability to maintain proliferative activity) after exposure to radiation is for the most part determined by the active processes of apoptosis, cell cycle checkpoint activation, DNA repair, specifically double strand breaks (DSBs), and senescence (Figure 2). The activation and implementation of each involves a cascade of sensor, signaling and effector molecules providing a rich source of potential targets for radiosensitization. Whereas each event includes specific molecular components, there are often interactions between the regulatory networks, which can be

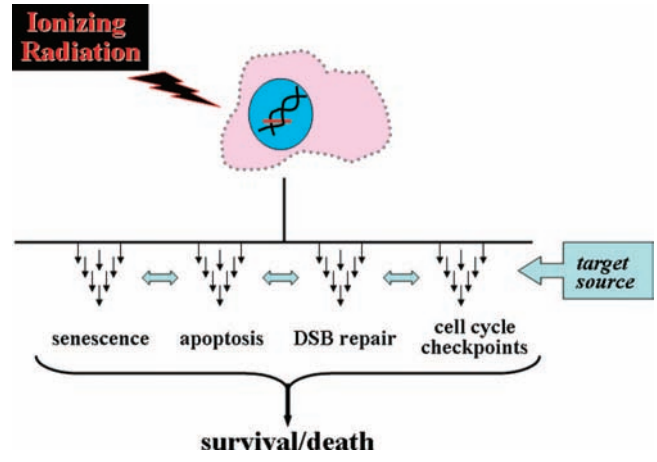


**Figure 2.** Critical processes determining cell death/survival.

exemplified by the relationship between cell cycle checkpoint activation and DNA repair, critical components of the DNA damage response. It should also be noted that whereas there have been significant advances toward defining the molecules and events mediating cell survival after irradiation, identifying those that are selectively operative in tumor cells remains a significant challenge.

Most studies aimed at delineating the molecular determinants of radiosensitivity have at least initially been performed using in vitro monolayer cultures. Although it is essential to the elucidation of the molecular components of the cellular response to radiation, the use of this model system is not without limitations. First, as for any in vitro model, its application is based on the assumption that the phenotype of tumor cells in culture recapitulates that of cells in an orthotopic in vivo setting, that is, the same targets are expressed and are operative. However, heterotypic signaling between normal and tumor cells has been implicated as a critical elements in tumorigenesis[4] and as recently reviewed by Witz, a variety of microenvironmental factors can influence tumor cell phenotype.[5] With respect to radioresponse, experiments using brain tumor cell lines have identified substantial differences in basal and radiation-induced gene expression signatures for cells grown in vitro and as intracerebral xenografts.[6,7] Thus, there is the possibility that a putative target identified in vitro is not expressed or operative in situ or other targets are available under orthotopic conditions. In addition, relying solely on in vitro models has a tendency to minimize the significance of the putative target molecule in the radioresponse of normal tissue. To address the issue of normal cells, a standard approach has been to compare tumor cells to normal fibroblasts in terms of the contribution of a given molecule to the regulation of radiosensitivity. Whereas these are critical initial experiments, whether the radioresponse of fibroblasts in vitro reflects that of any normal tissue is tenuous at best. With these caveats in mind, however, the experimental expedience of the monolayer culture system obviously qualifies it an indispensable model for identifying potential targets for radiosensitization and elucidating the critical mechanisms involved.



## 2.1. Signaling Molecules

A variety of molecules involved in transducing environmental signals through the cytoplasm to the nucleus have been shown to play a role in determining radiosensitivity. Such molecules include not only growth factor receptors but also the downstream components of their signaling pathways. Because many of these molecules are mutated, abnormally expressed, or have alternative functions in neoplastic cells, they have received considerable attention as tumor-specific targets for radiosensitization.

### *2.1.1. Ras*

These small GTP-binding proteins serve as a relatively early component in a number of signal transduction pathways and play critical roles in the regulation of cell proliferation, differentiation and oncogenic transformation.[8,9] Mutated Ras has been detected in approximately 30% of human tumors with aberrant activity present in many more tumors due to abnormal upstream signaling activity.[10] The expression of mutated Ras proteins has also been long associated with a reduction in cellular radiosensitivity. Sklar initially showed in 1988 that the resistance of NIH 3T3 cells to radiation was enhanced by the expression of a mutant H-ras or K-ras gene.[11] In subsequent experiments using human tumor cell lines, antisense oligonucleotides directed against H-ras increased the radiosensitivity of cells expressing mutant but not normal H-ras.[12,13] Bernhard et al. showed that the genetic inactivation of oncogenic N or K-ras in human colon tumor cell lines leads to increased radiosensitivity[14] and more recently, the siRNA mediated knockdown K-ras was reported to radiosensitize head and neck tumor cell lines.[15] Finally, an adenoviral vector encoding an anti-Ras single-chain antibody fragment was shown to enhance the radiosensitivity of human tumor cells containing mutated Ras, as well as cells in which Ras protein was constitutively active.[16]

While a causal link between aberrant Ras activity and tumor cell radioresistance has been well established, the specific mechanisms involved remain undefined. Investigations have for the most part been limited to cytoplasmic signaling molecules focusing on the Ras pathways proceeding through Raf1/MAPK and PI3 kinase/Akt. Data generated from both human and rodent models suggested that although Raf may be involved, Ras-mediated radioresistance does not involve MAPK.[17−19] PI3-kinase activity was found to be necessary for the radioresistance of Ras transformed cells implicating it as the critical effector pathway,[17,19] which further suggested an essential role for Akt. However, the molecules downstream from Akt participating in radioresistance have not been defined. Moreover, inhibition of Akt has been reported to modestly enhance the radiosensitivity of some tumor cell lines,[15] but not others.[20] Contributing to the limited understanding of the mechanisms of Ras-mediated radioresistance is the complexity of the Ras signaling network;[21] a simplified representation of which is shown in Figure 3. In addition to the frequently studied Raf1 and PI3K are a number of Ras effector molecules, as well various isoforms.[21] These effectors activate a variety of distinct, yet often interacting downstream signaling pathways ultimately influencing such fundamental processes as gene expression, second messenger activation, apoptosis, and cell cycle regulation. Whether these other effector molecules play a role in radiation response and under what cellular context remains to be determined.

Whereas research efforts have focused on the cytoplasmic signaling molecules, the downstream events through which Ras regulates radiosensitivity have not been clearly determined. That is, the question remains as to whether Ras-mediated radioresistance involves the modification of cell cycle checkpoints, DNA repair, apoptosis, senescence, or some other undefined process that ultimately determines cell survival. Defining the process responsible would not only be of mechanistic value but would also provide a framework for further investigations working upstream through the various Ras signaling pathways (Figure 3), which would likely lead to the identification of additional targets. Such information is of significance in that, although there is considerable data indicating that aberrant Ras activity provides a tumor-specific target for radiosensitization, Ras is not an enzyme and is thus a relatively more difficult molecule to “drug”.

To date, strategies aimed at exploiting Ras as a target for tumor radiosensitization have focused on farnesyltransferase and geranyl-geranyl transferase, which mediate the prenylation of the carboxy terminus of Ras proteins, an event necessary for their attachment to the cell membrane and subsequent activation.[22] Prenyl transferase inhibitors (PTIs)

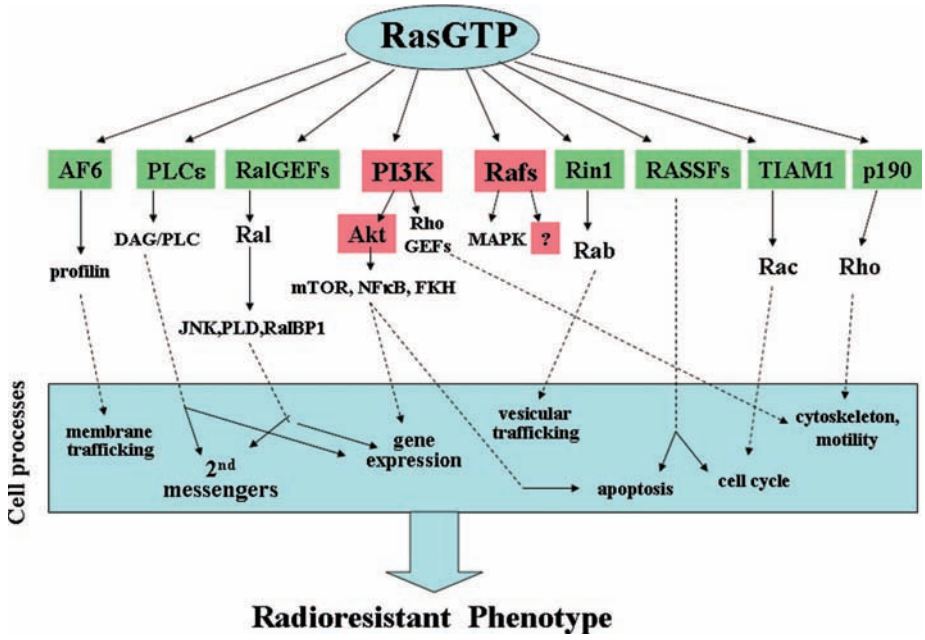


**Figure 3.** Ras-mediated signaling pathways.



were shown to enhance the in vitro radiosensitivity of a variety of human tumor cell lines including pancreatic,[23] lung, colon, and breast carcinomas,[24] as well as gliomas,[25] that expressed oncogenic forms of Ras. Importantly, PTIs had no effect on tumor cells that contained wild-type Ras or on normal fibroblasts.[18] With the use of leg tumor xenograft models, PTI delivery significantly enhanced the in vivo radiosensitivity of H-ras mutant T24 bladder carcinoma cells[26] and the K-ras mutant MiaPaca-2 and PSN-1 pancreatic carcinoma cells.[23]

On the basis of such results, PTIs have received considerable attention as "anti-Ras" drugs; a number have undergone clinical evaluation in combination with radiotherapy (see below). However, whereas PTIs inhibit Ras prenylation and activation, they also inhibit the prenylation of a variety of other proteins. More than 100 proteins have been identified as requiring the post-translational modification of prenylation.[27] Although the consequence of inhibiting the prenylation of these proteins on radiosensitivity has not been completely defined, among the proteins whose prenylation and activity are inhibited by PTIs are RhoB and Rheb. Inhibition of RhoB using a genetic approach and by PTIs enhanced the radiosensitivity of glioma cells.[25,28] Inhibiting Rheb prenylation by a PTI was reported to reduce its activity along with that of mTOR, which enhanced the antitumor activity of taxane and tamoxifen.[29] Although the effects of inhibiting Rheb on radioresponse have not been specifically tested, mTOR inhibition has been associated with an increase in radiosensitivity.[30] Thus, this lack of specificity of the PTIs for Ras clearly complicates the interpretation of experimental and clinical studies.

Clinical trials involving the combination of PTIs and radiotherapy have recently been reviewed by Rengan et al.[31] and will be briefly summarized here. The first trial reported combined Lovastatin with radiotherapy in patients with either anaplastic gliomas or glioblastoma multiforme,[32] which showed minimal enhancement of the effects of radiation alone. Multi-institutional phase I studies have also been completed using the PTI L-778,123 combined with radiotherapy for patients with locally advanced nonsmall cell lung cancer (NSCLC), locally advanced head and neck cancers, and locally advanced pancreatic tumors.[33,34] Although neither study was powered for efficacy, it appeared L-778,123 in combination with radiation had antitumor activity similar to standard radio-chemotherapy. Of significance, the combination of L-778,123 and radiation was associated with Q-T prolongation in EKG measurements, which halted further clinical evaluation of this agent. These results illustrate the potential for significant normal tissue toxicity that would not be predicted based on in vitro or even in standard investigations conducted using immuno-compromised rodents. A more recent trial combined a different PTI R115777, with radiation for patients with GBM.[35] The study was not powered for efficacy; in contrast to L-778,123, no unusual or excessive normal tissue reaction was detected.

While a number of additional, ongoing studies are aimed at evaluating the efficacy of the PTI/radiotherapy combination in cancer treatment (www.cancer.gov), to date, there has been little evidence that an enhancement of radiosensitivity occurred in any of the tumor subtypes studied. As put forth by Rengen et al. and supported by in vitro studies, Ras-mediated radioresistance is likely to be highly dependent on the genetic background of any given tumor.[31] This would appear to emphasize the limitations of conducting clinical trials in the absence of a more thorough understanding of the mechanisms through which a putative target molecule regulates radiosensitivity. However, an additional weakness in the clinical trials putatively targeting Ras has been the trials' sole reliance on PTIs. The large number of proteins other than Ras whose function may be affected is likely a complicating factor, as are the redundancies/interactions between the two forms of Ras prenylation, that is, those mediated by the farnesyltransferase and those mediated by geranyl-geranyl transferase. Thus, in addition to a thorough mechanistic understanding, a critical aspect in translating molecular target results from the laboratory to the clinic is the availability of a specific and effective targeting agent.

