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# Cell Cycle Checkpoints and Their Impact on Anticancer Therapeutic Strategies

**Alan Eastman***

Department of Pharmacology, and the Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, New Hampshire 03755

**Abstract** Cells contain numerous pathways designed to protect them from the genomic instability or toxicity that can result when their DNA is damaged. The p53 tumor suppressor is particularly important for regulating passage through $G_1$ phase of the cell cycle, while other checkpoint regulators are important for arrest in S and $G_2$ phase. Tumor cells often exhibit defects in these checkpoint proteins, which can lead to hypersensitivity; proteins in this class include ataxia–telangiectasia mutatated (ATM), Meiotic recanbination 11 (Mre11), Nijmegen breakage syndrome 1 (Nbs 1), breast cancer susceptibility genes 1 and 2 (BRCA1), and (BRCA2). Consequently, tumors should be assessed for these specific defects, and specific therapy prescribed that has high probability of inducing response. Tumors defective in p53 are frequently considered resistant to apoptosis, yet this defect also provides an opportunity for targeted therapy. When their DNA is damaged, p53-defective tumor cells preferentially arrest in S or $G_2$ phase where they are susceptible to checkpoint inhibitors such as caffeine and UCN-01. These inhibitors preferentially abrogate cell cycle arrest in p53-defective cells, driving them through a lethal mitosis. Wild type p53 can prevent abrogation of arrest by elevating levels of $p21^{waf1}$ and decreasing levels of cyclins A and B. During tumorigenesis, tumor cells frequently loose checkpoint controls and this facilitates the development of the tumor. However, these defects also represent an Achilles heel that can be targeted to improve current therapeutic strategies. J. Cell. Biochem. 91: 223–231, 2004. 

**Key words:** DNA damage; p53 tumor suppressor; $p21^{waf1}$; cyclin B; Chk1; UCN-01

## REFLECTIONS OF EDWARD BRESNICK

About every 6 years, Ed's fancy would turn to a sabbatical leave so that he could spend a year away from the administrative responsibilities that had taken over his life. Yet every time he was seduced by a new opportunity to take on a different challenge, and instead of going on a sabbatical leave, he moved to a new Institution to run this or that department, or direct a Cancer Center. Georgia, Vermont, Nebraska, New Hampshire, and Massachusetts, as well as the American Association for Cancer Research, all benefited from his administrative skills. Ed eventually realized that a sabbatical was not going to happen, but he found another way to get back into the lab; he retired. So in 1999, Ed retired to my laboratory where he began to challenge the students in my lab and throughout the Department. And they all loved him for it. Ed taught me that the best way to gain respect from students is to ask tough questions, make them think, and then to enjoy a joke with them. In return, they taught him how to pipette (not by mouth), make solutions, do cell culture, Western blots, clone and subclone genes, and how not to break glassware. Some of these lessons he learned better than others. Ed chose to work on one of our projects concerning the regulation of DNA damage-induced cell cycle checkpoints. He focussed on the role of p53 in regulating cell cycle arrest in S and $G_2$ phase, and in doing so created a cell line with a unique and fascinating phenotype. This cell line has now been named in his memory as MCF10A/EB. His latest venture was to subclone a checkpoint regulator Mre11 and reintroduce this into cells. He had transformed his bacteria and was selecting colonies on his last day in the lab. He was thoroughly enjoying this new postdoctoral research experience. All who knew Ed would say that, if given a choice, that was exactly how

*Correspondence to: Alan Eastman, Department of Pharmacology, Dartmouth Medical School, 7650 Remsen, Hanover, NH 03755. E-mail: alan.eastman@dartmouth.edu

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he would have choreographed it. It seems only fitting that this review should focus on the checkpoint pathways that had become such an important part of Ed's final days.

## CELL CYCLE CHECKPOINTS

The p53 tumor suppressor protein has been called the guardian of the genome. In the face of DNA damage, p53 can either trigger cell cycle arrest to permit time for adequate DNA repair, or it can trigger apoptosis to prevent the damaged cell from developing into a tumor. More than 50% of tumors are defective in p53, and this loss is frequently thought to prevent apoptosis. This idea is somewhat confounding as cell cycle progression that is permitted in the absence of p53 should lead to increased cytotoxicity. However, p53 is only one checkpoint regulator, and cells contain many other proteins that also trigger cell cycle arrest and thereby protect them from DNA damage-induced cytotoxicity. This review will summarize some of these checkpoint regulators, and suggest ways by which this knowledge is helping to develop tumor-targeted therapeutic strategies.

