DNA Structure and Integrity Checkpoints during the Cell Cycle and Their Role in Drug Targeting and Sensitivity of Tumor Cells to Anticancer Treatment

Andrzej Skladanowski,*,† Przemyslaw Bozko,‡ and Michal Sabisz†

Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland, and Institute of Experimental Internal Medicine, Medical Faculty, Otto von Guericke University Magdeburg, Magdeburg, Germany

Received January 20, 2009

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

Contents

1. Introduction 2951
2. Types of DNA Lesions Induced in Tumor Cells by Anticancer Treatment 2952
   2.1. Direct DNA Damage: Induction of DNA Strand Breaks and Covalent Binding 2952
   2.2. Indirect DNA Damage 2953
3. DNA Structure and Integrity Checkpoints during the Cell Cycle 2953
   3.1. Initiation Steps in DNA Damage Checkpoint Activation—General Mechanisms 2954
   3.2. G1/S Checkpoint 2955
   3.3. DNA Replication Checkpoint during S (intra-S Checkpoint) 2956
   3.4. G2 Checkpoint 2957
   3.5. Decatenation Checkpoint 2958
   3.6. DNA Damage during Mitosis and the Spindle Checkpoint 2958
   3.7. The G1 Postmitotic Checkpoint (Tetraploidy Checkpoint) 2959
4. Different Outcomes Initiated by DNA Damage Checkpoint Signaling 2959
5. Cross-talk between Cell Cycle Checkpoints DNA Repair and Other Intracellular Signaling Pathways 2960
   5.1. MAPK Stress Signaling and DNA Damage Checkpoints 2960
   5.2. NF-kB and DNA Damage Checkpoints 2960
   5.3. Survival Signaling during G2-M 2961
   5.4. DNA Damage Checkpoints and DNA Repair Pathways 2961
6. Defects in DNA Damage Checkpoints in Tumor Cells 2962
   6.1. Defects in DNA Damage Sensing and Signaling Pathways of the Cell Cycle Checkpoint Response in Tumor Cells 2962
   6.2. Deregulation of Checkpoint Maintenance Mechanisms in Tumor Cells 2963
   6.3. Defects of Effector and Regulatory Proteins Involved in the DNA Damage Checkpoint Response 2963
   6.4. Other Specific Changes in the DNA Damage Checkpoint Response in Tumor Cells 2963
   6.5. Cancer Stem Cells and DNA Damage Checkpoint Defects 2964
7. DNA Damage Checkpoint Functionality and Sensitivity of Tumors to DNA Damaging Agents 2964
8. Chemical Inhibition and Modulation of the DNA Damage Checkpoint Signaling and Sensitization of Tumor Cells to Anticancer Drugs 2964
   8.1. Chemical Inhibitors and Modulators of the DNA Damage Checkpoint Regulatory Proteins—Activity and Specificity 2965
   8.2. Checkpoint Inhibitors and Modulators—Current Developments and Perspectives 2966
9. Concluding Remarks and Perspectives 2966
   9.1. Possible Adverse Effects of DNA Damage Checkpoint-Targeted Chemotherapy 2967
   9.2. Future Directions in DNA Damage Checkpoint-Targeted Chemotherapy 2967
10. References 2968

* Phone: (4858) 3471749. Fax: (4858) 3471144. E-mail: as@chem.pg.gda.pl.
† Gdansk University of Technology.
‡ Otto von Guericke University Magdeburg.

1. Introduction

From all antitumor chemotherapeutics used in the treatment of human cancers, the most prominent group is constituted of agents which directly or indirectly induce DNA damage. Radiotherapy also primarily targets DNA and induces different DNA lesions. This may be surprising given the fact that DNA is not a perfect target for relatively unspecific small molecular weight drugs or γ-irradiation. However, the current view is that cellular response to DNA damage induced by antitumor agents, the so-called "cell context-driven effect", is responsible for their higher killing effect and relative specificity toward cancerous compared to normal cells.

DNA damaging agents with antitumor activity include compounds with divergent activities from drugs which directly or indirectly induce DNA strand breaks, covalently modify DNA bases, and change the chromatin structure and topology by inhibiting chromatin-modifying enzymes, such as DNA topoisomerases, histone deacetylases, or demethylases. Some drugs, e.g. DNA topoisomerase inhibitors, induce both DNA breaks and perturbations of the DNA structure. In addition, although inhibitors of tubulin polymerization are not classically recognized as DNA damaging agents, interference by these drugs with the mitotic spindle functions may also lead to production of chromosomal breaks during mitosis. This chromosomal damage could either kill tumor cells or induce additional genetic changes that may promote drug resistance.