### 2.1.2. ErbB Receptor Tyrosine Kinase

The activation of the ErbB family of receptor tyrosine kinases (RTKs), which include ErbB1 (EGFR), ErbB2

(Her2), ErbB3 and ErbB4, initiates a diverse set of signaling pathways that ultimately affect such fundamental processes as cell division, survival, and cell−cell interactions.[36] Moreover, the aberrant expression of these RTKs, particularly ErbB1 and ErbB2, has long been associated with malignant transformation and tumor cell survival.[37] Ligands for the ErbB receptors include EGF, TGFα, and amphiregulin, which bind ErbB1; epiregulin, heparin-binding EGF-like growth factor, which binds ErbB1 and 4; neuregulins 1−4 which bind to ErbB3 and 4.[36] Ligand binding results in ErbB receptor dimerization and the autophosphorylation of specific tyrosine residues of the intracellular tyrosine kinase domain, which in turn can activate a variety of signaling pathways including those mediated by Ras, PI3K, STAT3, PKC, and phospholipase D.[36] Whereas signaling through this receptor family is dependent on the formation of homodimeric and heterodimeric combinations, the biological activity of the ErbB receptors is primarily dependent on ErbB heterodimers with homodimers having significantly less activity.[36,38] Further emphasizing the importance of heterodimer formation, ErbB2 has no known ligand and ErbB3 has no intrinsic kinase activity.[36,38] Finally, an additional complexity to this network is derived from the selectivity of the individual dimers for activating the various signaling cascades.[36]

In contrast to the ligand specificity of ErbB receptor activation, radiation induces the activation of all ErbB receptors, which occurs within minutes after irradiation with clinically relevant doses in the range of 2 Gy.[39,40] Given the established significance of the ErbB receptors in tumor cell survival, this rapid activation suggested that these RTKs may play a cytoprotective role in cellular radioresponse and would thus provide a target for radiosensitization.[39,40] Investigations pursuing this hypothesis have focused on ErbB1 (EGFR). It should be noted that in vitro investigations of radiation-induced ErbB1activation have employed a variety of cell culture conditions including exponential growth, confluence arrested, and serum starved. Clearly, these are model systems; the relevance of each to human tumor cells in situ can be argued. However, to date, numerous reports have indicated that inhibition of ErbB1 enhances the in vitro radiosensitivity to varying degrees of a variety of tumor cell lines, consistent with this receptor serving as a target for radiosensitization. These studies have used several approaches to inhibiting ErbB1 and enhancing radiosensitivity, including (1) MAbs that compete with ligand binding to the receptor,[41] (2) small-molecule inhibitors of the intracellular tyrosine kinase domain,[42] and (3) genetic modulation of the receptor expression.[43] In addition to the radiosensitization of tumor cells in vitro, each strategy for inhibiting ErbB1 activity has also been shown to enhance the radiosensitivity of human tumor cells grown as xenografts in immuno-compromised mice.[44−46]

As noted above, ErbB1 serves as an upstream effector molecule for a variety of critical signaling pathways, providing a number of possible mechanisms for its role in modulating tumor cell radiosensitivity. Among the consequences of the ErbB1-initiated cytoplasmic signaling pathways is the stimulation of tumor cell proliferation, which is primarily the consequence of transcription factor activation and changes in gene expression. Inhibition of ErbB1 thus reduces tumor growth rate, an effect reported for a variety of in vivo tumor models. However, although perhaps inhibiting tumor repopulation after in vivo irradiation and contributing to an enhanced growth delay, the antiproliferative effect of ErbB1 inhibitors is unlikely to play a role in intrinsic radiosensitivity. ErbB1 signaling in some cell types also acts to inhibit apoptosis via Akt activation, as well as through modulation of gene expression,[41,47] which suggested that radiosensitization may be the result of enhanced radiation-induced apoptosis. However, whereas ErbB1 inhibitors have been reported to enhance radiation-induced apoptosis, the inhibitors alone significantly increase apoptotic death; the additional increase resulting from the combination with radiation was modest at best.[41,44] Moreover, the enhanced apoptosis occurs at 72 h after irradiation, which is not consistent with the activation of known apoptotic pathways and is likely the consequence of accumulated DNA damage.

Over the last several years, in addition to the activation of cytoplasmic signaling processes, studies have indicated that ErbB1 translocates to the nucleus in response to a variety of stimuli including such stresses as exposure to $H_2O_2$, heat shock, and ionizing radiation.[48] In the nucleus, ErbB1 directly interacts with DNA-PKcs, a molecule critical to the repair of DSBs via nonhomologous end-joining (NHEJ), facilitating its activation (Figure 4). Accordingly, exposure to pharmacological or biological inhibitors of ErbB1 prevents its translocation into the nucleus and decreases DNA-PKcs activation, which in turn is accompanied by a reduction in DSB repair and an enhancement in radiation-induced cell killing.[49−51] Identifying nuclear ErbB1 and delineating its interaction with a critical DNA repair enzyme provided a major advancement in understanding the molecular processes regulating radiation-induced cell death. However, as for most of tumor biology, the situation is more complicated; additional factors/mechanisms appear to be involved in the radiosensitization induced by ErbB1 inhibitors. While ErbB1 inhibition clearly results in the radiosensitization of a number of tumor cell lines, not all tumor cells are affected.[52−54] The lack of a radiosensitizing effect is not limited to cells that do not express ErbB1 but also pertains to tumor cells that robustly express the RTK suggesting the involvement of other ErbB signaling pathways. Thus, as a recurring theme applicable to signaling molecules in general, the role of ErbB1 as a determinant of radiosensitivity is dependent on the underlying cellular context, both genetic and epigenetic.

Because of the cooperation between ErbB receptors in signal transduction, an alternative approach to inhibiting individual receptors has been to use single agents to target more than one of the ErbB receptors.[55] The effects of 2 pan-ErbB inhibitors on tumor cell radiosensitivity have been reported. For Lapatinib, which inhibits ErbB1 and 2, exposure of two breast carcinoma cell lines that overexpress both RTKs resulted in a modest, at best, radiosensitization.[54] Nyati et al. investigated the effects of CI-1033, which inhibits all the ErbBs with kinase activity (ErbB1, 2 and 4), on the radiosensitivity of colon tumor cell lines.[56] They also showed modest to no radiosensitization in vitro, combined with previous results, suggesting that the pan-ErbB approach does not provide an advantage toward radiosensitization. However, in their study of CI-1033 Nyati et al. showed that when LoVo cells, which were not radiosensitized in vitro, were grown in vivo as flank tumor xenografts, the ErbB1/2/4 inhibitor induced a substantial increase in radiation-induced tumor growth delay. As noted,[56] these results were consistent with those obtained with specific inhibitors of ErbB1. The enhanced degree of radiosensitization detected in vivo may be caused by a number of effects. One possibility is that ErbB inhibition directly affects microenvironmental aspects of radioresponse such as angiogenesis;[57] inhibition of ErbB1

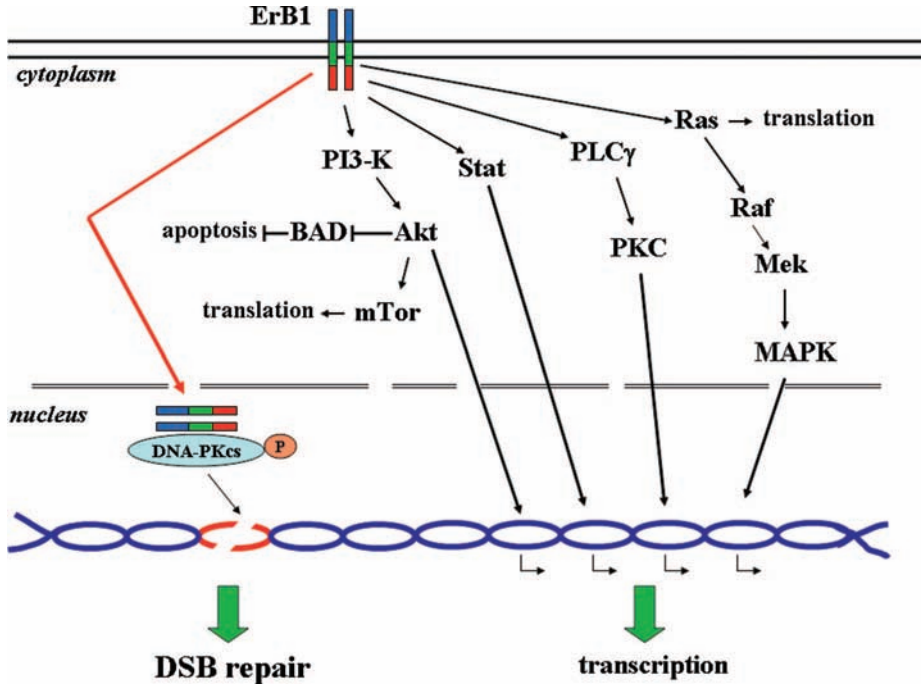


**Figure 4.** ErbB1 mediated signaling pathways.



has been reported to reduce VEGF expression.[42] Alternatively, the gene expression pattern/phenotype of the tumor cells may differ when grown under in vivo circumstances modulating the cellular context under which ErbB influences radioresponse. These results suggest that to fully define ErbB RTKs as a target for tumor radiosensitization it may be necessary to account for microenvironmental influences.

With respect to normal cells, radiation induces ErbB1 activation in fibroblasts in vitro,[58] yet their radiosensitivity is not affected by inhibition of ErbB1,[52] suggesting the potential for tumor-specific radiosensitization. Consistent with these in vitro results, Fehrmann and Dorr[59] showed that the pharmacological inhibition of ErbB1 in a mouse model had no effect on the radioresponse of acutely responding oral mucosa. In contrast, Suzuki et al. reported that the treatment of mice with the ErbB1 inhibitor ZD1839 enhanced the pulmonary fibrosis induced by the radiomimetic drug bleomycin.[60] Whether similar results would be obtained for radiation-induced pulmonary fibrosis remains to be determined. However, it should be noted that treatment of lung cancer patients with small-molecule inhibitors of ErbB1 has been associated with increased incidence of pulmonary fibrosis.[61] Thus, currently available data illustrate the potential for differential consequences of ErbB1 inhibition on acute (mucosal ulceration) and late (lung fibrosis) forms of radiation-induced normal tissue injury.

To date, there have been 16 published trials involving ErbB1 (EGFR)-targeted therapy in combination with radiotherapy (reviewed[62]). This has included a mixture of results ranging from negative trials with toxic side effects to positive trials.[63] The most positive study was conducted by Bonner et al. and was a phase III trial comparing once daily radiation to once daily radiation plus Cetuximab, an ErbB1 (EGFR)-targeting monoclonal antibody.[64] The results of this trial were an improvement in both local control and overall survival in the group of patients that had the anti-EFGR therapy in combination with standard radiotherapy. This led to FDA approval of Cetuximab as a radiationsensitizer (www.fda.gov). However, the therapy also had severe side effects including rash, dermatitis, and mucositis, all of which are commonly seen in patients receiving radiotherapy for head and neck tumors but not to the degree seen in the patients receiving the combination therapy. Although Cetuximab was a clinical success, because of the toxicity profile and the length of time to finish this study, the standard of care for treatment of advanced head and neck tumors evolved to either twice daily radiation or once daily radiation in combination with chemotherapy and thus, the combination of Cetuximab and radiotherapy is rarely used in the clinic. In an attempt to select patients that would benefit most from EGFR inhibition, studies of Cetuximab alone have demonstrated that patients whose tumors overexpress EGFR, as determined by immuno-histochemistry, have the greatest benefit from Cetuximab treatment.[65] Thus, pretherapy EGFR levels may stratify those patients for whom the combination therapy may be of most benefit. Likewise, recent biomarker data from patients with colon cancer treated with Cetuximab alone showed that patients with K-ras mutations had a worse prognosis.[66] Whether the EGFR and K-ras status of a patient's tumor will translate to clinically useful biomarkers of Cetuximab efficacy in combination with radiotherapy remains to be seen. Also complicating this type of specific molecular inhibition is that the ERB family is extremely complex, with numerous homo- and heterodimer formations and various ligand and downstream signaling pathways. Whether inhibiting a single pathway (antibody to ErbB1/EGFR) or multiple pathways (pan-Erb) would be more successful clinically in combination with radiotherapy also remains to be seen. Thus, additional studies are needed to understand the genetic and epigenetic background in which either specific or pan-Erb inhibition can lead to a meaningful improvement in clinical response in combination with radiotherapy.

