Improving gemcitabine-mediated radiosensitization using molecularly targeted therapy: A review

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Abstract

In the last three decades gemcitabine has progressed from the status of a laboratory cytotoxic drug to a standard clinical chemotherapeutic agent and a potent radiation sensitizer. In an effort to improve the efficacy of gemcitabine, additional chemotherapeutic agents have been combined with gemcitabine (both with and without radiation) but with toxicity proving to be a major limitation. Therefore, the integration of molecularly targeted agents, which potentially produce less toxicity than standard chemotherapy, with gemcitabine-radiation is a promising strategy for improving chemoradiation. Two of the most promising targets, described in this review, for improving the efficacy of gemcitabine-radiation are EGFR and Chk1.

Keywords

gemcitabine; radiation; EGFR; Chk1; molecularly targeted therapy

Introduction

Gemcitabine was first introduced into the clinic as a chemotherapeutic agent nearly 3 decades ago. Since then, both laboratory and clinical investigations have shown gemcitabine to be a potent radiation sensitizer. In this review we will begin with a discussion of gemcitabine biochemistry and its mechanisms of interaction with radiation, highlighting observations which may lead to improving the design of clinical trials combining gemcitabine with radiation. Previous attempts to improve the efficacy of gemcitabine-radiotherapy have included the addition of other chemotherapeutic agents (1-3) such as cisplatin (4) and oxaliplatin (5). More recent studies have focused on the addition of molecularly targeted therapies, to gemcitabine and radiation (6,7). In this review we will present our rationale for integrating checkpoint kinase

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Statement of translational relevance

Gemcitabine with radiation is a standard of care in the treatment of locally advanced pancreatic cancer. However, even with the best available care patient median survival is in the range of one year. Thus we have focused our efforts on improving therapy for pancreatic cancer by using multi-modality therapies including gemcitabine, radiation, and cisplatin or oxaliplatin. However, the use of multiple cytotoxic agents produces additional toxicity. Thus it seems likely that the future of pancreatic cancer therapy will integrate molecularly targeted therapies with standard chemoradiation regimens. We describe here two promising molecular targets: EGFR and Chk1. Our hope is that this review will stimulate rationally designed clinical trials combining molecularly targeted agents with gemcitabine-radiation with the ultimate goal of improving survival.

1 (Chk1)- and epidermal growth factor (EGFR)-molecularly targeted agents with gemcitabine-radiation therapy.

Gemcitabine biochemistry and radiosensitization

The antitumor activity of gemcitabine depends on a series of sequential phosphorylations. In the first rate limiting step, deoxycytidine kinase converts gemcitabine to the monophosphorylated metabolite, dFdCMP. (This has motivated the study of fixed-dose-rate infusion (10 mg/m^2/min), which increases intracellular metabolites compared to bolus treatment (8,9), but in the majority of trials does not significantly improve survival (10)). Subsequent phosphorylations lead to the accumulation of gemcitabine di- and triphosphate (dFdCDP and dFdCTP) which are both active metabolites (Figure 1). While dFdCTP can interfere with DNA synthesis by competing with endogenous dCTP for misincorporation into replicating DNA, dFdCDP is a potent inhibitor of ribonucleotide reductase, reducing the synthesis of deoxynucleoside triphosphates, primarily dATP (in solid tumor cells).[1]

The inhibition of ribonucleotide reductase by dFdCDP and subsequent depletion of dATP pools caused by gemcitabine suggested that it would be a good radiation sensitizer (Box 1) (12,13). Early preclinical studies showed that, as anticipated, gemcitabine radiosensitized both solid tumor cell lines (12,14-16) and mouse sarcoma (17). Subsequent studies showed that cells transduced with the active subunit of ribonucleotide reductase become relatively resistant to gemcitabine-mediated radiosensitization (18). Furthermore, radiosensitization does not correlate with intracellular concentrations of dFdCTP (19), suggesting that dATP pool depletion and not incorporation of dFdCMP into DNA underlies radiosensitization. Although gemcitabine-induced dATP pool depletion is necessary, it alone is not sufficient for radiosensitization. The ability of gemcitabine to cause redistribution of cells into S phase is also required for radiosensitization (20). Although high concentrations of gemcitabine cause near complete dATP pool depletion within just a few hours, cells irradiated at this time are minimally radiosensitized. Maximum sensitization requires both dATP pool depletion and sufficient time to permit redistribution of cells into early S-phase (15,21). Sensitization is maximized in vivo by a fixed-dose-rate exposure to gemcitabine, compared to a bolus administration (22), presumably due to the production of more intracellular metabolites, as alluded to above.