Successful progression through the cell cycle is controlled by a number of different regulatory mechanisms termed checkpoints.[1] There are specific cell cycle checkpoints which are activated by changes in DNA structure and integrity induced by drug treatment during progression of cells through G1, S, G2, and M. DNA damage checkpoints are frequently

10.1021/cr900026u CCC: $71.50 © 2009 American Chemical Society
Published on Web 06/12/2009

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

Andrzej Skladanowski received his M.Sc. (1978) and Ph.D. (1994) degrees in Biotechnology and Biochemistry from Gdansk University of Technology (GUT), Gdansk, Poland. He joined the group of Prof. Jerzy Konopa at the same university, working as a research associate (1984−1994) on covalent binding of anthracyclines to DNA. In 1994, he moved to Institute Gustave-Roussy (Villejuif, France) and joined the group of Dr. Annette K. Larsen, initially as a postdoctoral fellow and later as an international research fellow (1999−2001). After his return to Poland, he started his academic carrier as a Lecturer in Cell Biology and later in Anticancer and Antiviral Chemotherapeutics at Gdansk University of Technology. Prof. Andrzej Skladanowski and his group are actively involved in characterization of molecular targets of new antitumor agents. His research interests also include cellular response induced by antitumor drugs in tumor cells, including the role of cell cycle control in the induction of cell death after genotoxic stress, and mechanisms of inherent and drug-induced cellular resistance to anticancer treatment.

Przemyslaw Bozko was born in 1973. He received his M.Sc. degree at the University of Warsaw (Poland) in 1998 and his Ph.D. degree at the University of Gdansk in 2003. He spent two years at the Karolinska Institutet, Stockholm (Sweden), and one year at the University of Illinois, Chicago (USA), as a postdoctoral fellow. He is currently working at the University of Magdeburg (Germany). His research interests include interactions between tumor suppressors and oncogenes, during both tumor formation and anticancer therapy.

Michal Sabisz was born in 1978. He graduated in Food Chemistry and Technology from Gdansk University of Technology (GUT). He received his B.Sc. in 2001 and M.Sc. in Biotechnology and Pharmaceutical Technology from the same university in 2003. In 2008, he received his Ph.D. in Biotechnology, and he currently works as a research assistant at the Faculty of Chemistry of GUT. His research interests include induction of premature senescence by anticancer drugs and the role of cancer stem cells in the resistance of tumor cells to antitumor therapy.

defective in cancer cells and therefore play an important role in the sensitivity of these cells to DNA damage induced by anticancer treatment. Importantly, nonfunctional cell cycle checkpoints may greatly influence the efficacy of antitumor agents toward tumor cells and may be associated with both drug resistance and oversensitivity to antitumor drugs.

In this article, we will overview different types of DNA lesions produced by antitumor agents and present how these lesions could activate various cell cycle checkpoint responses. In particular, we will concentrate on how DNA damage checkpoint control mechanisms are influenced and cross-regulated by other intracellular signaling pathways such as DNA repair, stress, and survival signaling. We will also discuss how deregulation of checkpoint mechanisms observed in many tumor types could be exploited to specifically target tumor cells by anticancer treatment and how defects in checkpoint regulation could increase or decrease the sensitivity of tumor cells to DNA damaging agents. Finally, we will present how the activity of different checkpoint regulators could be modulated by chemical inhibitors and how these compounds could increase the efficacy of anticancer therapies, when applied in combination with DNA damaging agents to treat cancer patients. We believe that a more comprehensive understanding of the complexity of the DNA damage checkpoint response could help in the rational design of new checkpoint abrogators or DNA damaging agents to assist with the successful discovery of drugs or innovative combination strategies with improved activity and selectivity toward tumor cells.

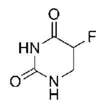
2. Types of DNA Lesions Induced in Tumor Cells by Anticancer Treatment

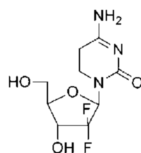
It is important to realize that various anticancer agents induce different types of DNA damage from direct or indirect breakage of DNA strands to effects on DNA structure, resulting from the inhibition of nuclear enzymes controlling DNA topology and/or condensation. The overall perception is that different types of DNA damage may activate various checkpoint signaling pathways, but the molecular mechanisms responsible for their activation are still not clear. The situation may be further complicated by the fact that many types of DNA damage are processed by DNA replication/transcription machinery or DNA repair enzymes.

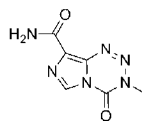
2.1. Direct DNA Damage: Induction of DNA Strand Breaks and Covalent Binding

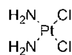
At least some antitumor drugs, such as bleomycin, produce direct DNA strand breaks by free radical-mediated cleavage of deoxyribose.[2] Direct DNA damage, leading to single- and double-stranded breaks, is also produced by ionizing irradiation during radiotherapy. Many drugs bind covalently to DNA bases, mostly purines, and form monofunctional or bifunctional DNA−DNA adducts as well as DNA−protein cross-links.[3] These include Temozolomide, cisplatin and its

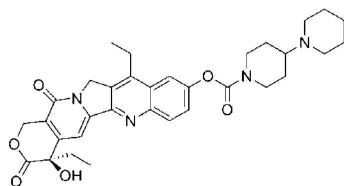
Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