### 2.1.3. Hsp90

As noted in the Introduction, there are numerous examples in which targeting a signaling molecule affects radiosensi-

tivity in a cell type dependent manner. For example, inhibition of ErbB1, Akt, p53, and NF$\kappa$B has been shown to enhance the radiosensitivity of some but not all tumor cells.[15,20,54,67,68] To overcome such cell type dependency and increase the probability or degree of radiosensitization a multitarget approach to radiosensitization has been suggested. Rather than developing a "cocktail" of targeted agents, research has primarily focused on Hsp90, a molecular chaperone. Hsp90, the 90-kd heat shock protein, modulates the degradation, folding and/or transport of a diverse set of critical cellular regulatory proteins.[69] Most Hsp90 clients, that is, those proteins that require its "chaperoning" activity for appropriate function, participate in some aspect of signal transduction including a wide variety of protein kinases, hormone receptors, and transcription factors.[70] Hsp90 can also stabilize mutated proteins allowing them to maintain normal function despite genetic abnormalities.[71] Regarding its potential as a target for radiosensitization, a number of Hsp90 client proteins have been associated with radioresponse (e.g., erbB2, Akt, Raf, Chk1),[72−76] albeit in a cell type dependent manner.

Investigations into Hsp90 as a potential determinant of tumor cell radiosensitivity have been aided by the availability of specific inhibitors geldanamycin and radicicol (natural products) and the clinically relevant geldanamycin analogs 17AAG and 17DMAG. There have been a number of reports indicating that nanomolar concentrations of 17AAG enhances the radiosensitivity of a wide variety of cell lines initiated from solid tumors with minimal cytotoxicity induced by drug treatment alone.[72,75,76] Another clinically relevant Hsp90 inhibitor 17DMAG enhanced the radiosensitivity of a variety of tumor cell lines in vitro, as well as the radiation-induced growth delay of a prostate tumor xenograft model.[73,74] The Hsp90 inhibitors geldanamycin and radicicol have also been shown to enhance the radiosensitivity of a variety of human tumor cell lines.[72,77,78] Thus, on the basis of results using a variety of compounds and a variety of human tumor cell lines, Hsp90 has been established as a determinant of tumor cell intrinsic radiosensitivity.

The mechanisms through which Hsp90 inhibition modifies tumor cell radiosensitivity have been traced down to two components of the DNA damage response − DNA double strand break repair and cell cycle checkpoint activation[79] (Figure 5). The inhibition of double strand break repair was attributed to the loss of ErbB2 (Her2/neu) in 17DMAG treated cells and the consequent reduction in ErbB1 activity, which leads to a reduction in the ErbB1 interaction with DNA-PKcs and the subsequent attenuation of DNA-PK activation after irradiation. The abrogation of G2 cell cycle checkpoint activation by 17DMAG was associated with a reduction in radiation-induced activation of ATM, which was the result of a reduced interaction between NBS1 and ATM. Whereas Hsp90 was not bound to ATM, it was found to interact with the MRN (MRE11/RAD50/NBS) complex, suggesting a novel client protein or protein complex. Thus, data indicate that the DSB repair inhibition and the abrogation of the G2 check point are independent events linked to different Hsp90 client proteins. It should be noted, however, that for optimal radiosensitization, both the DSB repair inhibition and the abrogation of the G2 check point were required.[79] While most studies regarding Hsp90 as a target for cancer treatment have focused on its cytoplasmic activities, it appears that the ultimate effects of this chaperone on radiosensitivity are mediated within the nucleus.

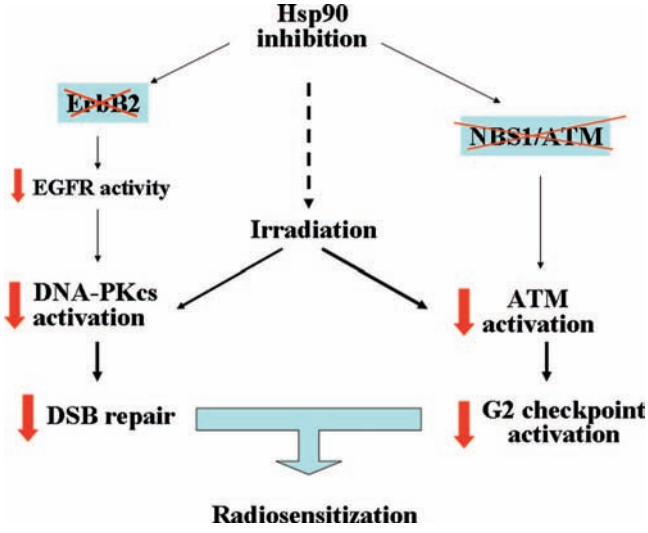


**Figure 5.** Two processes contributing to radiosensitization induced by Hsp90 inhibition.

Although effective against a wide variety of solid tumor cell lines, inhibition of Hsp90 was found not to enhance the radiosensitivity of the pancreatic carcinoma cell line, AsPC1.[74] Whereas exposure of AsPC1 cells to 17DMAG resulted in a loss of ErbB2, as well as the loss of the radioresponse associated proteins Raf and Akt, the Hsp90 inhibitor had no effect on ASPC1 radiosensitivity. In contrast to the tumor cell lines in which Hsp90 inhibition enhanced their radiosensitivity, ASPC1 cells expressed significant levels of ErbB3; other tumor cell lines that expressed ErbB3 were also found to be resistant to 17DMAG-induced radiosensitization.[74] Subsequent studies showed that the expression of ErbB3 compensates for the 17DMAG-mediated ErbB2 degradation via formation of the ErbB1/ErbB3 heterodimer and the maintenance of ErbB1 kinase activity, which then prevents the reduction in DNA-PKcs activation and consequent inhibition of DSB repair. These data indicate that ErbB3 expression predicts for tumor cell susceptibility to radiosensitization induced by Hsp90 inhibition in vitro.

When the same treatment protocol as used for tumor cells was employed, Hsp90 inhibitors had no effect on the radiosensitivity of a series of nonimmortalized, normal human diploid fibroblast cell lines.[72,75,76] Whether the lack of fibroblast radiosensitization in vitro is predictive of a lack of an enhancement in radiation-induced normal tissue toxicity certainly remains a critical question and subject to further investigation. The mechanism responsible for this selective sensitization of tumor cells over normal cells, however, remains unclear. Previous studies have suggested a difference in the biochemistry of Hsp90 in tumor versus normal cells.[80] However, this does not appear to be the case with respect to normal fibroblasts and radioresponse-associated proteins. Hsp90 inhibitors reduced the levels of the radioresistance-associated proteins Raf, Akt and ErbB2 in normal fibroblast cell lines,[76] in a manner similar to tumor cells and yet, in the fibroblasts these decreases were not accompanied by an increase in radiosensitivity. Moreover, each of the fibroblast cell lines (C29, MRC5 and MRC9) does not express detectable levels of ErbB3 suggesting that the resistance mechanism identified for tumor cells is not operative in these normal cells. Thus, results to date suggest that it is not Hsp90 function that differs between normal and tumor cells, but that the mechanisms through which solid tumor cell lines and normal fibroblasts respond to radiation are substantially

different. Although not explaining the inability of Hsp90 inhibitors to enhance fibroblast radiosensitivity, these results do support the existence of tumor specific targets for radiosensitization.

At present, there are no clinical trials open involving the combination of an Hsp90 inhibitor and radiotherapy. However, there are 15 open cancer trials of 17AAG including nine phase I and six phase II, with eight of those trials using 17AAG as a single agent and seven of those trials using 17AAG in combination with standard chemotherapy; there are four active phase I trials using 17DMAG (www.cancer.gov). In an attempt to overcome a number of the pharmaceutical and pharmacodynamic difficulties associated with these geldanamycin derivatives,[81] phase I studies of the synthetic Hsp90 inhibitors CNF2024,[81] SNX-5422[82] and IPI-504 [83]), all of which are single agent trials, are ongoing (www.cancer.gov). Information generated from these trials will clearly aid in the design of combination protocols involving radiotherapy.

A putative advantage of any clinical trial involving a targeted agent is the availability of a biomarker as a translational end point of drug efficacy. In trials involving Hsp90 inhibitors as single modalities, the evaluation of biomarkers indicative of drug effect has focused on increases in Hsp70 and/or the decrease in Raf-1 or cyclin-dependent kinase 4.[84] With respect to the potential design of clinical protocols combining an Hsp90 inhibitor and radiotherapy, the preclinical results suggest that ErbB3 expression may be a useful marker for patient stratification.[74] That is, patients with tumors that do not express ErbB3 would be predicted to respond best to this combined modality. Moreover, data from experimental models indicate that a mode of resistance to Hsp90 inhibitor induced tumor cell radiosensitization is via the maintenance of ErbB signaling.[74] Thus, while laboratory studies suggest that ErbB3 expression may be of use in selecting patients, they also suggest that the degree and/or probability of Hsp90 inhibitor-induced radiosensitization might be enhanced if the combined modality included an ErbB1 (EGFR) inhibitor. Clearly, such a combination awaits the initial clinical trials combining Hsp90 inhibitors with radiotherapy.



## 2.2. Epigenetic Targets

Genetic alterations involving a variety of oncogenes and tumor suppressor genes, as well as their associated molecular networks are well established as critical participants in cancer development and progression. More recently, epigenetic modifications have become increasingly recognized as a significant contributor to tumor initiation and biology, often working in concert with genetic alterations.[85] Epigenetics refers to heritable modifications in gene expression that are not the result of changes in primary DNA sequence. In contrast to genetic abnormalities, epigenetic changes are reversible and subject to pharmacological manipulation. Thus, given the prevalence of altered epigenetic signatures in human tumors, epigenetic modifiers have received considerable attention in cancer treatment.[86]

The most frequent and well studied of the epigenetic alterations in cancer cells are aberrant DNA methylation and histone deacetylation (HDAC). In a number of in vitro and in vivo experimental models inhibitors of DNA methylation or HDAC have been reported to induce tumor cell differentiation, apoptosis, or growth arrest.[87,88] Moreover, consistent with an aberrant epigenome being characteristic of neoplastic cells, experimental and clinical results suggest that these effects are tumor selective.[88,89] Indeed, for tumors originating from hematopoietic or lymphoid tissue epigenetic modifiers typically induce differentiation to a nonmalignant phenotype or apoptotic cell death.[87,88] However, for most cell lines generated from solid tumors, their primary result is reversible cytostasis, questioning their potential as a single modality treatment for cancers of nonhematopoietic origin. At the molecular level, the general effect of inhibiting DNA methylation or HDAC activity is a relaxation of chromatin structure and modification of gene expression; each of these processes individually has been implicated in the regulation of radiosensitivity. Along these lines, the orally active DNA methylation inhibitor zebularine has been shown to enhance the in vitro and in vivo radiosensitivity of human tumor cells, which was correlated with gene re-expression and associated with an inhibition of DNA repair.[90] Whereas the zebularine-induced radiosensitization is consistent with an epigenetic target for tumor cell radiosensitivitization, little additional information pertaining to its mechanism of action or translation to a treatment setting has been generated. However, as to the potential of epigenetic drugs serving as radiosensitizing agents, HDAC inhibitors have received considerable attention, and are described below.

### 2.2.1. Histone Deacetylase

Histone proteins play an intimate role in maintaining and regulating chromatin structure from nucleosomes to higher-order packaging and chromosome organization. Critical to the dynamic modulation of chromatin structure are post-translational histone modifications including acetylation, which is determined by the opposing actions of histone acetyltransferases (HAT) and histone deacetylases (HDAC).[91] Whereas oncogenesis has been associated with HAT inactivation, it is aberrant HDAC activity leading to the transcriptional repression of tumor suppressor and other genes that is generally considered a common event contributing to tumor formation.[88] Because it is easier to inhibit an enzyme rather than enhance its activity, HDAC inhibition has generated considerable interest as a potential form of cancer treatment.