Cellular effects of radiation and gemcitabine

DNA-directed effects

Based on the inhibition of dNTP synthesis by gemcitabine, it seemed likely that gemcitabine would have an effect on the repair of radiation-induced DNA damage, which may contribute in part to its radiosensitizing activity. When initial work showed that gemcitabine had no effect on the induction or repair of bulk DNA damage (23-25), individual repair pathways were explored. These studies found that DNA damage induced by ionizing radiation is primarily repaired by the non-homologous end joining (NHEJ) pathway and, to a lesser extent, through base-excision repair and homologous recombination repair (HRR) (26). While the NHEJ pathway is not required for gemcitabine-mediated radiosensitization (23) other studies suggest that HRR may be required. Whereas the radiation sensitivity of cells deficient in HRR is relatively unaffected by gemcitabine, cells that are HRR-competent, but unable to carry out base excision repair, are radiosensitized (27). The finding that ionizing radiation-induced

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[1]In solid tumor cells treated with gemcitabine, dATP pools are depleted. This is in contrast to leukemia cells, which in response to gemcitabine display a pronounced depletion of dCTP pools 11.
Heinemann V, Xu YZ, Chubb S, *et al.* Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. Molecular pharmacology 1990;38: 567−72..

Rad51 foci formation, a marker for HRR activity, is inhibited by gemcitabine pretreatment provides further evidence that gemcitabine inhibits this repair pathway in irradiated cells (27).

Another DNA repair pathway that may affect gemcitabine-mediated radiosensitization is the mismatch repair pathway (MMR). MMR-deficient cells display enhanced radiosensitization after a long exposure (24 hrs) to an IC_{50} concentration of gemcitabine (19,28). These data suggest that MMR may antagonize the radiosensitizing effects of relatively low-dose gemcitabine, perhaps by facilitating the repair of gemcitabine-induced errors in DNA caused by nucleotide pool imbalance.

Roles of apoptosis and p53 expression

One potential consequence of the increase in residual DNA damage after radiation in gemcitabine-treated cells is an increase in radiation-induced apoptosis. Initial studies suggested that the extent of apoptosis produced by the combination of gemcitabine and radiation correlated with radiosensitization, and that the inhibition of apoptosis substantially reduced sensitization (16). To test this hypothesis directly, we performed studies using MCF-7 cells overexpressing a dominant negative form of caspase-9. We found that caspase-9 dominant negative overexpression blocks apoptosis and inhibits gemcitabine-mediated radiosensitization (Figure 2). (However, overexpression of the pro-apoptotic protein bcl-x_S in fibroblasts, did not affect gemcitabine radiosensitization (29)). P53 does not seem to have a direct effect on gemcitabine-radiosensitization (30-32). Taken together these findings suggest that although apoptosis plays a role in radiosensitization by gemcitabine, this role depends on many factors, including the cell type and status of apoptotic regulators.

Cell cycle checkpoints

Because both gemcitabine-mediated cytotoxicity and radiosensitization depend on S-phase redistribution, efforts have been directed at understanding the mechanisms of gemcitabine-induced S-phase arrest. In response to DNA damage, ATM and ATR activate Chk1 and Chk2 kinases which result in Cdc25 phosphatase inhibition and cell cycle arrest (Figure 3). In general, gemcitabine treatment results in the accumulation of the phosphorylated forms of Chk1 and Chk2 and degradation of Cdc25A (33) (34-36). These observations led to the hypothesis that Chk1 and/or Chk2 activation were required for gemcitabine-induced early S-phase arrest. Initial studies found, however, that although Chk1 activity was required for gemcitabine-induced Cdc25A degradation, neither Chk1 nor Chk2 inhibition affected the gemcitabine-induced accumulation of cells in early S-phase (33,35,37). Instead, Chk1 inhibition abrogated the G2/M checkpoint, and permitted gemcitabine-treated cells with arrested DNA synthesis to enter mitosis with either a 4N DNA content (normal mitosis) or a sub-4N DNA content (premature mitosis). Thus, it appears that gemcitabine-induced Chk1 activation functions in part to coordinate cell cycle progression with DNA synthesis, preventing cells with stalled replication from prematurely entering mitosis (33).

The finding that gemcitabine activates Chk1 and Chk2 led to studies assessing the effects of checkpoint inhibition on gemcitabine-induced cytotoxicity. Inhibition of Chk1 by either siRNA-mediated Chk1 depletion (34,37) or by small molecule Chk1 inhibitors (35,36) enhanced gemcitabine cytotoxicity. Likewise, inhibition of other members of the Chk1 signaling pathway, such as Rad9, ATR, and ATM, enhanced gemcitabine cytotoxicity (34). Although, enhancement of gemcitabine cytotoxicity is accompanied by inhibition of Cdc25A degradation and induction of premature mitotic entry in some instances, we have found examples where these markers do not correlate with sensitization. Instead, our recent data demonstrate a stronger correlation between sensitization to gemcitabine by Chk1 inhibition and depletion of Rad51 protein, inhibition of Rad51 focus formation, and increased γ-H2AX

(36). These findings suggest that sensitization to gemcitabine by Chk1 inhibition is mediated by inhibition of the DNA damage response.