Cells incubated with $\gamma$-radiation, cisplatin, or other anticancer DNA damaging agents arrest their cell cycle progression in an attempt to repair the damage. The phase of the cell cycle where the cells arrest depends on their p53 status; cells with wild type p53 arrest predominantly in the $G_1$ phase, while cells with mutant p53 fail to arrest in $G_1$, but rather accumulate in the S and $G_2$ phases. Once repair is complete, cells may recover, proliferate, and divide. Premature progression through the cell cycle can be lethal.

Ataxia telangiectasia is an autosomal recessive trait in which cells fail to arrest when damaged (mutated gene = ATM) [Shiloh, 2003]. These cells are hypersensitive to $\gamma$-radiation and fail to arrest in S phase when irradiated; a phenomenon known as radioresistant DNA synthesis. More recently, other genetic disorders have been identified that also exhibit hypersensitivity and radioresistant DNA synthesis. These disorders include Nijmegen breakage syndrome (Nbs1) and ataxia telangiectasia-like disease (Mre11) [D'amours and Jackson, 2002]. Additionally, inherited breast cancer susceptibility (BRCA1 and BRCA2) and Fanconi's anemia also exhibit hypersensitivity to DNA damage; in the latter case, mutations affect eight different genes of which the FANCD1 gene is identical to BRCA2 [D'Andrea and Grompe, 2003]. These diseases emphasize the complexity of checkpoint regulation and its importance for genomic stability and cell survival. They further emphasize that many tumors may already have defects that should make them sensitive to appropriate therapy, or that these gene products are potential therapeutic targets to enhance anticancer therapies, particularly in the context of defective p53.

## CELL CYCLE CHECKPOINT INHIBITORS

It was reported in 1967 that caffeine sensitized cells to ultraviolet radiation [Rauth, 1967]. This sensitization was subsequently observed for many DNA damaging agents. It was initially thought that caffeine inhibited DNA repair, but in 1982, it was discovered that caffeine abrogated $G_2$ arrest, and drove cells through a lethal mitosis [Lau and Pardee, 1982]. Increased cytotoxicity resulted from an inadequate time for repair to occur prior to mitosis. Unfortunately, caffeine was not a viable clinical drug as patients can not tolerate the millimolar concentrations required.

The molecular mechanisms of checkpoint regulation took many years to define, hence identification of the molecular target for caffeine had to wait. Over the intervening time frame, other checkpoint inhibitors were identified including staurosporine [Tam and Schlegel, 1992], but this also was too toxic for administration to humans. A major advance occurred in 1996 when we discovered that a novel protein kinase C (PKC) inhibitor, 7-hydroxystaurosporine (UCN-01), was 100,000 more potent than caffeine at abrogating cell cycle arrest in human cells [Bunch and Eastman, 1996]. Importantly, the necessary concentration was well tolerated in murine tumor models where UCN-01 had already been shown to enhance the activity of DNA-damaging agents [Akinaga et al., 1993]. UCN-01 initially entered phase I clinical trials as a single agent because of evidence that it had some therapeutic activity when administered alone. This activity may be due to its inhibition of PKC, or perhaps to its inhibition of a more recently identified target, PDK1 [Sato et al., 2002]. Unfortunately, UCN-01 was found to bind avidly to $\alpha$1 acid glycoprotein in human plasma, leading to plasma concentrations in excess of 30 $\mu$M [Fuse et al., 1998; Sausville et al., 2001] whereas only 10 nM is required to abrogate cell cycle arrest [Kohn et al., 2002].

Although clinical trials of UCN-01 in combination with DNA-damaging agents continue, it has become evident that it will be difficult to obtain an adequate amount of free drug without compromising patient safety. Hence, the search for better inhibitors continues. One improved candidate is another PKC inhibitor, Gö6976, which is more potent as a checkpoint inhibitor, but abrogates arrest even in the presence of human plasma [Kohn et al., 2003]. Hence, this limitation should be resolvable, and there is optimism for development of effective checkpoint inhibitors.