Histone deacetylation is mediated by a series of histone deacetylases (HDACs). To date, 19 human genes that encode HDACs have been identified and are classified into to three subfamilies.[92] Class I HDACs (HDAC1, 2, 3, 8 and 11) are generally nuclear and associate with transcriptional repressors and cofactors. Class II HDACs (HDAC4, 5, 6, 7, 9, 10, and HDRP/MITR) are larger proteins and migrate between the cytoplasm and nucleus. Class III HDACs or sirtuins (SIRT1−7) are the most recently discovered HDACs, are NAD-dependent, and share homology with the yeast Sir2 gene. Currently, a relatively large number of compounds encompassing a variety of structural and pharmacological classifications have been shown to inhibit HDAC activity and result in histone hyperacetylation.[93] Although new inhibitors are being generated, at present, there are six general classes of HDAC inhibitors, most of which target the catalytic domains of class I and II HDACs.[88] These inhibitors for the most part are not specific for a given HDAC but show slight preferences to either class I or II HDAC.[94]

**HDAC and Radiosensitization.** Direct support of a role for HDAC in regulating radioresponse was initially provided by the HDAC inhibitor sodium butyrate. In a series of studies by Leith and colleagues, sodium butyrate at relatively

nontoxic concentrations increased the radiosensitivity on human colon carcinoma cell lines.[95] However, because of its very short half-life and low achievable serum concentration, sodium butyrate has limited clinical applicability.[96] Interest in HDAC as a target for cancer treatment in general has led to the development of a variety of inhibitors with more favorable in vivo pharmacokinetic and toxicity profiles. One of the first clinically applicable HDAC inhibitors with respect to radiosensitizing potential was the benzamide MS-275, which was found to enhance the in vitro radiosensitivity of two human tumor cell lines of different histological origins,[97] as well as the radiation-induced growth delay of human tumor xenografts.[98] Members of the short chain fatty acid class of HDAC inhibitors phenyl butyrate (PB), tributyrin,[99] and valproic acid (VA)[100] have also been shown to enhance the radiosensitivity of a variety of human tumor cell lines. The HDAC inhibitors, SAHA and CBHA (hydroxamates), have been reported by a number of groups to enhance the radiosensitivity of tumor cells corresponding to a variety of histologies [101−103] as has the cyclic peptide depsipeptide.[103,104] Thus, given the structural disparity among these compounds, it appears that a fundamental consequence of HDAC inhibition is the enhancement of tumor cell radiosensitivity.

Initial investigations into HDAC inhibitors as radiosensitizers were based on the assumption that the critical event is histone hyperacetylation. A correlation between the onset of hyperacetylation and radiosensitization has been established for a variety of HDAC inhibitors across a series of tumor cell lines. However, histone acetylation and deacetylation is a dynamic process with some species of acetylated histones having a half-life on the order of minutes.[105] The standard protocol testing an HDAC inhibitor in combination with radiation involves exposing cells to the inhibitor for 24 to 48 h irradiation, followed by trypsinization and seeding cells into HDAC inhibitor-free media for analysis of clonogenic survival. Following this treatment protocol often resulted in minor to no increase in radiosensitivity.[95,97,100] Subsequent studies using MS275 and VA revealed that the maximal histone hyperacetylation declined to control levels by 6 h after placing cells in inhibitor-free media indicating that the hyperacetylation depends on the continuous exposure to the HDAC inhibitor. To determine the consequence of maintaining histone hyperacetylation on radiosensitivity, cultures were exposed to the MS275 or VA both before and after irradiation, which resulted in a substantially greater degree of radiosensitization than that induced by preirradiation exposure only.[97,100] The maximum degree of radiosensitization induced by SAHA (hydroxamate class) and depsipeptide (cyclic peptide class) was also found with pre- and postradiation exposure.[104] These results suggested that to obtain maximal enhancement in radiosensitivity exposure to an HDAC inhibitor is required both before and after irradiation.

**HDAC Inhibitors and the Repair of DNA Double Strand Breaks.** Although establishing a correlation between histone hyperacetylation and radiosensitization, which may have implications regarding protocol design, the mechanism through which HDAC inhibitors enhance radiosensitivity remains uncertain. A critical event in determining radiosensitivity is the repair of DNA double strand breaks (DSBs). Over the last several years, $\gamma$H2AX expression has been established as a sensitive indicator of DSBs induced by clinically relevant doses of ionizing radiation.[106] At sites of radiation-induced DNA DSBs the histone H2AX becomes rapidly phosphorylated (the phosphorylated form is referred to as $\gamma$H2AX), forming readily visible nuclear foci.[106] Although the specific role of $\gamma$H2AX in the repair of DSBs has not been defined, recent reports indicate that the dephosphoryation of $\gamma$H2AX and dispersal of $\gamma$H2AX foci in irradiated cells correlates with the repair of DNA DSBs.[107] Moreover, Macphail et al. in their study of ten cell lines reported that the loss of $\gamma$H2AX correlates with clonogenic survival after irradiation.[108] The HDAC inhibitors MS-275,[97] VA,[100] SAHA (Vorinostat),[109] CBHA,[83] depsipeptide,[102] and NaB[99] have all been shown in a variety of human tumor cell lines to prolong the expression of radiation-induced $\gamma$H2AX, thus suggesting the inhibition of DNA DSB repair.

Thus, $\gamma$H2AX data generated to date suggest that radiosensitization induced by HDAC inhibitors is the result of an inhibition of DSB repair. However, complicating this scenario, HDAC inhibitors (VA and MS275) have no effect on the repair of radiation-induced DSBs as measured by the neutral comet assay.[110] Accounting for this discrepancy may be that the 2 assays reflect different manifestations of the DSB repair process. In the neutral comet assay repair is complete within 4−6 h after irradiation corresponding to the rejoining of strand breaks, an initial process in DSB repair also referred to as the "fast" component. The dispersal of $\gamma$H2AX foci, in contrast, requires 16−24 h after irradiation, which is more consistent with the re-establishment of chromatin structure and chromosome metabolism. It should be noted that in VA or MS-275 treated cells, there was little to no difference in $\gamma$H2AX expression at 1 and 6 h after irradiation, the effect is most prominent at 16−24 h.[97,100] Moreover, it was recently reported that addition of VA to culture medium at times out to 24 h after irradiation delayed the dispersal of $\gamma$H2AX foci and enhanced tumor cell radiosensitivity.[110] Thus, it appears that HDAC inhibitors affect the later stages of DSB repair, which involves chromatin restoration.

The molecular process responsible for this apparent inhibition of DSB repair was initially ascribed, as for the antitumor effects of HDAC inhibition in general, to modifications in gene expression. Along these lines and related to their putative effects on DNA repair, exposure of tumor cells to NaB[99] or SAHA[109] were reported to decrease the expression of the repair proteins of Ku70, Ku86, and DNA-PKcs. In addition, exposure of a prostate tumor cell line to SAHA also resulted in a decrease of DNA-PKcs levels.[101] Ku70, Ku86, and DNA-PKcs are critical components of the NHEJ DSB repair pathway; decreases in their expression would be expected to result in a decrease in the fast component of DSB repair, which is readily detectable in the neutral comet assay or some other analysis that measures DSB rejoining in mammalian cells. However, whereas $\gamma$H2AX foci analysis was performed in these studies, the neutral comet or other DSB rejoining assay was not reported. Thus, whether there is an inhibition of NHEJ in these cells after exposure to HDAC inhibitors remains to be defined.

Regarding transcription as a target, although essentially all HDAC inhibitors result in radiosensitization across a wide variety of tumor cell lines, the changes in gene expression induced are not consistent among the different inhibitors or cell lines.[111,112] According to DNA microarray analyses the expression of only 2−10% of genes are affected after tumor cell exposure to HDAC inhibitors.[111,113] Moreover, a direct comparison of 3 solid tumor cell lines indicated that, following HDAC inhibition, there were only 13 genes whose

expression was commonly affected in each of the cell lines.[111] Thus, on the basis of the broad capacity of HDAC inhibitors to enhance radiation response in multiple cell lines across tumor types and what appears to be a cell line specific control of gene transcription, it appears unlikely that the influence of HDAC inhibitors on transcription per se is the sole mechanism mediating the enhanced radiation response. In support of a nontranscription based process, is the observation that the addition of VA to culture media at times up to 24 h after irradiation resulted in a significant radiosensitization of glioma cell lines.[110]

Attempts to define the mechanisms through which HDAC inhibitors induce radiosensitization have also addressed direct interactions between HDACs and essential components in the DNA repair process itself.[114,115] ATM, a critical molecule in the initiation and regulation of the DNA damage response, was found to be associated with HDAC1 after irradiation.[116] Moreover, Kao et al. reported that HDAC4 interacts with 53BP1, another protein involved in the DNA damage response.[117] With respect to HDAC inhibitors, Chen et al. reported that TSA, SAHA and MS-275 exposure resulted in the acetylation of Ku70, a nonhistone protein critical to DSB repair.[118] Although it is unclear whether such interactions are involved in HDAC inhibitor-induced radiosensitization, these results illustrate that it is not only the acetylation status of histones that is affected by HDAC inhibition.

It has become increasingly well recognized that HDAC inhibitors induce the acetylation of a wide variety of nonhistone proteins. Kim et al. in their proteomic-based analysis, identified more than 190 nonhistone proteins that are subject to lysine acetylation and are thus potential targets for HDAC inhibitors.[119] Given that histone hyperacetylation, which is induced in both tumor and normal cells, cannot account for the tumor selectivity of HDAC inhibitors in terms of cell proliferation, apoptosis and differentiation, the authors speculate that the critical targets are actually nonhistone proteins. Clearly, the same situation may apply to the radiosensitization-induced by HDAC inhibitors. Thus, while an inhibition of DSB repair has been associated with HDAC inhibitor-induced radiosensitization, the specific mechanisms mediating this effect remain to be determined.

**Normal Tissue Radiosensitivity and HDAC Inhibition.** As for other molecularly targeted radiosensitizers, tumor selectivity over normal cells will be an essential characteristic for HDAC inhibitors. Munshi et al. reported that following an NaB exposure protocol that induced the radiosensitization of two melanoma cell lines, no effect on the radiosensitivity of a normal human fibroblast cell line was detected[99] Because the relationship of radiation-induced death of normal cells as detected in monolayer culture to the actual normal tissue injury induced by radiation is unclear, defining the effects of HDAC inhibitors on radiation-induced normal tissue injury would aid in the clinical application of these agents in combination with radiotherapy. Along these lines, Chung et al. described the consequences of PB, TSA, and VA administration on radiation-induced skin injury using a rat model.[120] They showed that delivery of each of the HDAC inhibitors reduced the severity of the acute radiation-induced skin reaction and inhibited the late fibrotic response, which was attributed to the suppression of aberrant expression of TGF$\beta$. It should be noted that HDAC inhibitors have also been reported to protect against traumatic[121] and ischemic injury[122] in the rodent brain. Thus, although the specific mechanisms have yet to be defined, laboratory evidence suggests that HDAC inhibitors selectively induce tumor radiosensitization and may actually protect against normal tissue injury.

**Clinical Application.** HDAC inhibitors have been extensively evaluated in single modality clinical trials as recently reviewed by Kim et al. [123] and at present there are numerous ongoing clinical studies evaluating HDAC inhibitors with chemotherapy (www.cancer.gov). In addition, there are four ongoing trials combining HDAC inhibitors with radiotherapy: two with SAHA and two with VA (www.cancer.gov). The initial trial opened involves the combination of VA with Temozolomide (TMZ) and radiotherapy for patients with newly diagnosed glioblastoma multiforme (http://bethesdatrials.cancer.gov/clinical-research/search_detail.aspx?ProtocolID=NCI-06-C-0112). VA is particularly attractive for brain tumor treatment because it has a long history as a safe and effective antiseizure medication with excellent blood brain barrier permeability.[124] The design of this trial was based on preclinical data as described above. That is, laboratory data indicated that maximum radiosensitizing effect was shown when VA was present both before and after the radiation. To accomplish this in the clinic, patients are treated with VA for 1 week before starting the radiotherapy with daily dosing during the entire 30 fractions of radiation. Moreover, to evaluate acetylation of H3 and H4, PBMCs are collected from patients before initiating VA and after 1 week of VA therapy. Nine patients have been accrued to date with each demonstrating an increase in PBMC histone acetylation after VA therapy. Although PBMC acetylation status is an indirect analysis, based on previous clinical[125] and experimental results,[100] it is suggestive of a positive tumor effect. The accrual goal in this study is 41 patients, which will allow for an evaluation of the efficacy of this regimen. More important, it is anticipated that the data generated will not only provide the basis for further clinical studies but also suggest additional questions to be addressed in the laboratory.

## 2.3. DNA Damage Response

Cell survival after exposure to radiation is dependent on a highly integrated series of sensor, mediator and effector molecules that comprise the DNA damage response (DDR).[126] The DDR is composed of a number of processes; two that have received considerable attention as possible targets for tumor radiosensitization are DNA strand break repair and the activation cell cycle checkpoints. Although these events play an essential role in the survival of normal cells after irradiation, the rationale for tumor selectivity is that cancer cells are by definition genomically unstable and consequently have defects in their DDR.[127−129] Indeed, this is likely the case for certain tumor cells such as those with BRCA1 or BRCA2 mutations. However, whether tumor cells in general have defective DNA repair or cell cycle checkpoint activation after irradiation as compared to normal cells has not been clearly demonstrated. Thus, as discussed below, the tumor selectivity to be achieved via targeting critical components of the DDR remains to be determined.