Chk1 may also play a role in radiosensitization by gemcitabine. Chk1 inhibitors such as PD-321852 (Pfizer) and AZD7762 (AstraZeneca; (38)) increase radiation sensitivity in a variety of model systems (39-42). Based on the ability of Chk1 inhibitors to sensitize to gemcitabine or radiation, we have initiated studies to examine whether Chk1 inhibition might enhance gemcitabine-mediated radiosensitization. PD-321852 (43) enhanced radiation sensitivity (Figure 4A) as well as gemcitabine-cytotoxicity (36) in pancreas tumor cell lines. Likewise, AZD7762 enhanced radiation sensitivity and further enhanced gemcitabine-mediated radiosensitization (Figure 4B). Chk1 inhibitors have now entered clinical trials (for a review see (44)).

EGFR signaling

EGFR is a transmembrane receptor tyrosine kinase that is activated in response to binding of ligands such as EGF, transforming growth factor-α, or amphiregulin (for a review see 42). Ligand binding results in receptor dimerization and activation of a number of downstream pathways (STAT, AKT, ERK, PKC) which promote survival, angiogenesis, cell cycle progression, and transformation. A recent Phase III clinical trial in metastatic pancreatic cancer demonstrated a statistically significant but clinically modest improvement in overall survival for patients treated with gemcitabine plus erlotinib versus gemcitabine alone (6.2 vs. 5.9 months) (45). There are several mechanisms (discussed below) through which EGFR inhibitors might interact with gemcitabine and/or radiation including EGF receptor activity, cell cycle, and DNA repair.

In addition to nucleotide pool depletion, S-phase arrest, and cell cycle checkpoint activation, gemcitabine stimulates phosphorylation of EGFR both in head and neck as well as in pancreas cancer cells (46,47). EGFR is also phosphorylated in response to a variety of other cytotoxic agents (48-51) and it is hypothesized that this phosphorylation may promote survival through stimulation of stress/survival response pathways as illustrated in Figure 3. This model provides an obvious rationale for the addition of EGFR inhibitors, such as the small molecule tyrosine kinase inhibitor, erlotinib or the anti-EGFR antibody, cetuximab to gemcitabine therapy. Initial studies in head and neck cancer xenografts demonstrated that gefitinib, which blocked gemcitabine-mediated EGFR phosphorylation, enhanced gemcitabine-mediated tumor growth delay (46). In other studies, both cetuximab and erlotinib were found to enhance pancreas tumor growth delay when combined with gemcitabine and radiation (7,47).

The ability of EGFR inhibitors to sensitize to gemcitabine is sequence dependent. In head and neck cancer cells as well as xenografts, the combination of gemcitabine followed by gefitinib is superior to the reverse sequence (46). This observation has been supported in pancreatic cancer cells as well where treatment with gemcitabine prior to gefitinib produced additive to synergistic effects but antagonistic effects in response to the reverse sequence (52,53). This schedule dependent cell killing may be attributable to the cell cycle effects of EGFR inhibitors since EGFR inhibitors upregulate the cyclin dependent kinase inhibitors, p27 (54,55) and p21 (56) and thus produce G1 cell cycle arrest.

EGFR also plays a role in DNA repair. Ionizing radiation and chemotherapeutic agents produce a variety of types of DNA damage including single- and double-strand DNA breaks, DNA adducts, and DNA crosslinks. EGFR can physically interact with DNA-dependent protein kinase (DNA-PK) (57). In response to radiation, EGFR translocates to the nucleus which is associated with increased DNA-PK activity (58,59). Inhibition of EGFR activation by cetuximab blocks nuclear EGFR import, DNA-PK activity, and radiation-induced DNA damage repair, and induces radiosensitization (60) (for a review see (61). Together, these

results suggest that EGFR inhibitors could potentiate the efficacy of gemcitabine-radiation through inhibition of DNA repair.

In addition to kinase activity, EGFR may have important structural functions to inhibit cell death (62). In head and neck cancer, treatment with gemcitabine results in degradation of EGFR (63). EGFR degradation in response to gemcitabine is accompanied by inhibition of downstream EGFR signaling molecules such as AKT and ERK as well as cell death. In preclinical studies, EGFR degradation in response to gemcitabine correlated with response. In contrast, gemcitabine does not cause EGFR degradation in pancreatic cancer models (47). These differences in EGFR degradation may at least in part account for the greater sensitivity to gemcitabine in head and neck cancer versus pancreatic cancer models.