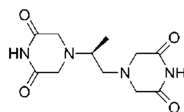


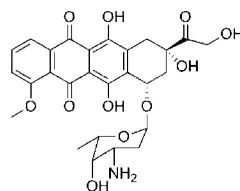












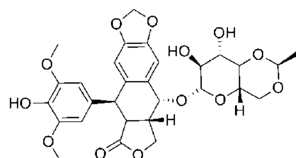


Figure 1. Typical anticancer drugs which induce different types of DNA lesions: antimetabolites (5-fluorouracil and gemcitabine), mono- and bifunctional DNA binding drugs (Temozolomide and cisplatin), as well as inhibitors of DNA topoisomerase I (irinotecan) and II (dexrazoxane, doxorubicin, and etoposide).

analogues, mitomycin C, nitrosoureas, and others. Incorporation of nucleoside analogues (5-fluorouracil, cytarabine; gemcitabine, troxacitabine, etc.) also leads to chemical changes and structural perturbations in DNA. In Figure 1, chemical structures of typical anticancer drugs, which induce different types of DNA lesions, are presented.

Mono- and bifunctional covalent modifications of DNA bases by antitumor drugs lead to local perturbations of the DNA helix structure, including changes in DNA curvature (DNA bending) or mispairing between DNA bases. Some of DNA binding drugs, such as mitomycin C and doxorubicin, possess planar multiring chromophores and are also able to bind noncovalently to DNA by intercalation between base pairs. Other drugs, such as the duocarmycin analogues Adozelesin and Bizelesin, bind into the minor groove of DNA.[4] This type of DNA binding also introduces local changes in DNA structure, e.g. may lead to DNA unwinding.

Structural changes induced by covalent and noncovalent binding of drugs to DNA have important functional consequences, such as changed affinity of different enzymes or regulatory proteins to DNA, including transcription factors and DNA structure/topology regulators (DNA topoisomerases, histone deacetylases—HDACs or methylases). These DNA structure perturbations may, in turn, activate or inactivate regulatory regions of genes and consequently change gene expression profiles in tumor cells. Covalent modifications also lead to mispairing between DNA bases during DNA transcription and replication, resulting in erroneous RNA transcripts and unreplicated DNA regions due to premature termination of DNA replication.

2.2. Indirect DNA Damage

Many clinically important antitumor drugs such as anthracyclines (daunorubicin, doxorubicin, and analogues), podophyllotoxins (etoposide), and camptothecin analogues (topotecan and irinotecan) inhibit type I and/or type II DNA topoisomerases (see Figure 1 for chemical structures of the typical DNA topoisomerase I and II inhibitors), which control DNA topology and structure by regulating DNA supercoiling, catenation, and chromatin condensation. The so-called "classical inhibitors" of DNA topoisomerase I and II stabilize transient intermediates, where DNA is covalently linked to topoisomerase I or II (called cleavable complexes). That leads to DNA strand breaks due to the collision of drug—DNA—enzyme complexes with DNA replication and transcription machinery. Alternatively, the catalytic activity of topoisomerase II is inhibited by antitumor drugs such as ICRF compounds (e.g., ICRF-187 or dexrazoxane) by stabilizing a closed clamp DNA—enzyme complex (for review see ref 5). Inhibition of both type I and II DNA topoisomerases leads to changed local supercoiling, which may directly regulate the expression of genes with supercoiling-sensitive promoter elements.[6] In the absence of topoisomerase II activity, progression of the transcription complex is also inhibited by excessive DNA supercoiling, and DNA transcription is inhibited specifically at the elongation step.[7] In addition, type II topoisomerases, in particular the α isoform, in cooperation with cohesins play a key role to establish chromatid cohesion, which is essential for the faithful separation of chromosomes during mitosis.[8] Changed DNA cohesion, induced for example by the inhibition of topoisomerase II, may greatly influence the efficiency of DNA repair (for a recent review, see ref 9).

DNA topoisomerases are part of chromatin modifying complexes, together with histone acetylases/deacetylases or methylases/demethylases. Recently, several compounds have been approved or entered clinical trials, which act by inhibition of different HDAC enzymes (e.g., SAHA, valproic acid),[10,11] or demethylases (5-azacytidine).[12] Inhibition of HDAC enzymes or demethylases leads to changes in local DNA structure and activation of cell cycle checkpoints in early mitosis.[13]

Finally, antimitotics or drugs that interfere with tubulin polymerization are usually not perceived as DNA damaging agents; however, stabilization or destabilization of microtubules by antitumor drugs also induces indirect DNA damage. This is associated with perturbation of chromosome separation and segregation during mitotic division in cells with nonfunctional microtubules.