### *2.3.1. DNA Double Strand Break Repair*

The critical lesion leading to cell death after irradiation is the double strand break (DSB). DSBs are repaired via 2 pathways: nonhomologous end joining (NHEJ) and homologous repair (HR). The specific molecules and events involved

in each DSB repair process have recently been reviewed in detail by Shrivastav et al.[130] HR utilizes a homologous template and consequently is only operative in S and G2, although a recent report suggests that it only occurs in S phase.[131] It is generally considered that HR plays a minor role in repairing DSBs in mammalian cells as suggested by the modest increase in radiosensitivity of cell lines with defective HR.[132] NHEJ, in contrast, is operative throughout the cell cycle; mutant cells (tumor or normal) lacking components of NHEJ are dramatically radiosensitive.[132] DNA-PKcs is an essential molecule in NHEJ;[133] its loss is responsible for the extreme radiosensitivity of the SCID mouse.[134] In contrast to other essential components of NHEJ such as KU70 and KU80, DNA PKcs is an enzyme and consequently has been suggested as a target for drug development. To date there have been a number of DNA-PKcs inhibitors developed and reported to enhance the radiosensitivity of tumor cell lines.[135−137] Whereas there are cytoplasmic signaling pathways that can influence DNA-PKcs efficiency selectively in some tumor cells (see ErbB1 above), at present there is no evidence indicating that DNA-PKcs or other core components of the NHEJ machinery differ between tumor and normal cells. Thus, the potential for tumor selective radiosensitization by DNA-PKcs inhibitors remains unclear.

An additional DDR target for radiosensitization is poly-(ADP-ribose) polymerase (PARP) 1, a nuclear protein involved in sensing and signaling the presence of DNA damage, specifically single strand breaks (SSBs). Activation of PARP1 leads to the addition of poly(ADP-ribose) branched chains from $NAD^+$ onto damaged DNA leading to the relaxation of the chromatin structure and recruitment of additional repair proteins, including XRCC1, Pol $\beta$, and DNA ligase. PARP1 inhibition thus inhibits SSB repair; a number of PARP1 inhibitors have been reported to induce radiosensitization.[138] Whereas SSBs in themselves are not critical lesions contributing to radiation-induced cell death, the mechanism of radiosensitization appears to involve the conversion of the accumulated SSBs resulting from PARP1 inhibition into DSBs.[138] Supporting a compromise of DSB repair by PARP1 inhibitors is data generated using the neutral comet assay[137] and the delay the dispersion of radiation-induced $\gamma$H2AX foci.[139] Whereas there is some selectivity for PARP1 inhibitors alone as cytotoxic agents against BRCA1 and BRCA2 deficient cells,[140] they appear to be effective radiosensitizers against tumor cells in general; their effects on the radiosensitivity of normal cells and tissue remains to be fully defined.

### 2.3.2. Cell Cycle Checkpoints

After irradiation, cells are transiently arrested in G1, within S phase and at the G2/M border, providing an increase in time for DNA repair before progression into or through the critical phases of DNA synthesis (S phase) or mitosis. The activation of such cell cycle checkpoints is an essential feature of the DDR and has long been considered to provide a radioprotective effect. With respect to targets for radiosensitization, the intra-S phase and G2 checkpoints have been the primary focus. CHK1 is a critical molecule in the activation of the S-phase and G2/M checkpoints. Knockdown of CHK1 using siRNA was reported to enhance the radiosensitivity of human colon tumor cells.[141] Exposures of human tumor cell lines to chemical inhibitors of CHK1 have also been reported to result in radiosensitization.[142,143] These types of data have led to the development of clinically applicable CHK1 inhibitors, which at present are being evaluated in phase 1 trials in combination with chemotherapy.[144]

The ATM (ataxia telangiectasia mutated) protein kinase plays a pivotal role in the activation of the DDR after irradiation and is essential in activating each of the cell cycle checkpoints.[145] More recent data indicates that it also is critical in the repair of a subset of DSBs.[146] These molecular aspects of ATM function are consistent with the extreme radiosensitivity of cells isolated from patients with ataxia telangeictasia. Caffiene and wortmannin are long established inhibitors of ATM, however, these agents have significant off-target effects and are not clinically applicable. Recently, specific and more potent ATM inhibitors have been identified, which have been shown to enhance tumor cell radiosensitivity and have been suggested for potential clinical application as radiosensitizing agents.[147,148] Given the established role of ATM in regulating cellular radiosensitivity, whether these agents affect normal cell or tissue radiosensitivity remains a critical question.

## 3. Microenvironmental Targets

Solid tumors in situ are composed not only of cancer cells but also of a variety of normal tissue components including structural (e.g., fibroblasts), vascular (e.g., endothelial cells), and immune (e.g., macrophages) elements. As noted in section 2, interactions between normal and tumor cells have been implicated as critical components of tumorigenesis[4] with data also suggesting that microenvironmental factors play a significant role in determining tumor cell phenotype.[5,6] With respect to radiosensitivity, the potential significance of the microenvironment can be illustrated by considering glioblastomas. Primary glioblastomas are highly variable in terms of their gene expression profiles and genetic abnormalities.[149,150] Yet, despite this extensive heterogeneity, although some glioblastomas respond better than others, they all essentially fail radiotherapy. This relatively "homogeneous" clinical response in a background of intertumor heterogeneity suggests that the microenvironment, which all glioblastomas have in common, plays a significant role in determining their radiosensitivity.

An environmental factor that has long been causally associated with the radioresistance of tumor cells in vitro and when grown as tumor xenografts is hypoxia, specifically oxygen levels below ~1%. Such information led to numerous clinical trials combining radiotherapy with nitroimadizole-based hypoxic cell radiosensitizers, which have to date met with little clinical usage.[151] However, more recent laboratory investigations have begun to describe the specific molecules or cells involved in heterotypic in situ interactions that can play a role in tumor radioresponse, which include the presence of critical immune cells,[152] $\beta$1 integrin-mediated signaling between tumor cells and the extracellular matrix,[153] and VEGF signaling in endothelial cells after irradiation.[154] While defining the interactions between tumor and normal tissue that influence radioresponse would appear to provide a novel source of targets for radiosensitization, such investigations are difficult to model. Traditionally, in vivo models have relied on human tumor xenografts grown in nude or SCID mouse models. Although vasculature and stromal cells may be present there is little active immune system in these animals. An alternative approach is to grow murine tumors in immuno-competent syngeneic host animals. However,

significant differences exist between rodent and human cells regarding the specific mechanisms and molecules mediating DNA repair (summarized in [155]), suggesting that putative molecular targets for radiosensitization may also differ. A third strategy that has recently been expanded is to use spontaneous tumors in other mammalian species. The Comparative Oncology Program (http://ccr.cancer.gov/resources/cop/public.asp) is studying the use of molecularly targeted agents in dogs with cancer. This approach is relatively expensive and whether the response of canine tumors is comparable to the response of human tumors to either drugs or radiation remains to be determined. However, it does provide a unique population for potentially more relevant "clinical trials". Even with these noted deficiencies experimental data generated to date supports a critical role for the microenvironment in determining tumor radioresponse. With respect to targeted radiosensitization, the major advances have involved angiogenesis, which is addressed below.



## 3.1. Angiogenesis

For tumors to grow beyond a microscopic size, they must establish a network of vessels to provide nutrients and oxygen, a process termed angiogenesis. Angiogenesis is a balance between expression of numerous endogenous pro- and antiangiogenic factors. A tip toward the pro side, the angiogenic switch, leads to the ordered recruitment, migration, or proliferation of components of the vessel wall. Endothelial cells are normally arranged in a monolayer with tight junctions that limit vessel permeability. In larger capillaries and venules, the endothelial monolayer is closely associated with pericytes, cells that play an important role in vascular stabilization. The endothelial and pericyte cells are surrounded by a basement membrane. The tumor vasculature is an attractive target for various reasons. First, though the sensitivity of tumor cells can vary within and between tumors because of an accumulation of mutations, the tumor vasculature provides a more homogeneous and stable target because it is less genetically variable. Second, the tumor vasculature is in direct contact with the bloodstream allowing uncomplicated delivery of agents to the target cells. Third, numerous pro-angiogenic growth factors are involved in the formation and stabilization of new blood vessels including VEGF, PDGF, HGF, and bFGF, and because it is easier to inhibit an activity than to promote one, these growth factors and the pathways they stimulate have been the focus of much study into antiangiogenic therapy. However, as with other tumor physiology the multitude of pro-angiogenic molecules and redundancy of their actions may make the inhibition of any one pathway ineffective. This has rapidly led to the development of multitargeted antiangiogenic agents.

### *3.1.1. Angiogenesis and Radiosensitization*

In preclinical models, radiation can induce the expression of pro-angiogenic factors including VEGF, a potent pro-survival factor, which may lead to radiation resistance.[154] For this reason, delivery of antiangiogenic agents that block the release of VEGF or its downstream effects may lead to enhanced tumor response to radiation. Conversely, the presence of oxygen is the most important molecule in stabilizing the DNA damage induced by radiation. Thus, if angiogenesis inhibitors were to prevent the formation of new blood vessels, thereby, decreasing oxygen levels, it seems counterintuitive that combining an angiogenesis inhibitor with radiation would result in an improved antitumor effect. However, in multiple preclinical models treatment with inhibitors of angiogenesis has been clearly shown to increase the oxygenation of tumors [154,156] and, thus, the response to radiation.[157] Preclinical data indicates that antiangiogenic therapy in combination with radiation leads to a decreased microvessel density (MVD) yet, paradoxically, an increase in tumor $pO_2$. Geng et al. demonstrated that although the overall MVD had decreased in their model the number of larger functioning vessels remained constant possibly leading to the increase in the $pO_2$.[158] Thus, two explanations for the antiangiogenic-induced radiosensitivity are possible: the first is that by elimination of the small nonfunctional or blunt-ended microvessels the actual $pO_2$ of a tumor increases making the radiation more effective because of an improvement in tumor oxygen status. The second is that the effects of the combination therapy are unrelated to the oxygenation status of the tumor and are related to some other process.

Numerous antiangiogenic agents have been tested in the laboratory in combination with radiation (reviewed in ref 157). These can be divided into molecules that are found endogenously and those that are not. Several endogenous agents have been used in combination with radiation in preclinical models, including Angiostatin (a plasminogen fragment)[159] and endostatin (a collagen 18 fragment).[160] Several nonendogenous antiangiogenic agents have also been used in preclinical models in combination with radiation, including anti-VEGF antibodies,[154] small VEGF tyrosine kinase inhibitors,[158] and nonspecific TKi.[161] In general, these studies have been conducted on relatively small tumors (<1 cm). This may be an important variable to consider in evaluating these experiments since tumor size at the time of therapy affects the response to both radiation[162] and angiogenesis inhibitors.[163] This maximal sensitivity to radiation and angiogenesis inhibitors appears to correspond to the rapid phase of tumor growth. It also corresponds to the highest proliferation rates of the tumor vasculature. Thus, growing tumors with rapidly proliferating tumor vessels may be more sensitive to angiogenesis inhibitors than tumors with slower growth rates and a larger percentage of established vessels.

The timing of antiangiogenic drug delivery in relation to radiation may also have significant implications. Delivery of the agent prior to radiation may lead to "normalization" of the tumor vasculature, improving blood flow and oxygenation, thus, improving the response to radiation.[164] However, delivery of an antiangiogenic agent that is effective against a molecule that is up-regulated by irradiation at the time of or shortly after radiation, may allow inhibition of the angiogenic activity that is induced by the radiation.[154] In both the clinic and the laboratory, much work is underway attempting to develop markers for either vascular normalization (dynamic enhanced magnetic resonance[165,166]) or radiation-induced biomarkers (reviewed in ref 167). Thus, specific antiangiogenic agents, biomarker development, and the timing of the drug/radiation combination may be agent specific, and much work remains.

### *3.1.2. Clinical Trials*

Though successes have been reported with the combination of antiangiogenic agents and chemotherapy, few studies have been completed using angiogenesis inhibitors with radiation. The only reported trial of an endogenous inhibitor is a phase

I study of Angiostatin in combination with radiation in patients with solid tumors that was completed at Thomas Jefferson Hospital (Dicker AP 38th ASCO meeting abstract). Patients with varied histologies were included on this study and received daily Angiostatin infusions prior to radiotherapy. No additional toxicity was appreciated in these patients within the radiation portal, but a mild skin rash was seen in three patients. Since the data have only been presented in abstract form, no definitive conclusions can be drawn from this trial.

Using anti-VEGF antibody therapy in combination with radiation, Willet et al. reported a phase I trial in patients with locally advanced adenocarcinoma of the rectum that were treated with a combination of radiation, 5-fluorouracil, and bevacizumab before surgical resection.[168] The patients received a bevacizumab infusion and multiple biomarkers were assessed prior to initiation of the combined therapy (imaging, serum markers). The patients were then treated with bevacizumab and radiation and then taken to surgery. A marked response was observed in all patients with only microscopic disease identified in 5 of the 6 patients. However, since this was a phase I trial it was not powered to evaluate clinical efficacy. Three additional bevacizumab/RT phase 1 studies have been conducted in patients with pancreatic, rectal, and head and neck tumors.[169−171] Overall, the addition of bevacizumab to radio-chemotherapy was well tolerated. However, there is some concern that the addition of bevacizumab may lead to ulceration or fistula formation, thus caution must be maintained as phase II trials are initiated.