The finding that EGFR inhibitors produce much greater effects in head and neck versus pancreatic cancer tumor models (47,64) illustrates the importance of the cellular context of EGFR activation or inhibition. One plausible explanation for the relative insensitivity of pancreatic cancers to EGFR inhibitors is the presence of mutant Ras in more than 85% of pancreatic cancers (65). Mutant Ras confers resistance to EGFR inhibition (66,67). While Ras mutation confers resistance to EGFR inhibitor monotherapy and combination EGFR inhibitor-chemotherapy, some preclinical models have demonstrated radiosensitization by EGFR inhibitors in Ras mutant cell types, which could be explained by inhibition of EGFR/H-Ras (7,68-70). While the role of Ras mutation status in patients treated with radiation and EGFR inhibitor therapies has not yet been determined, the consensus of the existing clinical data is that Ras mutation confers resistance to both EGFR inhibitor monotherapy as well as combination EGFR inhibitor-chemotherapy. Recent clinical studies in colorectal cancer and non-small-cell lung cancer demonstrated a lack of efficacy of EGFR inhibitors (as monotherapy and in combination with chemotherapy) against tumors with Ras mutations (71-73) Since Ras mutation is present in the majority of pancreatic cancers and EGFR inhibitors have produced limited benefit, retrospective studies to determine the influence of Ras mutation on EGFR inhibitor sensitivity should be conducted. Together, these studies indicate that the influence of EGFR inhibition on survival is influenced by the presence of other activated pathways, such as Ras.

Combining molecularly targeted agents with gemcitabine-radiotherapy in the clinic

Gemcitabine and radiation have been used in combination to treat a variety of solid tumors types including lung, head and neck, cervix, bladder, and breast (for a review see (74,75). Based on its two distinct mechanisms of action (incorporation into DNA and ribonucleotide reductase inhibition), gemcitabine has been used clinically both as a chemotherapeutic agent and as a radiation sensitizer, effects separable by concentration. For example, early clinical trials in pancreatic cancer investigated low dose gemcitabine concurrent with standard radiation (50.4 Gy in 1.8 Gy fractions) in patients with locally advanced pancreatic cancer (76) and determined the maximum tolerated dose of gemcitabine to be approximately 40 mg/m^2 given twice a week. In latter trials patients treated with 350−500 mg/m^2 gemcitabine weekly and 30−33 Gy in 3 Gy fractions (77) experienced unacceptable toxicities (fatigue, anorexia, vomiting, etc.). It has been speculated that the relatively large standard radiation fields including clinically uninvolved regional lymph nodes increased the toxicity of the combination therapy. Our study used a standard chemotherapeutic dose of gemcitabine (1000mg/m^2), which should maximize systemic control, with dose-escalated 3D conformal radiotherapy administered to the gross disease only, with exclusion of clinically uninvolved regional lymph nodes (78). We found this treatment was tolerable and produced a favorable objective response rate (10/33 patients) and median survival (11.6 months). The great majority of the recurrences were systemic, suggesting that the most important need was better systemic therapy. Subsequent preclinical

and clinical trials have been carried out adding cisplatin or oxaliplatin to gemcitabine-radiation (4,5,79,80).

Unfortunately, neither cisplatin-gemcitabine nor oxaliplatin-gemcitabine significantly prolong survival compared to gemcitabine alone in the treatment of metastatic disease (81,82), suggesting that these combinations will only modestly improve the treatment of locally advanced, non-metastatic disease. Likewise, adding capecitabine to gemcitabine marginally improved the survival of patients with metastatic disease in one study (median 6 months to 7.4 months (83)) but not in another (84).

Therefore, we have turned to integrating targeted agents with gemcitabine-radiation with the goal of improving systemic disease control while maintaining or improving local radiosensitization. This has led us to combine EGFR or Chk1 inhibitors with gemcitabine-radiation. Because both preclinical and clinical studies have demonstrated that erlotinib plus gemcitabine is superior to gemcitabine alone, we have initiated studies combining EGFR inhibitors with gemcitabine and radiation. While clinical trials combining Chk1 inhibitors with gemcitabine are underway, a variety of preclinical models have demonstrated enhanced gemcitabine-mediated radiosensitization as well as cytotoxicity in response to Chk1 inhibitors (Figure 4) (36). These studies have prompted our ongoing investigation of Chk1 inhibitors in combination with gemcitabine-radiation.

Looking into the future

One of our current goals in gemcitabine-radiation therapy for pancreatic cancer is to integrate a third agent to gemcitabine-radiation therapy that improves systemic disease control (cytotoxicity) without reducing local tumor control (radiosensitization). In the previous decade we have successfully added other standard chemotherapeutic agents (i.e. cisplatin, oxaliplatin) to gemcitabine-radiation therapy. However, trials combining agents such as oxaliplatin, cisplatin, irinotecan, and 5-fluorouracil have not significantly improved survival (although capecitabine may). Likewise, targeted therapies such as marimastat (matrix metalloproteinase inhibitor), and tipifarnib (farnesyltransferase inhibitor) with gemcitabine have not produced significant survival improvements over gemcitabine alone (85). Thus, the finding that the addition of erlotinib to gemcitabine produced a significant (yet modest) improvement in survival (0.3 months) compared to gemcitabine alone is of interest (45).