The molecular target for caffeine was eventually identified in 1999 as ATM, as well as the AT–related homolog ATR [Blasina et al., 1999; Sarkaria et al., 1999], while the target for UCN-01 was identified in 2000 as Chk1 [Busby et al., 2000; Graves et al., 2000]. Recent results have suggested that UCN-01 also inhibits Chk2 [Yu et al., 2002].

## REGULATION OF S AND $G_2$ CELL CYCLE CHECKPOINTS

Cell cycle progression is primarily regulated by cyclin-dependent kinases (Cdk). Each Cdk usually exists in a phosphorylated form, associated with a cyclin and inactive in the cell. At an appropriate time in the cell cycle, the cyclin/Cdk complex is dephosphorylated by Cdc25 and activated. Cdc25A acting on cyclin E/cdk2 is primarily responsible for S phase progression, while Cdc25C acting on cyclin B/Cdk1 is responsible for $G_2 \rightarrow M$ progression (Fig. 1). The checkpoint regulatory proteins Chk1 and Chk2 function by phosphorylating and inhibiting these Cdc25 homologs. Phosphorylation of Cdc25A leads to its degradation [Mailand et al., 2000]. Phosphorylation of Cdc25C maintains it inactive in the cytosol complexed with 14-3-3 [Peng et al., 1997]. Cdc25C is also constitutively phosphorylated by C-TAK1 to prevent premature mitosis [Peng et al., 1998].

In the presence of DNA damage, Chk1 is preferentially phosphorylated and activated by ATR, while Chk2 is preferentially phosphorylated by ATM, although crosstalk occurs between these pathways. ATM appears more responsive to $\gamma$-radiation-induced damage, while ATR is more responsive to ultraviolet radiation and antimetabolites. The topoisomerase I inhibitor SN38 activates both pathways. When a cell is arrested in S phase, Cdc25A is degraded. Inhibition of ATM/ATR or Chk1/2 by caffeine or UCN-01, respectively, causes reaccumulation of Cdc25A, activation of cyclin E/Cdk2 and S phase progression. Cdc25C remains inactive in S phase because of the action of C-TAK1. Once a cell reaches $G_2$, C-TAK1 must be switched off and the cell then relies on Chk1 and Chk2 to prevent the onset of mitosis if the DNA is damaged. At this point, caffeine and UCN-01 can induce a lethal mitosis.

The MRN complex (Mre11, Rad50, Nbs1) also regulates S phase arrest but not $G_2$ arrest. The complex binds to DNA double-strand breaks, thereby activating the S phase checkpoint and in turn is activated by the checkpoint as seen in the ability of ATM to phosphorylate Nbs1. It is not yet know how the MRN complex prevents S phase progression but it likely acts on Chk1/2, Cdc25A, or cyclin E/Cdk2. BRCA1 is thought to act as a scaffold for the MRN complex. BRCA1 has additional activities at the $G_2$ checkpoint presumably by complexing with other proteins.

## MISINFORMATION IN THE CHECKPOINT FIELD

It is frequently stated that Chk1/2 phosphorylate serine 216 of Cdc25C. Although correct, this ignores the fact that Cdc25C is constitutively phosphorylated on this site even in the absence of activated Chk1. There is evidence that Chk1 has some constitutive activity even when not phosphorylated, and this is required for normal turnover of Cdc25A [Zhao et al., 2002b]. However, when Chk1 is inhibited by UCN-01, and Cdc25A accumulates, Cdc25C remains phosphorylated on serine 216. This occurs because this site is also a substrate for C-TAK1. It is believed that C-TAK1 is required to prevent activation of Cdc25C at the wrong phase of the cell cycle. Only at the onset of mitosis is Cdc25C activated, presumably because C-TAK1 is inactivated, although the mechanism for this remains to be determined. Only at this point in the cell cycle are Chk1/2, required for phosphorylation of Cdc25C; they keep it in an inactive state to avoid mitosis of a damaged cell.