Thalidomide has been evaluated in combination with radiation or radio-chemotherapy in a number of reported trials.[172,173,74,175] In each of the trials, there was no benefit to the addition of thalidomide compared to the standard radiotherapy or radio-chemotherapy. However, because of the toxicity profile from Thalidomide, including but not limited to the CNS toxicity (severe drowsiness), many of the patients in the Thalidomide arms of the trials did not receive all of the prescribed drug. Thus, thalidomide plus radiotherapy may have been ineffective because adequate drug was not given to the patients. However, newer thalidomide analogs without the CNS toxicity are currently under development.



## 4. Conclusions

The promise of target-based radiosensitizing strategies lies in the potential for tumor selectivity and the availability of molecular indicators of tumor susceptibility (biomarkers). While initial studies focused on defining causal relationships between a given molecular target and radiosensitivity, it has become increasingly apparent that the translation of such laboratory information to a clinical treatment setting is considerably more complicated than initially envisioned. As evidenced from initial clinical trials, effective and specific targeting agents are essential. Moreover, it appears unlikely that a single molecule that does not play a role in normal cell radioresponse will serve as target for the radiosensitization of all tumor cells. Thus, an additional requirement appears to be a more thorough understanding of the specific cellular context under which a putative target actually serves as a determinant of tumor radiosensitivity. Such information should provide the basis for the identification of biomarkers of susceptibility and move target-based radiosensitization into the era of personalized medicine.

## 5. References

(1) Steel, G. G.; Peckham, M. J. *Int. J. Radiat. Oncol., Biol., Phys.* **1979**, *5*, 85.
(2) Willett, C. G.; Safran, H.; Abrams, R. A.; Regine, W. F.; Rich, T. A. *Int. J. Radiat. Oncol., Biol., Phys.* **2003**, *56*, 31.
(3) Tannock, I. F. *J. Clin. Oncol.* **1996**, *14*, 3156.
(4) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
(5) Witz, I. P. *Adv. Cancer Res.* **2008**, *100*, 203.
(6) Camphausen, K.; Purow, B.; Sproull, M.; Scott, T.; Ozawa, T.; Deen, D. F.; Tofilon, P. J. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 8287.
(7) Camphausen, K.; Purow, B.; Sproull, M.; Scott, T.; Ozawa, T.; Deen, D. F.; Tofilon, P. J. *Cancer Res.* **2005**, *65*, 10389.
(8) Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779.
(9) Shields, J. M.; Pruitt, K.; McFall, A.; Shaub, A.; Der, C. J. *Trends Cell Biol.* **2000**, *10*, 147.
(10) Bos, J. L. *Cancer Res.* **1989**, *49*, 4682.
(11) Sklar, M. D. *Science* **1988**, *239*, 645.
(12) Rait, A.; Pirollo, K.; Will, D. W.; Peyman, A.; Rait, V.; Uhlmann, E.; Chang, E. H. *Bioconjug. Chem.* **2000**, *11*, 153.
(13) Pirollo, K. F.; Hao, Z.; Rait, A.; Ho, C. W.; Chang, E. H. *Biochem. Biophys. Res. Commun.* **1997**, *230*, 196.
(14) Bernhard, E. J.; Stanbridge, E. J.; Gupta, S.; Gupta, A. K.; Soto, D.; Bakanauskas, V. J.; Cerniglia, G. J.; Muschel, R. J.; McKenna, W. G. *Cancer Res.* **2000**, *60*, 6597.
(15) Kim, I. A.; Bae, S. S.; Fernandes, A.; Wu, J.; Muschel, R. J.; McKenna, W. G.; Birnbaum, M. J.; Bernhard, E. J. *Cancer Res.* **2005**, *65*, 7902.
(16) Russell, J. S.; Lang, F. F.; Huet, T.; Janicot, M.; Chada, S.; Wilson, D. R.; Tofilon, P. J. *Cancer Res.* **1999**, *59*, 5239.
(17) Grana, T. M.; Rusyn, E. V.; Zhou, H.; Sartor, C. I.; Cox, A. D. *Cancer Res.* **2002**, *62*, 4142.
(18) Gupta, A. K.; Bakanauskas, V. J.; Cerniglia, G. J.; Cheng, Y.; Bernhard, E. J.; Muschel, R. J.; McKenna, W. G. *Cancer Res.* **2001**, *61*, 4278.
(19) Gupta, A. K.; Bernhard, E. J.; Bakanauskas, V. J.; Wu, J.; Muschel, R. J.; McKenna, W. G. *Radiat. Res.* **2000**, *154*, 64.
(20) de la Pena, L.; Burgan, W. E.; Carter, D. J.; Hollingshead, M. G.; Satyamitra, M.; Camphausen, K.; Tofilon, P. J. *Mol. Cancer Ther.* **2006**, *5*, 1504.
(21) Rodriguez-Viciana, P.; Sabatier, C.; McCormick, F. *Mol. Cell. Biol.* **2004**, *24*, 4943.
(22) Adjei, A. A. *J. Natl. Cancer Inst.* **2001**, *93*, 1062.
(23) Brunner, T. B.; Cengel, K. A.; Hahn, S. M.; Wu, J.; Fraker, D. L.; McKenna, W. G.; Bernhard, E. J. *Cancer Res.* **2005**, *65*, 8433.
(24) Bernhard, E. J.; McKenna, W. G.; Hamilton, A. D.; Sebti, S. M.; Qian, Y.; Wu, J. M.; Muschel, R. J. *Cancer Res.* **1998**, *58*, 1754.
(25) Delmas, C.; Heliez, C.; Cohen-Jonathan, E.; End, D.; Bonnet, J.; Favre, G.; Toulas, C. *Int. J. Cancer* **2002**, *100*, 43.
(26) Cohen-Jonathan, E.; Muschel, R. J.; Gillies McKenna, W.; Evans, S. M.; Cerniglia, G.; Mick, R.; Kusewitt, D.; Sebti, S. M.; Hamilton, A. D.; Oliff, A.; Kohl, N.; Gibbs, J. B.; Bernhard, E. J. *Radiat. Res.* **2000**, *154*, 125.
(27) Lane, K. T.; Beese, L. S. *J. Lipid Res.* **2006**, *47*, 681.
(28) Ader, I.; Delmas, C.; Bonnet, J.; Rochaix, P.; Favre, G.; Toulas, C.; Cohen-Jonathan-Moyal, E. *Oncogene* **2003**, *22*, 8861.
(29) Basso, A. D.; Mirza, A.; Liu, G.; Long, B. J.; Bishop, W. R.; Kirschmeier, P. *J. Biol. Chem.* **2005**, *280*, 31101.
(30) Cao, C.; Subhawong, T.; Albert, J. M.; Kim, K. W.; Geng, L.; Sekhar, K. R.; Gi, Y. J.; Lu, B. *Cancer Res.* **2006**, *66*, 10040.
(31) Rengan, R.; Cengel, K. A.; Hahn, S. M. *Cancer Metastasis Rev.* **2008**, *27*, 403.
(32) Larner, J.; Jane, J.; Laws, E.; Packer, R.; Myers, C.; Shaffrey, M. *Am. J. Clin. Oncol.* **1998**, *21*, 579.
(33) Hahn, S. M.; Bernhard, E. J.; Regine, W.; Mohiuddin, M.; Haller, D. G.; Stevenson, J. P.; Smith, D.; Pramanik, B.; Tepper, J.; DeLaney, T. F.; Kiel, K. D.; Morrison, B.; Deutsch, P.; Muschel, R. J.; McKenna, W. G. *Clin. Cancer Res.* **2002**, *8*, 1065.
(34) Martin, N. E.; Brunner, T. B.; Kiel, K. D.; DeLaney, T. F.; Regine, W. F.; Mohiuddin, M.; Rosato, E. F.; Haller, D. G.; Stevenson, J. P.; Smith, D.; Pramanik, B.; Tepper, J.; Tanaka, W. K.; Morrison, B.; Deutsch, P.; Gupta, A. K.; Muschel, R. J.; McKenna, W. G.; Bernhard, E. J.; Hahn, S. M. *Clin. Cancer Res.* **2004**, *10*, 5447.
(35) Moyal, E. C.; Laprie, A.; Delannes, M.; Poublanc, M.; Catalaa, I.; Dalenc, F.; Berchery, D.; Sabatier, J.; Bousquet, P.; De Porre, P.; Alaux, B.; Toulas, C. *Int. J. Radiat. Oncol. Biol. Phys.* **2007**, *68*, 1396.
(36) Yarden, Y.; Sliwkowski, M. X. *Nat. Rev. Mol. Cell. Biol.* **2001**, *2*, 127.
(37) Holbro, T.; Civenni, G.; Hynes, N. E. *Exp. Cell Res.* **2003**, *284*, 99.
(38) Olayioye, M. A.; Neve, R. M.; Lane, H. A.; Hynes, N. E. *EMBO J.* **2000**, *19*, 3159.