How can laboratory studies help us improve on these results? One obvious strategy is better patient selection. For example, it is conceivable that the efficacy of the combination of gemcitabine with EGFR inhibitors could be improved upon by identifying populations of patients most sensitive to EGFR inhibition, such as those who lack Ras activation (71-73) or who develop a rash in response to EGFR inhibitor therapy (86). Another approach to improve the clinical efficacy of molecularly targeted agents in combination with gemcitabine or gemcitabine-radiation is through preclinical determination of the optimal sequence of gemcitabine, radiation, and EGFR inhibitor. For instance, in the aforementioned clinical trial, EGFR inhibitor was given concurrently with gemcitabine and produced a modest survival advantage. It seems possible that survival might have been improved if the most effective preclinical schedule (gemcitabine prior to EGFR inhibitor) had been used. Other targets, such as Chk1, need to be explored in combination with gemcitabine-radiation therapy. The utilization of better preclinical models such as tumor xenografts derived from primary human tumors will be crucial in order to translate results directly to the clinic. In addition, the effects of therapy combinations on tumor stem cells versus gross tumor (87) may provide insight into potential therapeutic efficacy. This decade will focus on preclinical studies in the best available model systems, combining molecularly targeted therapies with gemcitabine-radiation with the goal of producing better patient responses.

> ### BOX 1. Mechanisms of radiosensitization by gemcitabine
>
> - Requires dATP pool depletion and S phase redistribution (15)
> - Correlates with Chk1 and Chk2 activation (33)
> - Interferes with homologous end rejoining (27)
> - Increased in mismatch repair deficient cells (19)
> - Partially mediated by apoptosis (16)