Interestingly, UCN-01 also inhibits C-TAK1 but at considerably higher concentrations [Busby et al., 2000]. This can be seen in undamaged cells by dephosphorylation of Cdc25C at $>100$ nM UCN-01. Many papers use con-

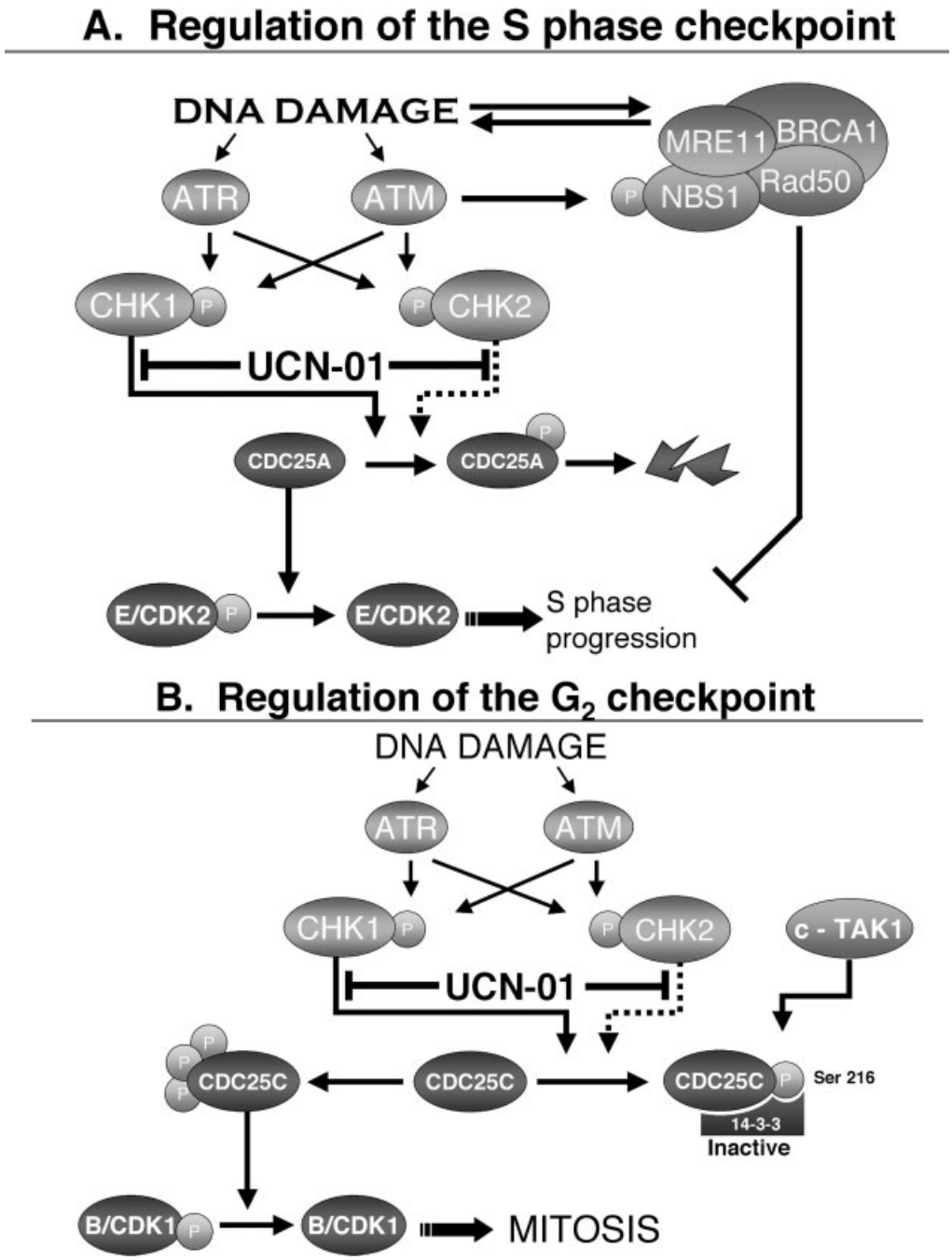


**Fig. 1.** Models for the regulation of (**A**) S phase arrest and (**B**) $G_2$ arrest in cells exposed to DNA-damaging agents.

centrations in this range believing they are selective for Chk1, when this is clearly not the case. Indeed at these concentrations, S phase-arrested cells can be driven directly into mitosis without completion of DNA synthesis. At low concentrations of UCN-01, DNA-damaged cells will progress from S phase arrest into $G_2$ before undergoing mitosis. At high concentrations, this S phase progression is not required. This can be demonstrated by addition of the DNA polymerase inhibitor aphidicolin during S phase abrogation. Aphidicolin protects cells at low concentrations of UCN-01, but not at high concentrations where C-TAK1 is inhibited and the cells undergo rapid S → M transition [Kohn et al., 2002].