3. DNA Structure and Integrity Checkpoints during the Cell Cycle

DNA damage, if left unrepaired, leads to genomic instability and cell death. Cells have evolved complex signaling networks that monitor if the genome was correctly replicated during the S phase and whether mitotic division was successful and produced two viable daughter cells, with

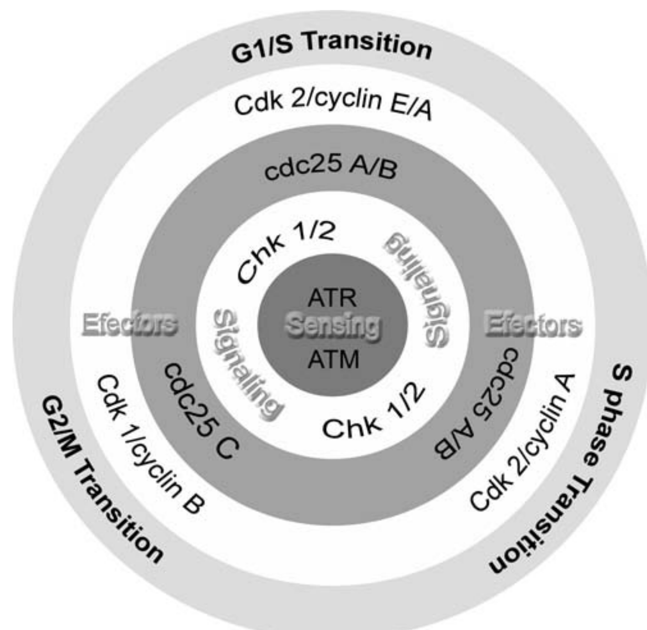


Figure 2. General mechanism of cell cycle checkpoints induced by DNA damage. A common sensing mechanism based primarily on the activation of ATM/ATR kinases phosphorylates and activates signaling Chk1/2 kinases. DNA damage signaling is then translated to different effector proteins, such as Cdc25 phosphatases and cyclin-dependent kinases, that culminates in arrest of the cell cycle progression in G1, S, or G2 phases.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

unchanged genetic information. If errors in any of these processes occur, this initiates a cascade of signaling pathways, called DNA damage checkpoints, which arrest cell cycle progression, activate repair of DNA damage, or initiate cell death responses. During carcinogenesis, tumor cells acquire many genetic changes, which may relieve at least some of the restraints imposed by cell cycle checkpoint mechanisms on cell proliferation. These changes in the functionality of the checkpoint response sensitize tumor cells to DNA damaging agents but can also lead to cellular resistance to anticancer drugs.

All checkpoints activated by DNA lesions have similar functional components that include early damage sensors, PIKK kinases (ATM, ATR, and DNA-PK), mediator or signal transducer proteins (Chk1 and Chk2 signaling kinases), and effector proteins (phosphatases Cdc25A/B/C and cyclin-dependent kinases Cdks). It follows that DNA damage is first recognized and then signals are transmitted to cell cycle machinery that temporarily arrest cell cycle progression and allow DNA repair. Finally, DNA lesions are repaired, cell cycle arrest signaling is switched off, and if repair of DNA damage is successful, cells resume proliferation. Many molecular regulators are shared between different DNA damage checkpoints. Therefore, all checkpoints induced by DNA damage (G1/S, intra-S, G2, and early M checkpoints) could be viewed as only one general response mechanism to genotoxic stress (schematically presented in Figure 2), where different sensors and regulatory complexes are assembled, such as ATR/ATRIP, ATM/MRN (Mre11-Rad50-Nbs1 complex), and the replication factor C (RFC/Rad17) or Rad9-Rad1-Hus1 (9-1-1 complex), described in section 3.1. These complexes promote recruitment of other proteins to the damaged DNA. Some of these proteins are activated and immediately released from the site of damage, such as Chk1 and Chk2 kinases and, by phosphorylating other protein substrates, activate soluble signals to be transmitted to cell cycle machinery. Many more proteins remain at or in the vicinity of the damaged DNA and form multimeric complexes (so-called foci), which protect/stabilize the site of damage and promote its repair. Depending on when in the cell cycle different DNA damage sensors are activated, various cell cycle regulatory mechanisms are switched off and cell proliferation is temporarily arrested at the G1/S border, during the S phase or before the onset of mitosis (G2 and early M). From this perspective, these are *different outcomes* of the initial activation of DNA damage sensors, that define respective checkpoints, and *not molecular mechanisms* which are activated by DNA damage (Figure 2). This is partially related to the availability of different checkpoint regulators during the cell cycle. As an example, checkpoint signaling kinase Chk1 is present in cells in significant quantities only during S and G2 phases, whereas Chk2 is expressed throughout the cell cycle.[14,15] In addition, the ATM protein level follows that of Chk1, whereas ATR levels remain unchanged even after treatment with various DNA damaging agents or DNA replication inhibitors.[16−18]