(39) Bowers, G.; Reardon, D.; Hewitt, T.; Dent, P.; Mikkelsen, R. B.; Valerie, K.; Lammering, G.; Amir, C.; Schmidt-Ullrich, R. K. *Oncogene* **2001**, *20*, 1388.
(40) Schmidt-Ullrich, R. K.; Valerie, K.; Fogleman, P. B.; Walters, J. *Radiat. Res.* **1996**, *145*, 81.
(41) Huang, S. M.; Bock, J. M.; Harari, P. M. *Cancer Res.* **1999**, *59*, 1935.
(42) Huang, S. M.; Li, J.; Armstrong, E. A.; Harari, P. M. *Cancer Res.* **2002**, *62*, 4300.
(43) Contessa, J. N.; Reardon, D. B.; Todd, D.; Dent, P.; Mikkelsen, R. B.; Valerie, K.; Bowers, G. D.; Schmidt-Ullrich, R. K. *Clin. Cancer Res.* **1999**, *5*, 405.
(44) Bianco, C.; Tortora, G.; Bianco, R.; Caputo, R.; Veneziani, B. M.; Caputo, R.; Damiano, V.; Troiani, T.; Fontanini, G.; Raben, D.; Pepe, S.; Bianco, A. R.; Ciardiello, F. *Clin. Cancer Res.* **2002**, *8*, 3250.
(45) Lammering, G.; Hewit, T. H.; Hawkins, W. T.; Contessa, J. N.; Reardon, D. B.; Lin, P. S.; Valerie, K.; Dent, P.; Mikkelsen, R. B.; Schmidt-Ullrich, R. K. *J. Natl. Cancer Inst.* **2001**, *93*, 921.
(46) She, Y.; Lee, F.; Chen, J.; Haimovitz-Friedman, A.; Miller, V. A.; Rusch, V. R.; Kris, M. G.; Sirotnak, F. M. *Clin. Cancer Res.* **2003**, *9*, 3773.
(47) Contessa, J. N.; Hampton, J.; Lammering, G.; Mikkelsen, R. B.; Dent, P.; Valerie, K.; Schmidt-Ullrich, R. K. *Oncogene* **2002**, *21*, 4032.
(48) Dittmann, K.; Mayer, C.; Fehrenbacher, B.; Schaller, M.; Raju, U.; Milas, L.; Chen, D. J.; Kehlbach, R.; Rodemann, H. P. *J. Biol. Chem.* **2005**, *280*, 31182.
(49) Dittmann, K.; Mayer, C.; Rodemann, H. P. *Radiother. Oncol.* **2005**, *76*, 157.
(50) Toulany, M.; Kasten-Pisula, U.; Brammer, I.; Wang, S.; Chen, J.; Dittmann, K.; Baumann, M.; Dikomey, E.; Rodemann, H. P. *Clin. Cancer Res.* **2006**, *12*, 4119.
(51) Friedmann, B. J.; Caplin, M.; Savic, B.; Shah, T.; Lord, C. J.; Ashworth, A.; Hartley, J. A.; Hochhauser, D. *Mol. Cancer Ther.* **2006**, *5*, 209.
(52) Toulany, M.; Dittmann, K.; Baumann, M.; Rodemann, H. P. *Radiother. Oncol.* **2005**, *74*, 117.
(53) Contessa, J. N.; Bhojani, M. S.; Freeze, H. H.; Rehemtulla, A.; Lawrence, T. S. *Cancer Res.* **2008**, *68*, 3803.
(54) Zhou, H.; Kim, Y. S.; Peletier, A.; McCall, W.; Earp, H. S.; Sartor, C. I. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *58*, 344.
(55) Britten, C. D. *Mol. Cancer Ther.* **2004**, *3*, 1335.
(56) Nyati, M. K.; Maheshwari, D.; Hanasoge, S.; Sreekumar, A.; Rynkiewicz, S. D.; Chinnaiyan, A. M.; Leopold, W. R.; Ethier, S. P.; Lawrence, T. S. *Clin. Cancer Res.* **2004**, *10*, 691.
(57) Huang, S. M.; Harari, P. M. *Clin. Cancer Res.* **2000**, *6*, 2166.
(58) Gueven, N.; Dittmann, K.; Mayer, C.; Rodemann, H. P. *Int. J. Radiat. Biol.* **1998**, *73*, 157.
(59) Fehrmann, A.; Dorr, W. *Int. J. Radiat. Biol.* **2005**, *81*, 437.
(60) Suzuki, H.; Aoshiba, K.; Yokohori, N.; Nagai, A. *Cancer Res.* **2003**, *63*, 5054.
(61) Tsuboi, M.; Le Chevalier, T. *Med. Oncol.* **2006**, *23*, 161.
(62) Magne, N.; Chargari, C.; Castadot, P.; Ghalibafian, M.; Soria, J. C.; Haie-Meder, C.; Bourhis, J.; Deutsch, E. *Eur. J. Cancer* **2008**, *44*, 2133.
(63) Nyati, M. K.; Morgan, M. A.; Feng, F. Y.; Lawrence, T. S. *Nat. Rev. Cancer* **2006**, *6*, 876.
(64) Bonner, J. A.; Harari, P. M.; Giralt, J.; Azarnia, N.; Shin, D. M.; Cohen, R. B.; Jones, C. U.; Sur, R.; Raben, D.; Jassem, J.; Ove, R.; Kies, M. S.; Baselga, J.; Youssoufian, H.; Amellal, N.; Rowinsky, E. K.; Ang, K. K. *N. Engl. J. Med.* **2006**, *354*, 567.
(65) Bonner, J. A.; Buchsbaum, D. J.; Russo, S. M.; Fiveash, J. B.; Trummell, H. Q.; Curiel, D. T.; Raisch, K. P. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *59*, 2.
(66) Lievre, A.; Bachet, J. B.; Le Corre, D.; Boige, V.; Landi, B.; Emile, J. F.; Cote, J. F.; Tomasic, G.; Penna, C.; Ducreux, M.; Rougier, P.; Penault-Llorca, F.; Laurent-Puig, P. *Cancer Res.* **2006**, *66*, 3992.
(67) Bristow, R. G.; Benchimol, S.; Hill, R. P. *Radiother. Oncol.* **1996**, *40*, 197.
(68) Russell, J. S.; Raju, U.; Gumin, G. J.; Lang, F. F.; Wilson, D. R.; Huet, T.; Tofilon, P. J. *Cancer Res.* **2002**, *62*, 2318.
(69) Young, J. C.; Moarefi, I.; Hartl, F. U. *J. Cell Biol.* **2001**, *154*, 267.
(70) Cullinan, S. B.; Whitesell, L. *Semin. Oncol.* **2006**, *33*, 457.
(71) Sangster, T. A.; Lindquist, S.; Queitsch, C. *Bioessays* **2004**, *26*, 348.
(72) Bisht, K. S.; Bradbury, C. M.; Mattson, D.; Kaushal, A.; Sowers, A.; Markovina, S.; Ortiz, K. L.; Sieck, L. K.; Isaacs, J. S.; Brechbiel, M. W.; Mitchell, J. B.; Neckers, L. M.; Gius, D. *Cancer Res.* **2003**, *63*, 8984.
(73) Bull, E. E.; Dote, H.; Brady, K. J.; Burgan, W. E.; Carter, D. J.; Cerra, M. A.; Oswald, K. A.; Hollingshead, M. G.; Camphausen, K.; Tofilon, P. J. *Clin. Cancer Res.* **2004**, *10*, 8077.
(74) Dote, H.; Cerna, D.; Burgan, W. E.; Camphausen, K.; Tofilon, P. J. *Cancer Res.* **2005**, *65*, 6967.
(75) Noguchi, M.; Yu, D.; Hirayama, R.; Ninomiya, Y.; Sekine, E.; Kubota, N.; Ando, K.; Okayasu, R. *Biochem. Biophys. Res. Commun.* **2006**, *351*, 658.
(76) Russell, J. S.; Burgan, W.; Oswald, K. A.; Camphausen, K.; Tofilon, P. J. *Clin. Cancer Res.* **2003**, *9*, 3749.
(77) Harashima, K.; Akimoto, T.; Nonaka, T.; Tsuzuki, K.; Mitsuhashi, N.; Nakano, T. *Int. J. Radiat. Biol.* **2005**, *81*, 63.
(78) Machida, H.; Matsumoto, Y.; Shirai, M.; Kubota, N. *Int. J. Radiat. Biol.* **2003**, *79*, 973.
(79) Dote, H.; Burgan, W. E.; Camphausen, K.; Tofilon, P. J. *Cancer Res.* **2006**, *66*, 9211.
(80) Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. *Nature (London)* **2003**, *425*, 407.
(81) Taldone, T.; Gozman, A.; Maharaj, R.; Chiosis, G. *Curr. Opin. Pharmacol.* **2008**, *8*, 370.
(82) Chandarlapaty, S.; Sawai, A.; Ye, Q.; Scott, A.; Silinski, M.; Huang, K.; Fadden, P.; Partdrige, J.; Hall, S.; Steed, P.; Norton, L.; Rosen, N.; Solit, D. B. *Clin. Cancer Res.* **2008**, *14*, 240.
(83) Sydor, J. R.; Normant, E.; Pien, C. S.; Porter, J. R.; Ge, J.; Grenier, L.; Pak, R. H.; Ali, J. A.; Dembski, M. S.; Hudak, J.; Patterson, J.; Penders, C.; Pink, M.; Read, M. A.; Sang, J.; Woodward, C.; Zhang, Y.; Grayzel, D. S.; Wright, J.; Barrett, J. A.; Palombella, V. J.; Adams, J.; Tong, J. K. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 17408.
(84) Workman, P. *Mol. Cancer Ther.* **2003**, *2*, 131.
(85) Jones, P. A. *Oncogene* **2002**, *21*, 5358.
(86) Sigalotti, L.; Fratta, E.; Coral, S.; Cortini, E.; Covre, A.; Nicolay, H. J.; Anzalone, L.; Pezzani, L.; Di Giacomo, A. M.; Fonsatti, E.; Colizzi, F.; Altomonte, M.; Calabro, L.; Maio, M. *J. Cell. Physiol.* **2007**, *212*, 330.
(87) Lubbert, M. *Curr. Top. Microbiol. Immunol.* **2000**, *249*, 135.
(88) Marks, P. A.; Richon, V. M.; Miller, T.; Kelly, W. K. *Adv. Cancer Res.* **2004**, *91*, 137.
(89) Cheng, J. C.; Yoo, C. B.; Weisenberger, D. J.; Chuang, J.; Wozniak, C.; Liang, G.; Marquez, V. E.; Greer, S.; Orntoft, T. F.; Thykjaer, T.; Jones, P. A. *Cancer Cell* **2004**, *6*, 151.
(90) Dote, H.; Cerna, D.; Burgan, W. E.; Carter, D. J.; Cerra, M. A.; Hollingshead, M. G.; Camphausen, K.; Tofilon, P. J. *Clin. Cancer Res.* **2005**, *11*, 4571.
(91) Thiagalingam, S.; Cheng, K. H.; Lee, H. J.; Mineva, N.; Thiagalingam, A.; Ponte, J. F. *Ann. N. Y. Acad. Sci.* **2003**, *983*, 84.
(92) Gray, S. G.; Ekstrom, T. J. *Exp. Cell Res.* **2001**, *262*, 75.
(93) Dokmanovic, M.; Marks, P. A. *J. Cell. Biochem.* **2005**, *96*, 293.
(94) Drummond, D. C.; Noble, C. O.; Kirpotin, D. B.; Guo, Z.; Scott, G. K.; Benz, C. C. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 495.
(95) Arundel, C. M.; Glicksman, A. S.; Leith, J. T. *Radiat. Res.* **1985**, *104*, 443.
(96) Miller, A. A.; Kurschel, E.; Osieka, R.; Schmidt, C. G. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1283.
(97) Camphausen, K.; Burgan, W.; Cerra, M.; Oswald, K. A.; Trepel, J. B.; Lee, M. J.; Tofilon, P. J. *Cancer Res.* **2004**, *64*, 316.
(98) Camphausen, K.; Scott, T.; Sproull, M.; Tofilon, P. J. *Clin. Cancer Res.* **2004**, *10*, 6066.
(99) Munshi, A.; Kurland, J. F.; Nishikawa, T.; Tanaka, T.; Hobbs, M. L.; Tucker, S. L.; Ismail, S.; Stevens, C.; Meyn, R. E. *Clin. Cancer Res.* **2005**, *11*, 4912.
(100) Camphausen, K.; Cerna, D.; Scott, T.; Sproull, M.; Burgan, W. E.; Cerra, M. A.; Fine, H.; Tofilon, P. J. *Int. J. Cancer* **2005**, *114*, 380.
(101) Chinnaiyan, P.; Vallabhaneni, G.; Armstrong, E.; Huang, S. M.; Harari, P. M. *Int. J. Radiat. Oncol. Biol. Phys.* **2005**, *62*, 223.
(102) Zhang, Y.; Adachi, M.; Zhao, X.; Kawamura, R.; Imai, K. *Int. J. Cancer* **2004**, *110*, 301.
(103) Zhang, Y.; Jung, M.; Dritschilo, A. *Radiat. Res.* **2004**, *161*, 667.
(104) Cerna, D.; Camphausen, K.; Tofilon, P. J. *Curr. Top. Dev. Biol.* **2006**, *73*, 173.
(105) Duncan, M. R.; Robinson, M. J.; Dell'Orco, R. T. *Biochim. Biophys. Acta* **1983**, *762*, 221.
(106) Rogakou, E. P.; Pilch, D. R.; Orr, A. H.; Ivanova, V. S.; Bonner, W. M. *J. Biol. Chem.* **1998**, *273*, 5858.
(107) Rothkamm, K.; Kruger, I.; Thompson, L. H.; Lobrich, M. *Mol. Cell. Biol.* **2003**, *23*, 5706.
(108) MacPhail, S. H.; Banath, J. P.; Yu, T. Y.; Chu, E. H.; Lambur, H.; Olive, P. L. *Int. J. Radiat. Biol.* **2003**, *79*, 351.
(109) Munshi, A.; Tanaka, T.; Hobbs, M. L.; Tucker, S. L.; Richon, V. M.; Meyn, R. E. *Mol. Cancer Ther.* **2006**, *5*, 1967.
(110) Chinnaiyan, P.; Cerna, D.; Burgan, W. E.; Beam, K.; Williams, E. S.; Camphausen, K.; Tofilon, P. J. *Clin. Cancer Res.* **2008**, *14*, 5410.
(111) Glaser, K. B.; Staver, M. J.; Waring, J. F.; Stender, J.; Ulrich, R. G.; Davidsen, S. K. *Mol. Cancer Ther.* **2003**, *2*, 151.
(112) Johnstone, R. W.; Licht, J. D. *Cancer Cell* **2003**, *4*, 13.
(113) Peart, M. J.; Smyth, G. K.; van Laar, R. K.; Bowtell, D. D.; Richon, V. M.; Marks, P. A.; Holloway, A. J.; Johnstone, R. W. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 3697.