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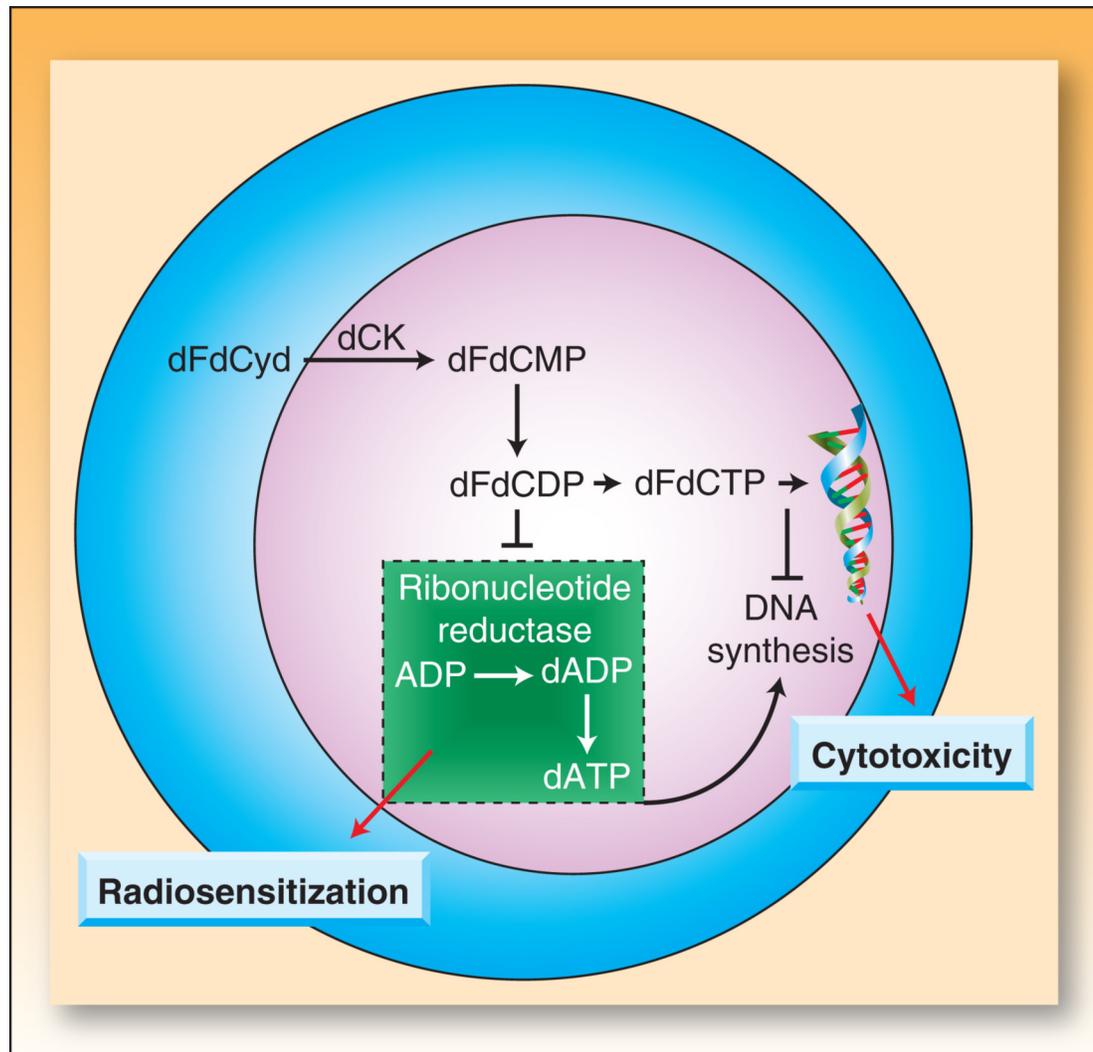
Figure 1. Gemcitabine mechanisms of action
Following cellular incorporation, gemcitabine (dFdCyd) undergoes a series of sequential phosphorylations mediated by deoxycytidine kinase. dFdCDP is a direct inhibitor of ribonucleotide reductase which results in inhibition of deoxyribonucleotide triphosphate synthesis, specifically deoxyadenosine triphosphate (dATP). Depletion of dATP pools is crucial for radiosensitization (19). dFdCTP is incorporated into DNA during synthesis and contributes to cytotoxicity (88-90).
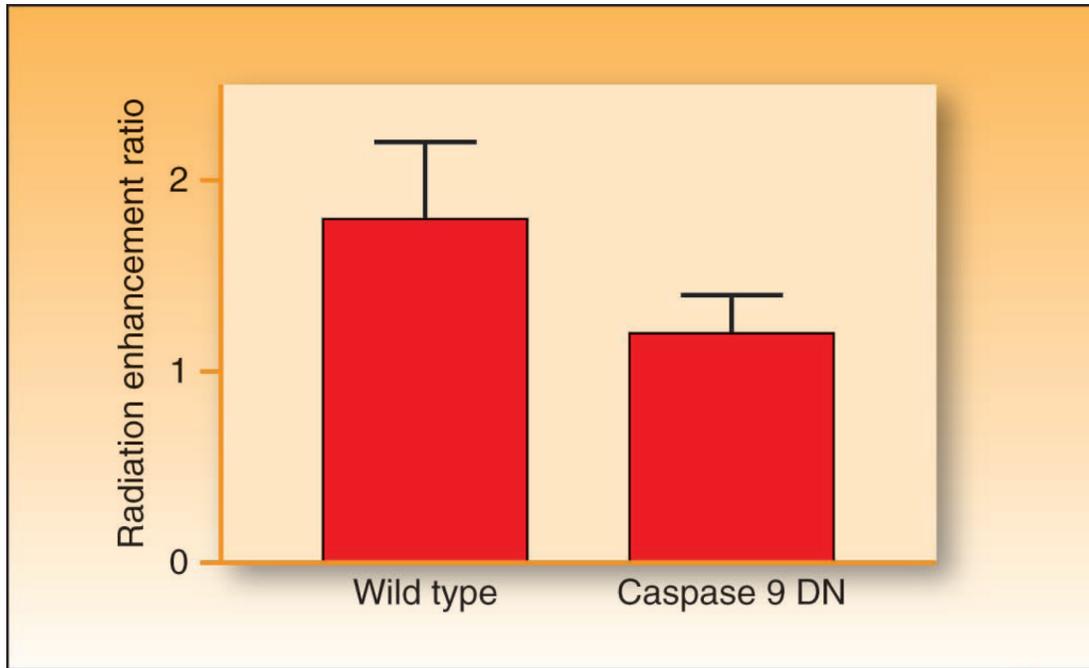
Figure 2. The effect of caspase 9 on gemcitabine-mediated radiosensitization
Wild type or caspase 9 dominant negative (DN) MCF-7 cells were exposed to equicytotoxic concentrations of gemcitabine for 24 hours prior to treatment with $0 - 8$ Gy radiation. Survival was determined by a clonogenic survival assay as previously described (91). Data are expressed as the radiation enhancement ratio which was calculated as the ratio of the mean inactivation dose for drug treated cells to non-drug treated cells (ER = 1). Data are from the mean of 3 independent experiments ± standard error.
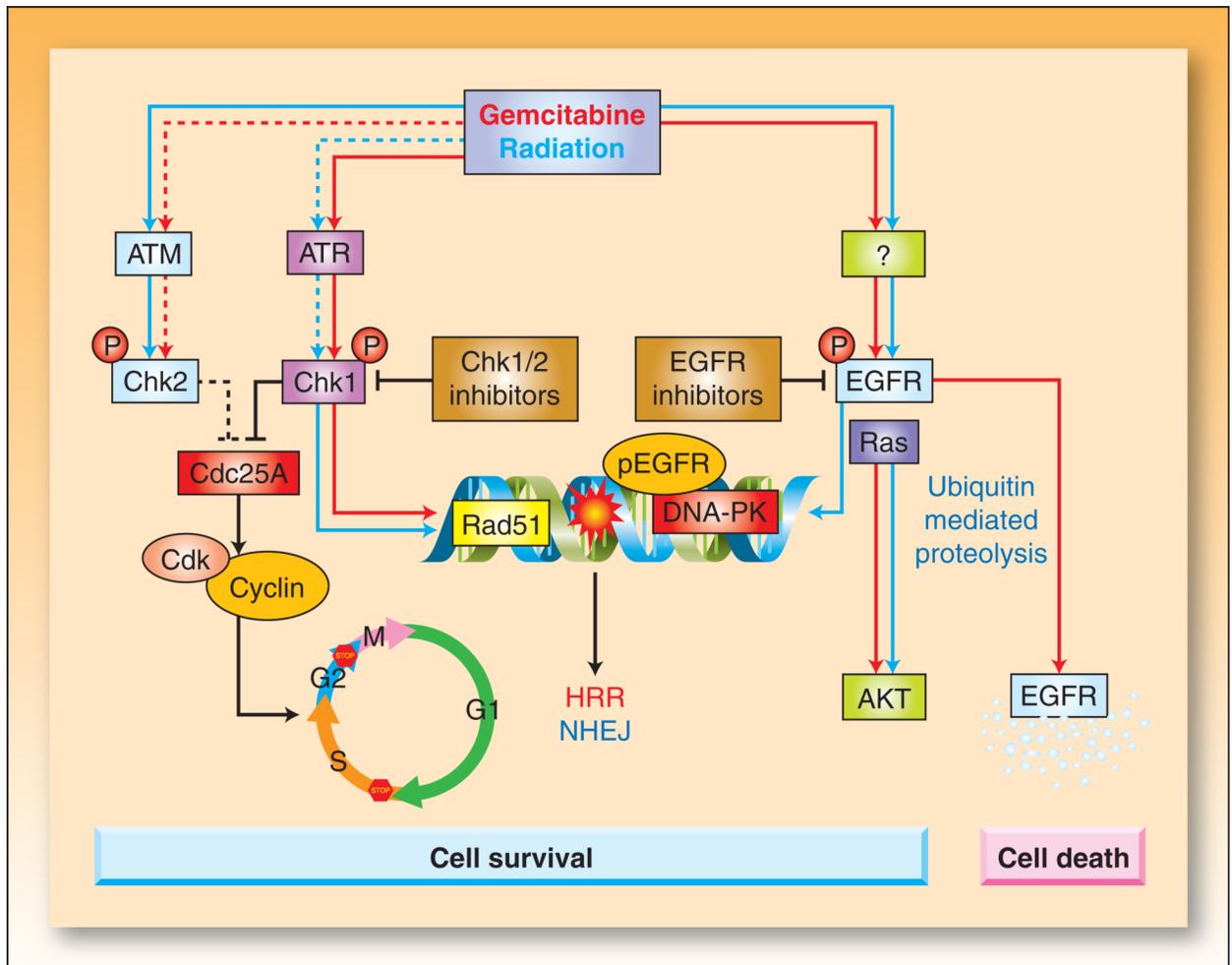
Figure 3. The effects of gemcitabine and radiation on cell cycle checkpoints and EGFR signaling
Radiation-induced double strand breaks or gemcitabine-induced replication stress trigger the
activation of ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) kinases,