3.1. Initiation Steps in DNA Damage Checkpoint Activation—General Mechanisms

In the most widely accepted model for the DNA damage response, there are two partially independent pathways involved in the initiation of different cell cycle checkpoints that are controlled by PIKK kinases, ATR and ATM. It is believed that ATM and ATR are activated by different types of DNA damage. ATM is primarily responsible for controlling proper responses to agents that induce double-stranded DNA breaks (DSB), whereas ATR is activated in response to a variety of damaging agents: UV light, alkylating agents such as methyl methanesulfonate (MMS), and chemical inhibitors of DNA replication.[19,20]

Sensing of DNA damage by both kinases and their subsequent activation require additional cofactors. ATM localization to the damage sites occurs in response to DNA double-stranded breaks and is facilitated by the MRN complex.[21−27] ATR responds to a more complex array of stimuli which induce single-stranded DNA breaks and gaps that cause functional uncoupling of the MCM helicase and DNA polymerases.[28] For its efficient binding to damaged DNA and activation, ATR requires a cofactor ATRIP, an ATR interacting protein,[29−31] but also other factors such as Rad17/RFC, that promote the loading of the Rad9-Rad1-Hus1 (9-1-1) clamp complex onto the DNA template.[32,33] There is also an interplay between ATM and ATR activation as DSBs could be processed by the MRN complex into single stranded breaks, which upon binding of RPA (replication protein A) form RPA−ssDNA structures. These structures facilitate recruitment and activation of ATR and its target, Chk1 signaling kinase. Activated ATR may also directly phosphorylate and activate ATM and strengthen checkpoint signaling by activation of Chk2 (see Figure 3).[34]

It should be stressed that activation of ATM and ATR kinases following DNA damage does not occur on the catalytic activity level but rather leads to increased ability of these kinases to bind DNA damage sites, bind other regulatory proteins, and recognize their substrates. For example, following binding to damaged DNA, ATM undergoes autophosphorylation, which induces a dimer-to-monomer transition.[35,36] This is an important kinase-activating step, as in the absence of DNA damage ATM is predominantly present as inactive dimers. The molecular mechanism

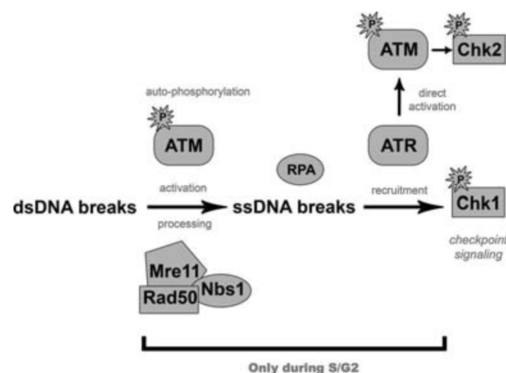


Figure 3. Cross-talk between ATR by ATM pathways in DNA damage checkpoint activation. Double-stranded DNA breaks are sensed by ATM kinase, which leads to its activation but could also be processed by the MRN complex (Mre11-Rad10-Nsb1) to single-stranded DNA breaks, which upon binding of RPA proteins form ssDNA-RPA structures. These structures facilitate recruitment and activation of ATR and subsequent activation of the signaling kinase Chk1. Activated ATR may also directly phosphorylate and activate ATM and strengthen checkpoint signaling by activation of Chk2 kinase.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

that leads to the dimer-to-monomer transition of ATM is not known, but it was found that ATM autophosphorylation *per se* does not appreciably change its catalytic activity.[26,37,38] Similarly, it was found that ATR catalytic activity does not increase in response to various DNA damaging agents or DNA replication inhibitors.[39,40]

Similarly to Chk1/2 kinases, there are major differences in the cellular levels of ATM and ATR kinases throughout the cell cycle. ATR protein levels do not change appreciably during the cell cycle, and all its cofactors and substrates are always present, especially when cells are undergoing DNA replication.[16] This is in contrast to ATM, which is expressed in a cell cycle dependent manner, and significant levels of this kinase accumulate only during late S and G2/M.[18,41]

It also should be mentioned that although ATM and ATR share many of the same substrates in tissue culture cells, drastically different phenotypes are observed if the gene for either kinase is disrupted, especially in the whole organism. Mutations in the *ATM* gene in humans are responsible for the genetic disorder ataxia telangiectasia, which is characterized by devastating and progressive neurodegeneration, increased susceptibility to the development of cancer, and oversensitivity to radiation.[42] On the other hand, disruption of the *ATR* gene in mice leads to early embryonic cell death that is associated with extensive chromosomal fragmentation.[40] A similar effect is observed when somatic cells are induced to prematurely undergo mitosis, also known as "mitotic catastrophe".[43] This suggests more fundamental cellular functions for ATR than ATM kinase.