Aphidicolin as well as hydroxyurea have been commonly used as models of S phase arrest, but the above experiment establishes that aphidicolin can prevent checkpoint abrogation. An important difference between most DNA-damaging agents and aphidicolin is the mechanism of S phase arrest. Aphidicolin prevents DNA synthesis by directly inhibiting the DNA polymerase, while hydroxyurea prevents DNA synthesis by preventing synthesis of the essential deoxyribonucleotides. Hence, it is impossible for these cells to synthesize DNA even if the S phase checkpoint is inhibited. They can however transition directly into mitosis as DNA synthesis is not required. This is frequently considered a checkpoint, but as discussed above, it may be due to inhibition of C-TAK1, which is a constitutive kinase rather than a checkpoint-specific kinase.

The following is a recent example of how this problem can yield misleading conclusions. It was shown that deletion of both ATM and ATR prevented arrest induced by γ-irradiation, but cells incubated with aphidicolin still arrested [Brown and Baltimore, 2003]. It was therefore proposed that an additional kinase must regulate the S phase arrest induced by aphidicolin. Unfortunately, this conclusion ignored the mechanism of action of aphidicolin. It is impossible to abrogate S phase arrest in the absence of functional DNA polymerase. Therefore, there is no need to propose that another checkpoint kinase is involved.

UCN-01 is also an inhibitor of Cdk1/2. In vitro analysis has shown that UCN-01 is almost as effective at inhibiting Cdk2 as it is at inhibiting Chk1 [Zhao et al., 2002a]. However, this relationship does not hold in cells. Cdk2 is required for abrogation of S phase arrest, and if both Chk1 and Cdk2 were inhibited, a cell would not progress through S phase. Using another Cdk2 inhibitor, roscovitine, we have found that inhibition of Cdk2 does block UCN-01-mediated abrogation of S phase arrest. On analysis of a series of UCN-01 analogs, we have found only one, staurosporine, that can antagonize S phase progression; that is, staurosporine abrogates S phase arrest at 3 nM, but inhibits the abrogation when the concentration is raised to 100 nM. In contrast, UCN-01 abrogates S phase arrest at 5 nM but this is not antagonized at even 1 μM. The important conclusion is that inhibition of Cdk activity in vitro does not extrapolate to inhibition in cells. This certainly raises concern for any in vitro kinase assay, and emphasizes the need to establish inhibitory activity inside cells.

## IMPACT OF p53 ON CHECKPOINT ABROGATION

It is important to emphasize that caffeine and UCN-01 have no effect on cells arrested in $G_1$, but only on cells arrested in S and $G_2$. Considering that DNA damage leads primarily to $G_1$ arrest in p53 wild type cells, and S or $G_2$ arrest in p53 mutant cells, it can be envisioned that caffeine plus a DNA-damaging agent might preferentially target tumor cells. However, if p53 wild type cells have passed the $G_1$ checkpoint before they are damaged, they will arrest in S and $G_2$ and might be susceptible to caffeine. The next major breakthrough came in 1995, when three papers simultaneously reported that caffeine, and another methylxanthene, pentoxifylline, failed to abrogate DNA damage-induced S and $G_2$ arrest in p53 wild type cells; it only abrogated arrest and enhanced cytotoxicity in p53 defective cells [Fan et al., 1995; Powell et al., 1995; Russell et al., 1995]. Similar results have been obtained with UCN-01 [Wang et al., 1996]. This has led to the very exciting possibility that DNA-damaging agents plus UCN-01 might represent a therapeutic strategy that selectively targets a tumor because of its lack of p53.