respectively (92). Active ATM/ATR phosphorylate and activate Chk1 and Chk2 (93-95) which
phosphorylate Cdc25 phosphatases, leading to their inactivation through degradation
(Cdc25A) or cytoplasmic sequestration (Cdc25C) (93,96). In the absence of Cdc25
phosphatase activity, cyclin dependent kinases (Cdk1 and Cdk2) remain bound by inhibitory
phosphorylations, resulting in arrest of the cell cycle at G1/S, intra-S, or G2/M. Treatment of
cells with gemcitabine prior to radiation results in radiosensitization that can be attributed to
a number of events (Box 1), including dATP depletion and S-phase arrest. Inhibition of Chk1
sensitizes cells to gemcitabine and radiation by a number of potential mechanisms including
abrogation of cell cycle arrest, premature mitotic entry, and inhibition of Rad 51 focus
formation resulting in impaired homologous recombination repair (HRR).
EGFR is phosphorylated in response to radiation or gemcitabine by an unknown mechanism
(s) (97). Radiation triggers translocation of EGFR into the nucleus (58,60). This process
coincides with transport of Ku70/80 and protein phosphatase 1 into the nucleus, resulting in
increases in DNA-PK, repair of DNA-strand breaks (NHEJ; nonhomologous endjoining), and
cell survival. Activation of EGFR in response to gemcitabine can also result in activation of
the survival signal AKT (46). Activating Ras mutations can result in activation of Ras-
dependent pathways, such as PI3K/AKT, even in the presence of EGFR inhibitors. EGFR
inhibitors prevent gemcitabine and/or radiation-mediated EGFR signaling and are thought to