The role of another PIKK kinase, DNA-dependent protein kinase (DNA-PKcs) and its regulators Ku70/Ku80 proteins, in DNA damage signaling has been debated for some time. Although DNA-PK is involved mainly in DNA repair by nonhomologous end joining, it also acts on damaged DNA in a similar way as ATM and ATR.[44,45] Therefore, it is possible that, in addition to ATM and ATR, DNA-PK is involved in sensing the DNA damage to checkpoint mechanisms. Accordingly, it has been shown that Chk2 can be effectively phosphorylated by DNA-PKcs *in vitro* and in cells exposed to camptothecin or ionizing radiation.[46,47] Similarly, H2AX phosphorylation after exposure to ionizing irradiation may be mediated by both ATM and DNA-PK.[48] It suggests

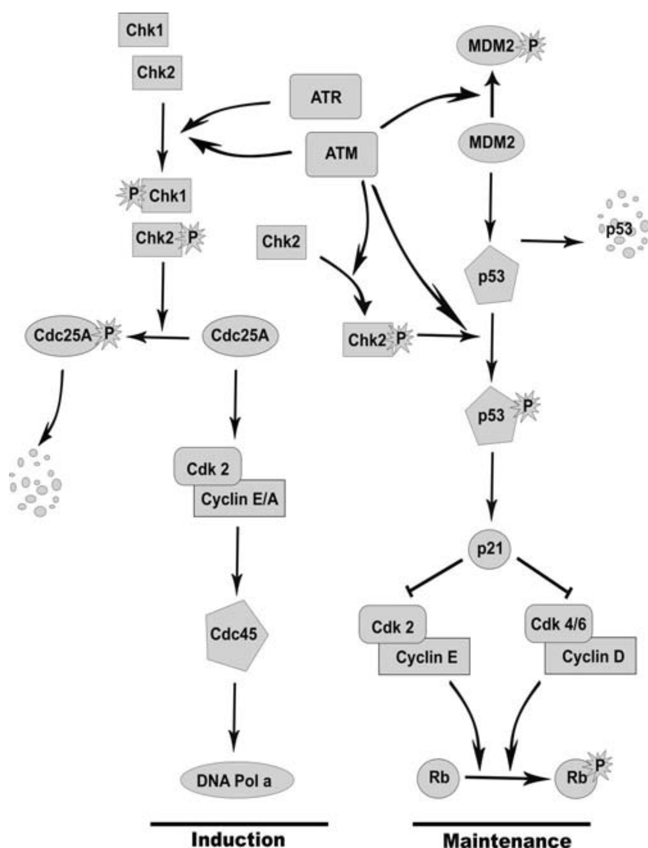


Figure 4. Activation of G1/S and intra-S checkpoints in response to genotoxic stress. DNA damage is sensed by ATM/ATR kinases which activate checkpoint signaling kinases Chk1/2. This leads to phosphorylation of Cdc25A phosphatase and its degradation, which, in turn, precludes the activation of S-phase specific Cdks, Cdk2/cyclin E, and Cdk2/cyclin A. Consequently, DNA replication in the S phase is prevented by the inhibition of Cdc45-dependent loading of DNA polymerase α onto chromatin. In parallel, phosphorylation of p53 by ATM/Chk2 kinases leads to its stabilization, by preventing its Mdm2-mediated degradation, and increased expression of p21, an inhibitor of Cdk2/cyclin E and Cdk4/6/cyclin A activity. Decreased phosphorylation of pRb protein by Cdks blocks the activation of S-phase specific DNA expression.

that DNA-PK, at least in some situations, may augment ATM and ATR kinases in the activation of the DNA damage checkpoint regulators such as Chk2.

3.2. G1/S Checkpoint

In the presence of DNA damage, the entry of cells into the S phase of the cell cycle is prevented. Transition from G1 to S is regulated positively by G1-specific cyclin-dependent kinases (Cdks) and negatively by the product of the retinoblastoma tumor suppressor gene, pRb. Current evidence suggests the sequence of events for the G1/S checkpoint activation in mammalian cells, illustrated in Figure 4. If the DNA damage is double-strand breaks, ATM kinase is activated and phosphorylates many of its target molecules, notably p53 and Chk2. These phosphorylations result in the activation of two signal transduction pathways, one to initiate and the other to maintain the cell cycle arrest at the G1/S border. The reaction that initiates the G1/S arrest is phosphorylation of the checkpoint kinase Chk2, which in turn phosphorylates Cdc25A phosphatase, causing its inactivation by nuclear exclusion and ubiquitin-mediated proteolytic degradation.[49,50] In addition, phosphorylation of Cdc25A by Chk2 inhibits its catalytic activity.[51] Lack of

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

active Cdc25A results in accumulation of the phosphorylated (inactive) form of Cdk2, that is incapable of initiating DNA replication. Alternatively, when the DNA damage signal is sensed by another PIKK kinase, ATR, this leads to phosphorylation of Chk1 checkpoint kinase. The activated Chk1 also phosphorylates Cdc25A and initiates G1 arrest by a similar mechanism as for Chk2. However, given very low cellular levels of Chk1 in the early phases of the cell cycle,[14] the role of Chk1 in the activation of the G1 checkpoint may be less important compared to Chk2 kinase.