(114) Masumoto, H.; Hawke, D.; Kobayashi, R.; Verreault, A. *Nature (London)* **2005**, *436*, 294.
(115) Hassa, P. O.; Hottiger, M. O. *Biochem. Cell. Biol.* **2005**, *83*, 270.
(116) Kim, G. D.; Choi, Y. H.; Dimtchev, A.; Jeong, S. J.; Dritschilo, A.; Jung, M. *J. Biol. Chem.* **1999**, *274*, 31127.
(117) Kao, G. D.; McKenna, W. G.; Guenther, M. G.; Muschel, R. J.; Lazar, M. A.; Yen, T. J. *J. Cell. Biol.* **2003**, *160*, 1017.
(118) Chen, C. S.; Wang, Y. C.; Yang, H. C.; Huang, P. H.; Kulp, S. K.; Yang, C. C.; Lu, Y. S.; Matsuyama, S.; Chen, C. Y.; Chen, C. S. *Cancer Res.* **2007**, *67*, 5318.
(119) Kim, S. C.; Sprung, R.; Chen, Y.; Xu, Y.; Ball, H.; Pei, J.; Cheng, T.; Kho, Y.; Xiao, H.; Xiao, L.; Grishin, N. V.; White, M.; Yang, X. J.; Zhao, Y. *Mol. Cell* **2006**, *23*, 607.
(120) Chung, Y. L.; Wang, A. J.; Yao, L. F. *Mol. Cancer Ther.* **2004**, *3*, 317.
(121) Zhang, B.; West, E. J.; Van, K. C.; Gurkoff, G. G.; Zhou, J.; Zhang, X. M.; Kozikowski, A. P.; Lyeth, B. G. *Brain Res.* **2008**, *1226C*, 181.
(122) Kim, H. J.; Rowe, M.; Ren, M.; Hong, J. S.; Chen, P. S.; Chuang, D. M. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 892.
(123) Kim, T. Y.; Bang, Y. J.; Robertson, K. D. *Epigenetics* **2006**, *1*, 14.
(124) Perucca, E. *CNS Drugs* **2002**, *16*, 695.
(125) Chavez-Blanco, A.; Segura-Pacheco, B.; Perez-Cardenas, E.; Taja-Chayeb, L.; Cetina, L.; Candelaria, M.; Cantu, D.; Gonzalez-Fierro, A.; Garcia-Lopez, P.; Zambrano, P.; Perez-Plasencia, C.; Cabrera, G.; Trejo-Becerril, C.; Angeles, E.; Duenas-Gonzalez, A. *Mol. Cancer* **2005**, *4*, 22.
(126) Harper, J. W.; Elledge, S. J. *Mol. Cell* **2007**, *28*, 739.
(127) O'Connor, M. J.; Martin, N. M.; Smith, G. C. *Oncogene* **2007**, *26*, 7816.
(128) Lieberman, H. B. *Curr. Med. Chem* **2008**, *15*, 360.
(129) Lord, C. J.; Garrett, M. D.; Ashworth, A. *Clin. Cancer Res.* **2006**, *12*, 4463.
(130) Shrivastav, M.; De Haro, L. P.; Nickoloff, J. A. *Cell Res.* **2008**, *18*, 134.
(131) Mao, Z.; Bozzella, M.; Seluanov, A.; Gorbunova, V. *Cell Cycle* **2008**, *7*, 2902.
(132) Pierce, A. J.; Stark, J. M.; Araujo, F. D.; Moynahan, M. E.; Berwick, M.; Jasin, M. *Trends Cell Biol.* **2001**, *11*, S52.
(133) Hartley, K. O.; Gell, D.; Smith, G. C.; Zhang, H.; Divecha, N.; Connelly, M. A.; Admon, A.; Lees-Miller, S. P.; Anderson, C. W.; Jackson, S. P. *Cell* **1995**, *82*, 849.
(134) Kirchgessner, C. U.; Patil, C. K.; Evans, J. W.; Cuomo, C. A.; Fried, L. M.; Carter, T.; Oettinger, M. A.; Brown, J. M. *Science* **1995**, *267*, 1178.
(135) Shinohara, E. T.; Geng, L.; Tan, J.; Chen, H.; Shir, Y.; Edwards, E.; Halbrook, J.; Kesicki, E. A.; Kashishian, A.; Hallahan, D. E. *Cancer Res.* **2005**, *65*, 4987.
(136) Ismail, I. H.; Martensson, S.; Moshinsky, D.; Rice, A.; Tang, C.; Howlett, A.; McMahon, G.; Hammarsten, O. *Oncogene* **2004**, *23*, 873.
(137) Veuger, S. J.; Curtin, N. J.; Richardson, C. J.; Smith, G. C.; Durkacz, B. W. *Cancer Res.* **2003**, *63*, 6008.
(138) Schreiber, V.; Dantzer, F.; Ame, J. C.; de Murcia, G. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7*, 517.
(139) Albert, J. M.; Cao, C.; Kim, K. W.; Willey, C. D.; Geng, L.; Xiao, D.; Wang, H.; Sandler, A.; Johnson, D. H.; Colevas, A. D.; Low, J.; Rothenberg, M. L.; Lu, B. *Clin. Cancer Res.* **2007**, *13*, 3033.
(140) McCabe, N.; Turner, N. C.; Lord, C. J.; Kluzek, K.; Bialkowska, A.; Swift, S.; Giavara, S.; O'Connor, M. J.; Tutt, A. N.; Zdzienicka, M. Z.; Smith, G. C.; Ashworth, A. *Cancer Res.* **2006**, *66*, 8109.
(141) Carrassa, L.; Broggini, M.; Erba, E.; Damia, G. *Cell Cycle* **2004**, *3*, 1177.
(142) Wang, Q.; Fan, S.; Eastman, A.; Worland, P. J.; Sausville, E. A.; O'Connor, P. M. *J. Natl. Cancer Inst.* **1996**, *88*, 956.
(143) Syljuasen, R. G.; Sorensen, C. S.; Nylandsted, J.; Lukas, C.; Lukas, J.; Bartek, J. *Cancer Res.* **2004**, *64*, 9035.
(144) Janetka, J. W.; Ashwell, S.; Zabludoff, S.; Lyne, P. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 473.
(145) Kastan, M. B.; Lim, D. S. *Nat. Rev. Mol. Cell. Biol.* **2000**, *1*, 179.
(146) Kuhne, M.; Riballo, E.; Rief, N.; Rothkamm, K.; Jeggo, P. A.; Lobrich, M. *Cancer Res.* **2004**, *64*, 500.
(147) Hickson, I.; Zhao, Y.; Richardson, C. J.; Green, S. J.; Martin, N. M.; Orr, A. I.; Reaper, P. M.; Jackson, S. P.; Curtin, N. J.; Smith, G. C. *Cancer Res.* **2004**, *64*, 9152.
(148) Rainey, M. D.; Charlton, M. E.; Stanton, R. V.; Kastan, M. B. *Cancer Res.* **2008**, *68*, 7466.
(149) Bredel, M.; Bredel, C.; Juric, D.; Harsh, G. R.; Vogel, H.; Recht, L. D.; Sikic, B. I. *Cancer Res.* **2005**, *65*, 4088.
(150) Mischel, P. S.; Cloughesy, T. F.; Nelson, S. F. *Nat. Rev. Neurosci.* **2004**, *5*, 782.
(151) Overgaard, J. *J. Clin. Oncol.* **2007**, *25*, 4066.
(152) Demaria, S.; Formenti, S. C. *Int. J. Radiat. Biol.* **2007**, *83*, 819.
(153) Cordes, N.; Park, C. C. *Int. J. Radiat. Biol.* **2007**, *83*, 753.
(154) Gorski, D. H.; Beckett, M. A.; Jaskowiak, N. T.; Calvin, D. P.; Mauceri, H. J.; Salloum, R. M.; Seetharam, S.; Koons, A.; Hari, D. M.; Kufe, D. W.; Weichselbaum, R. R. *Cancer Res.* **1999**, *59*, 3374.
(155) Banuelos, C. A.; Banath, J. P.; MacPhail, S. H.; Zhao, J.; Eaves, C. A.; O'Connor, M. D.; Lansdorp, P. M.; Olive, P. L. *DNA Repair* **2008**, *7*, 1471.
(156) Teicher, B. A.; Holden, S. A.; Ara, G.; Dupuis, N. P.; Liu, F.; Yuan, J.; Ikebe, M.; Kakeji, Y. *Int. J. Cancer* **1995**, *61*, 732.
(157) Wachsberger, P.; Burd, R.; Dicker, A. P. *Clin. Cancer Res.* **2003**, *9*, 1957.
(158) Geng, L.; Donnelly, E.; McMahon, G.; Lin, P. C.; Sierra-Rivera, E.; Oshinka, H.; Hallahan, D. E. *Cancer Res.* **2001**, *61*, 2413.
(159) Mauceri, H. J.; Hanna, N. N.; Beckett, M. A.; Gorski, D. H.; Staba, M. J.; Stellato, K. A.; Bigelow, K.; Heimann, R.; Gately, S.; Dhanabal, M.; Soff, G. A.; Sukhatme, V. P.; Kufe, D. W.; Weichselbaum, R. R. *Nature (London)* **1998**, *394*, 287.
(160) Hanna, N. N.; Seetharam, S.; Mauceri, H. J.; Beckett, M. A.; Jaskowiak, N. T.; Salloum, R. M.; Hari, D.; Dhanabal, M.; Ramchandran, R.; Kalluri, R.; Sukhatme, V. P.; Kufe, D. W.; Weichselbaum, R. R. *Cancer J.* **2000**, *6*, 287.
(161) Griffin, R. J.; Williams, B. W.; Wild, R.; Cherrington, J. M.; Park, H.; Song, C. W. *Cancer Res.* **2002**, *62*, 1702.
(162) Shipley, W. U.; Stanley, J. A.; Steel, G. G. *Cancer Res.* **1975**, *35*, 2488.
(163) Bergers, G.; Javaherian, K.; Lo, K. M.; Folkman, J.; Hanahan, D. *Science* **1999**, *284*, 808.
(164) Fukumura, D.; Jain, R. K. *J. Cell. Biochem.* **2007**, *101*, 937.
(165) Cheng, H. L. *Curr. Clin. Pharmacol.* **2007**, *2*, 111.
(166) Batchelor, T. T.; Sorensen, A. G.; di Tomaso, E.; Zhang, W. T.; Duda, D. G.; Cohen, K. S.; Kozak, K. R.; Cahill, D. P.; Chen, P. J.; Zhu, M.; Ancukiewicz, M.; Mrugala, M. M.; Plotkin, S.; Drappatz, J.; Louis, D. N.; Ivy, P.; Scadden, D. T.; Benner, T.; Loeffler, J. S.; Wen, P. Y.; Jain, R. K. *Cancer Cell* **2007**, *11*, 83.
(167) Brown, A. P.; Citrin, D. E.; Camphausen, K. A. *Cancer Metastasis Rev.* **2008**, *27*, 415.
(168) Willett, C. G.; Boucher, Y.; di Tomaso, E.; Duda, D. G.; Munn, L. L.; Tong, R. T.; Chung, D. C.; Sahani, D. V.; Kalva, S. P.; Kozin, S. V.; Mino, M.; Cohen, K. S.; Scadden, D. T.; Hartford, A. C.; Fischman, A. J.; Clark, J. W.; Ryan, D. P.; Zhu, A. X.; Blaszkowsky, L. S.; Chen, H. X.; Shellito, P. C.; Lauwers, G. Y.; Jain, R. K. *Nat. Med.* **2004**, *10*, 145.
(169) Crane, C. H.; Ellis, L. M.; Abbruzzese, J. L.; Amos, C.; Xiong, H. Q.; Ho, L.; Evans, D. B.; Tamm, E. P.; Ng, C.; Pisters, P. W.; Charnsangavej, C.; Delclos, M. E.; O'Reilly, M.; Lee, J. E.; Wolff, R. A. *J. Clin. Oncol.* **2006**, *24*, 1145.
(170) Czito, B. G.; Bendell, J. C.; Willett, C. G.; Morse, M. A.; Blobe, G. C.; Tyler, D. S.; Thomas, J.; Ludwig, K. A.; Mantyh, C. R.; Ashton, J.; Yu, D.; Hurwitz, H. I. *Int. J. Radiat. Oncol. Biol. Phys.* **2007**, *68*, 472.
(171) Seiwert, T. Y.; Haraf, D. J.; Cohen, E. E.; Stenson, K.; Witt, M. E.; Dekker, A.; Kocherginsky, M.; Weichselbaum, R. R.; Chen, H. X.; Vokes, E. E. *J. Clin. Oncol.* **2008**, *26*, 1732.
(172) Chang, S. M.; Lamborn, K. R.; Malec, M.; Larson, D.; Wara, W.; Sneed, P.; Rabbitt, J.; Page, M.; Nicholas, M. K.; Prados, M. D. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *60*, 353.
(173) Hsu, W. C.; Chan, S. C.; Ting, L. L.; Chung, N. N.; Wang, P. M.; Ying, K. S.; Shin, J. S.; Chao, C. J.; Lin, G. D. *Jpn. J. Clin. Oncol.* **2006**, *36*, 93.
(174) Turner, C. D.; Chi, S.; Marcus, K. J.; MacDonald, T.; Packer, R. J.; Poussaint, T. Y.; Vajapeyam, S.; Ullrich, N.; Goumnerova, L. C.; Scott, R. M.; Briody, C.; Chordas, C.; Zimmerman, M. A.; Kieran, M. W. *J. Neurooncol.* **2007**, *82*, 95.
(175) Knisely, J. P.; Berkey, B.; Chakravarti, A.; Yung, A. W.; Curran, W. J., Jr.; Robins, H. I.; Movsas, B.; Brachman, D. G.; Henderson, R. H.; Mehta, M. P. *Int. J. Radiat. Oncol. Biol. Phys.* **2008**, *71*, 79.

CR800504X