To investigate the mechanism by which wild type p53 prevents checkpoint abrogation, we generated a p53-defective subline of the immortalized breast cell line MCF10A; this cell line, MCF10A/EB, expresses a fragment of the p53 protein that prevents tetramerization. Incubation of both cell lines with the topoisomerase I inhibitor SN38 caused arrest in S and $G_2$ phase. In MCF10A cells, this arrest was associated with accumulation of p53 and $p21^{waf1}$; p53 was also phosphorylated on serines 15 and 20. In MCF10A/EB cells, arrest was also associated with p53 accumulation and phosphorylation, but with no detectable increase in p21. Hence, these cells have a defective response to p53 activation. UCN-01 was added to SN38-arrested cells. MCF10A cells were unaffected by UCN-01 and remained arrested in S and $G_2$ for 24 h. In contrast, UCN-01 drove S phase-arrested MCF10A/EB cells into $G_2$, but surprisingly, it did not drive them through mitosis. These results suggest that $p21^{waf1}$ may prevent abrogation of S phase arrest, but another mech-

anism is required to explain the failure to abrogate $G_2$ arrest. Analysis of cyclins A and B expression by flow cytometry showed that both cell lines repressed expression of these cyclins, and accordingly were unable to undergo mitosis [Kohn et al., 2002; and unpublished data].

We next compared an immortalized human mammary epithelial cell line, IMEC, to its isogenic derivative in which p53 was inhibited by siRNA (IMECΔp53). Similar to MCF10A, the IMEC cells arrested in S and $G_2$ in response to SN38 and were unaffected by UCN-01. In contrast, the IMECΔp53 cells responded to UCN-01 by abrogation of both S and $G_2$ arrest. These cells were inhibited for p53 transactivation as reflected by lack of induction of $p21^{waf1}$, but they significantly accumulated cyclin B showing that they had also lost the p53 repression response. The differences observed between IMECΔp53 and MCF10A/EB cells with respect to cyclin B expression suggest the latter cell line has lost the p53 transactivation function but retained the repression function. This is possible as transactivation requires tetramerization of p53, which is blocked in MCF10A/EB, but p53 is still present and phosphorylated in these cells, suggesting that repression can result from monomeric p53. Hence, this cell line will provide an excellent model to dissect the mechanisms of p53-mediated gene repression. There may be much more to this story as greater than 85% of tumors reportedly exhibit deregulated cyclin B [Gorczyca et al., 1997], including some that express wild type p53, suggesting that other defects must exist in this pathway.

These observations may resolve some conflicting reports as to whether the action of UCN-01 is truly selective for p53-defective cells [Husain et al., 1997; Hirose et al., 2001]. For example, it is important to establish that a p53-dependent checkpoint has been fully activated before adding UCN-01. This may require using a higher concentration of DNA-damaging drug or incubating longer to ensure all cells have fully arrested. Furthermore, it is important to establish that $p21^{waf1}$ is induced and cyclin B is repressed if one expects to see a block to abrogation. Using MCF10A cells, we have seen subpopulations that abrogate S phase arrest, yet these appear to be the few percent that have not fully induced $p21^{waf1}$. We have also observed at least one tumor cell line that has lost its p53 repression function even though transactivation still occurs. Hence, different conclusions may be obtained within a single cell line depending on drug, concentration, time, and method of analysis, while results may vary in different cell lines because of other defects in the pathway.

## TUMORS HAVE MULTIPLE DEFECTS IN CHECKPOINT REGULATION

In all our experiments, we routinely incubate cells with a range of drug concentrations initially to establish the pattern of growth arrest. For example, low concentrations of SN38 arrest cells in $G_2$ while increasing concentrations arrest cells in late, mid, and early S phase [Kohn et al., 2002]. This pattern has been remarkably reproducible in many cell lines. Hence, we were surprised when one cell line exhibited a very different pattern. Specifically, the p53 wild type HCT116 colon cell line failed to arrest in S phase but arrested in $G_2$ at all concentrations. This cell line is well known to be mismatch repair defective, but correction of this defect only partially restored S phase arrest. Mismatch repair-defective HCT116 cells have frequently been shown to be resistant to cisplatin [Aebi et al., 1996], yet we were unable to confirm this. However, there was one very disconcerting observation that two apparently identical wild type HCT116 cells obtained from two different sources exhibited approximately fivefold different sensitivity to cisplatin. This leaves us concerned that conclusions on the role of mismatch repair may have resulted from different clonal variants. It is worth noting that a $p21^{waf1}$-deleted variant was markedly more sensitive to cisplatin consistent with the ability of $p21^{waf1}$ to normally arrest and protect cells.