Irrespective of whether the initial proliferation arrest in G1 is caused by the ATM-Chk2-Cdc25A pathway or the ATR-Chk1-Cdc25A pathway, this rapid response is followed by the p53-mediated G1/S checkpoint maintenance signaling pathway (reviewed in refs 52 and 53). In the maintenance stage, ATM or ATR phosphorylates directly the N-terminus of p53 on Ser15,[54,55] or indirectly by activated Chk1 or Chk2 on Ser20,[56,57] which inhibits Mdm2-mediated p53 degradation. Moreover, phosphorylation of p53 on Ser15 or Ser20 increases its interaction with transcription coactivator p300, that stimulates acetylation of p53 at Lys320 and Lys382 within the C-terminal domain. Both types of post-transcriptional modifications of p53 inhibit the ability of its C-terminal domain to negatively regulate sequence-specific DNA binding either by inducing a conformational change in the protein or by inhibiting its nonsequence-specific DNA binding. To add to the complexity, modifications of the C-terminal part of p53 modulate also the oligomerization of p53 and may promote nuclear import/export by ATM-dependent dephosphorylation of serine 376 at the carboxyl terminus of p53 and exposure of the 14-3-3 consensus binding site.[58] In parallel, a safeguard mechanism exists to inactivate p53 inhibitors, Mdm2 and Mdmx. Phosphorylation of both Mdm2 and Mdmx, that is also mediated by ATM,[59−64] attenuates their interaction with the ubiquitin protease HAUSP and promotes degradation of both p53 regulators that results in nuclear accumulation of p53 following DNA damage.[65−67]

Increased levels of p53 and activation of its transcriptional activity lead to upregulation of inhibitors of cyclin-dependent kinase (CKIs) such as p21/CDKN1A.[68,69] A newly synthesized p21 binds to and inhibits the S-phase-promoting Cdk2−Cyclin E complex and prevents it from phosphorylating pRb.[70−73] Hypophosphorylated Rb binds to the E2F transcription factor and blocks the expression of genes required for S-phase progression, including Cyclin E itself.[74−78] This negative signaling loop is further strengthened by another CKI, p16CDKN2A, that binds to and inactivates Cdk4/6-Cyclin D complexes.[79]

Much less is known about how the G1-S checkpoint signaling is turned off. It could be speculated that, following successful repair of DNA lesions, ATM or ATR dependent phosphorylation of Chk1/2 is no longer sustained and Cdc25A phosphatase levels and activity gradually increase, which leads to removal of the inhibitory phosphate groups from Cdk2, inactivation of Rb, and eventually alleviation of G1/S arrest. Similarly, in the absence of ATM activation, p53 and Mdm2 interact in the resting conditions mode, which finally leads to deactivation of a p53-dependent maintenance mechanism of the G1/S arrest.

3.3. DNA Replication Checkpoint during S (intra-S Checkpoint)

Double-stranded DNA breaks or drug-induced replication fork stalling can transiently inhibit progression through the S phase by activation of the intra-S checkpoint. Molecular machinery is very similar to that activated during the G1/S checkpoint and involves ATM/ATR sensing kinases and Chk1 and Chk2 checkpoint signaling kinases and leads to the phosphorylation promoted degradation of Cdc25A phosphatase.[50] This again prevents dephosphorylation (activation) of Cdk2 and leads to a transient blockage of S phase progression (presented in Figure 4). In addition, inhibition of Cdk2 activity prevents loading of Cdc45 onto replication origins, a prerequisite for recruitment of DNA polymerase α, and thereby restart of DNA synthesis is delayed when the DNA template is damaged.[80] The kinase activity of Cdk2 and replicon initiation could be inhibited by both ATM/Chk2 and ATR/Chk1 dependent mechanisms.[81]

Many proteins are sequentially recruited to DNA damage foci, including ATM/ATR, claspin, BRCA1/2, and other proteins with BRCT repeats (53BP1, MDC1), Nbs1, histone H2AX, or FANCD2, and this multicomponent protein complex promotes the local concentration of ATM/ATR kinases and their targets. It also triggers structural changes within the DNA damage site, resolves some of the topological problems produced by stalled replication forks, facilitates repair of DNA damage, and enables reinitiation of DNA synthesis (for a review, see ref 82). ATR is recruited to DNA lesions through its association with ATR-interacting protein (ATRIP), which in turn interacts with the single-stranded DNA binding protein RPA (replication protein A). In vitro studies have shown that RPA stimulates the binding of ATRIP to ssDNA. On the other hand, the binding of ATRIP to RPA-coated ssDNA enables the ATR-ATRIP complex to associate with DNA and stimulates phosphorylation of Chk1 and Rad17.[83] ATR kinase activity could also be stimulated by binding of topoisomerase binding protein 1 (TopBP1) to ATRIP-ATR, and it can occur independently of the interaction of ATRIP with RPA.[84]