impair cell survival signals and DNA repair. EGFR inhibition blocks nuclear transport of EGFR and DNA-PK activity (60,98). In some instances, phosphorylation of EGFR by gemcitabine promotes ubiquitination of the receptor leading to degradation along a proteosome/lysosome pathway (63). EGFR degradation results in down-regulation of the survival signal pAKT, leading to apoptosis. Blocking EGFR degradation at various steps of this pathway reduces gemcitabine-mediated cytotoxicity. Whether an EGFR-activating insult leads to cell survival or cell death may ultimately be determined by the severity and duration of the stress. The colored arrows indicate the effects mediated by gemcitabine (red) versus radiation (blue). Dotted lines indicate less pronounced effects.
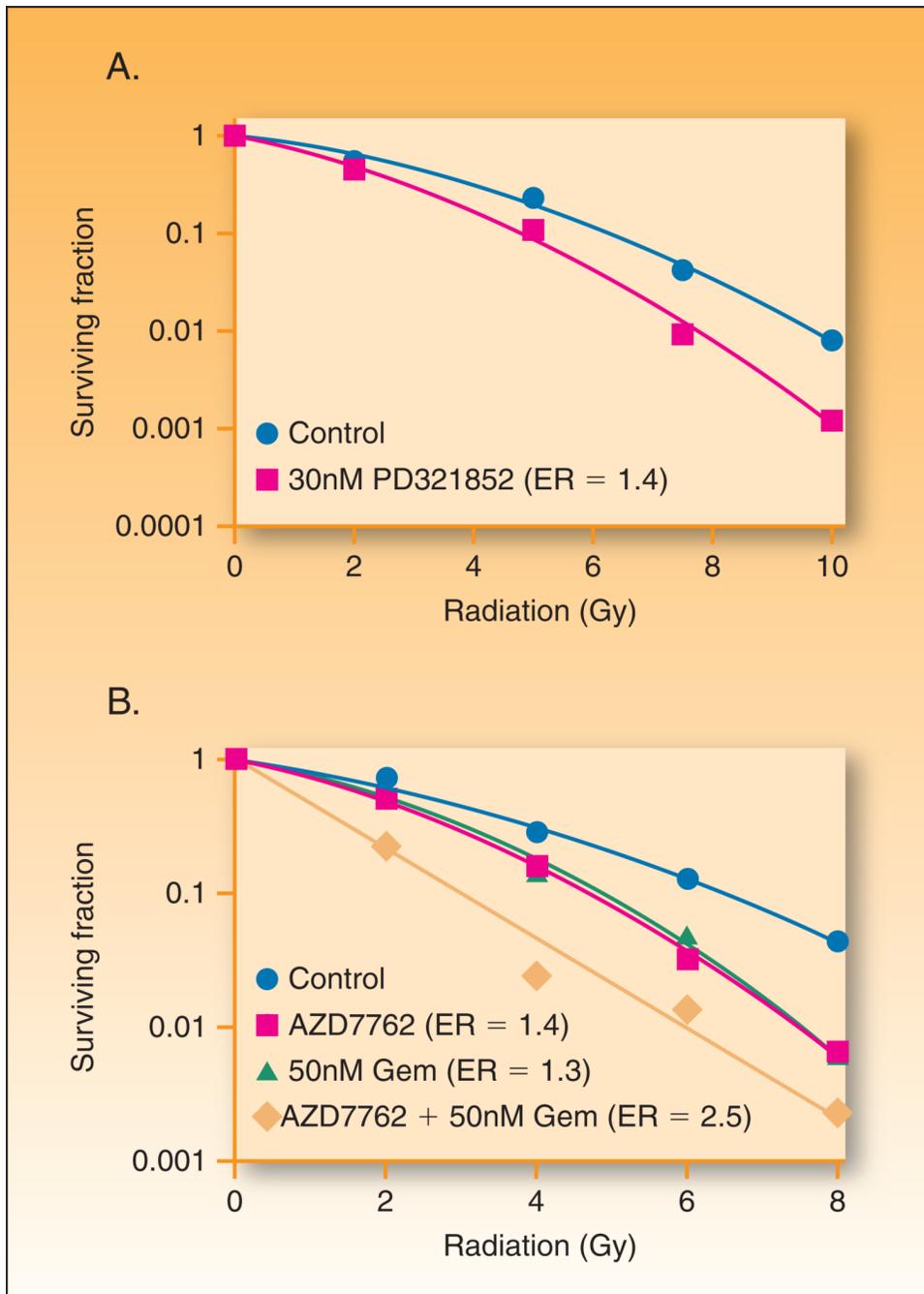
Figure 4. The effects of Chk1 inhibition on radiation and chemoradiation sensitivity
MiaPaca-2 cells were treated with 30 nM PD-321852 for 24 hrs pre- and post-ionizing radiation (0 −10 Gy) (A) or for 2 hours with gemcitabine (50nM) and then with AZD7762 (100nM) for 1 hour pre- and 24 hours post-irradiaiton (B). Cells were then plated at cloning densities and grown for 10 days to determine the surviving fraction, which represents the fraction of cells surviving radiation treatment relative to un-irradiated controls. Cell survival curves were then fitted using the linear quadratic equation, and the mean inactivation dose was calculated according to the method of Fertil et al. (99). The radiation enhancement ratio was calculated by dividing the mean inactivation dose under control conditions by the mean inactivation dose of Chk1 inhibitor-treated cells.