The reason why HCT116 cells failed to arrest in S phase on SN38 became apparent with the publication of a report showing a defect in the MRN pathway [Giannini et al., 2002]. Specifically, it was shown that a frame shift mutation had occurred in intron 4 of both alleles of the Mre11 gene leading to misplicing of the mRNA and production of a truncated protein. One function of Mre11 appears to be stabilization of the MRN complex, and as a consequence, Nbs1 and Rad50 are also markedly reduced. This mutation likely arose because of the mismatch defect in these cells. Further analysis showed that a similar defect occurred in many mismatch repair-deficient colorectal tumors [Giannini

et al., 2002]. Correction of the mismatch repair defect can not correct this checkpoint defect.

As discussed above, there are many traits that can be tracked to inherited defects in cell cycle checkpoints, but the observations on Mre11 suggest spontaneous defects can also occur in these genes. This is certainly true with BRCA1 whose expression appears to be defective in 35% of invasive ductal carcinomas [Wilson et al., 1999]; this is far more than can be accounted for by inherited defects in this gene. Cells that have lost BRCA1 also exhibit radioresistant DNA synthesis. The question arises as to how frequently do defects occur in these checkpoint pathways, and can these defects be exploited to selectively target cell death to tumors, thereby improving a patient's response to therapy.

## FUTURE DIRECTIONS: CUSTOMIZE DRUGS TO PATIENTS BASED ON A KNOWLEDGE OF THEIR CHECKPOINT DEFECTS

Cells with checkpoint defects and radioresistant DNA synthesis should be hypersensitive to many DNA-damaging agents. We envision that S phase checkpoint-defective cells should be particularly sensitive to topoisomerase I inhibitors. These drugs elicit toxicity when the replication fork collides with a topoisomerase cleavable complex leading to a DNA double-strand break. In the absence of the S phase checkpoint, replication will continue thereby generating many more double-strand breaks and a much greater cell kill. Similar arguments can be made for sensitivity to topoisomerase II inhibitors and alkylating agents. It seems important to determine which tumors have defects in these pathways and then to design therapeutic strategies that take advantage of this increased sensitivity. In designing appropriate drug combinations, it is important to realize that many drugs might be antagonistic to this strategy. Consider, for example, antimetabolites, such as 5-flourouracil, which deplete thymidine such that replication can not occur. This arrest would limit the cytotoxic potential of S phase-dependent DNA-damaging drugs, and in particular, override the potential hypersensitivity of checkpoint-defective cells.

On the one hand, understanding these cell cycle interactions seems highly logical in developing effective therapeutic drug combinations. However, drug interactions and schedules have rarely been a major consideration in designing clinical trials. Perhaps this is not surprising, as experiments with many cell and animal models have not extrapolated to a clinical observation. Indeed, the combination of 5-flourouracil plus the topoisomerase I inhibitor irinotecan has become widely accepted in the USA as a first line therapy for metastatic colorectal cancer, and the schedule involves simultaneous administration of these drugs [Saltz et al., 2000; Moehler et al., 2003]. The addition of irinotecan to 5-flourouracil increased survival from 12.6 months to 14.8 months. So this contradicts the hypothesis that 5-flourouracil should antagonize the response rate to irinotecan. Or does it, particularly as this seems a marginal increase in survival. Drug combinations can work by one of two ways: (a) they can function independently with each drug killing different cells; or (b) they can have a combined action on each individual cell. The truth is likely to be that both mechanisms work, but the predicted antagonism would only be true of the latter mechanism. The question that can not be resolved at this time is whether a better dosing schedule of these two drugs might have a much greater effect. And of critical importance is the question of whether there are sub-sets of tumors, those defective in cell cycle checkpoints, that might show dramatically improved response with an improved drug combination or schedule.

This is how we envision future therapeutic approaches. A patient presenting with a tumor will be screened for potential defects in cell cycle checkpoints. Those that have defects in S or $G_2$ checkpoint proteins will be stratified to receive DNA-damaging drugs that take advantage of this hypersensitivity. Other tumors that show defects in p53 pathways will be treated initially with a DNA-damaging agent followed by a checkpoint inhibitor such as UCN-01 (but one that does not bind human plasma proteins). This will still leave some tumors for which we would not be able to predict a therapeutic benefit. Fortunately, we are not fighting this battle alone, and many other investigators have identified novel targets that can be used to stratify patients for alternate therapies. Hopefully, we will soon end the crude approach of prescribing the same toxic drug concentration to every patient with a particular disease simply because a few may benefit. Eventually, we will know what works in advance and prescribe drugs accordingly.

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