Another protein that binds very early after induction of DNA damage is claspin, a ring shaped protein with high affinity to branched DNA structures. Claspin binds to damaged DNA and becomes phosphorylated in a ATR-dependent manner that results in recruitment and phosphorylation of BRCA1 and activation of Chk1.[85] Similarly, structural maintenance of chromosome (SMC) proteins, which are components of the cohesin complex, is an important effector of the intra-S phase checkpoint. Chromatid cohesion is established during DNA replication in the S phase and is required for DNA repair during S and G2 (for a recent review, see ref 86). SMC proteins, specifically SMC1 and SMC3, associate with BRCA1 and are phosphorylated in a NBS1- and ATM-dependent manner.[87−89]

In contrast to the G1/S checkpoint, activation of the intra-S checkpoint does not require p53 function.[90] It seems that there is no p53-dependent signaling loop which prolongs S phase arrest after DNA damage. For this reason, growth arrest mediated by the intra-S checkpoint after treatment with DNA damaging agents is usually relatively short, on the order of 2−4 h.[91] However, one study strongly suggests the important role of the transactivation activity of p53 in the maintenance of the intra-S checkpoint activated in immortalized breast cells by SN38. The authors showed that p53 transactivated the expression of p21, and this led not only to Cdk2 inhibition but also to decreased Cdc25A transcription.[92]

Termination of the intra-S checkpoint signaling is mediated by Plk-1 dependent phosphorylation of claspin followed by its removal from DNA and Chk1 inactivation.[93] Moreover,

successful recovery from S-phase arrest induced by inhibitors of DNA replication is critically dependent on DNA helicases such as BLM and WRN. BLM protein, a member of a ReQ helicase family, physically associates with ATM and ATR and is phosphorylated by these kinases.[94,95] Since BLM protein accumulates to high levels in the S phase, persisting in G2/M and sharply declining during mitosis, it suggests a possible implication of BLM not only in the intra-S phase but also in the G2 phase checkpoint signaling.[96] Another DNA helicase WRN is also a substrate of ATM and ATR.[97] Both BLM and WRN colocalize with components of DNA damage-induced foci, including H2AX, BRCA1, Nbs1, RPA, and Rad51 and are required for the recovery to normal fork topology, resolution of recombination intermediates, that arises from replication arrest, and effective repair of DNA damage induced during unperturbed or stressed S phase progression.[94,95,98,99]

3.4. G2 Checkpoint

If DNA damage produced during the S phase is not effectively repaired, it activates the DNA damage checkpoint in G2 and postreplicative DNA repair. Interestingly, when DNA lesions are induced during G2, only some types of DNA damage are able to activate the G2 checkpoint. Elegant studies with a psolaren derivative, that directly binds DNA and forms DNA−DNA cross-links after UV irradiation, showed that the G2 checkpoint is activated only after progression through the next S phase.[100] On the other hand, interference with chromatin condensation/decatenation during G2 by catalytic inhibitors of DNA topoisomerase II (e.g., ICRF compounds) effectively arrests cell cycle progression in the same G2.[101]

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

The major players which regulate induction and maintenance of the G2 checkpoint are very similar to those involved in the G1/S and intra-S checkpoints. These include the sensor kinase complexes ATR/ATRIP and ATM/MRN and the checkpoint kinases Chk1/Chk2 and p53 (see Figure 5). Generally, there are two partially independent pathways of the G2 checkpoint induced by DNA damage. One, that is independent of p53 function and relatively fast, is responsible for the initiation of checkpoint signaling. Both sensor kinases activate Chk1/Chk2 signaling kinases which phosphorylate Cdc25C phosphatase, that promotes its 14-3-3ε-dependent nuclear exclusion.[102] Active cdc2/cyclin B1 complexes can also be exported out of the nucleus by a Crm1-mediated mechanism. It requires inhibition of Plk1-dependent phosphorylation of Ser 147 in the nuclear export sequence (NES) region of cyclin B1, that promotes its nuclear export.[103] Activation of Cdk1 may be delayed by nuclear retention of inactive cyclinB1/Cdk1 complexes, promoted by their association with p21.[104] Chk1 leads to hyperphosphorylation of Wee1, and this results in maintenance of Tyr 15 phosphorylation and hence G2 delay.[105] Similarly, Myt1, another tyrosine kinase that inhibits Cdc2, is positively regulated by the DNA damage checkpoint by Chk1-dependent phosphorylation and stabilization of this protein.[106] All these mechanisms delay activation (dephosphorylation) or nuclear localization of catalytically active Cdk1 and prevent initiation of G2 to M transition.

There is also a p53-dependent G2 checkpoint maintenance mechanism that is relatively slow and inhibits mitotic onset through regulation of gene expression, similarly as during the G1/S checkpoint (see section 3.2). Transcription of the Cdk1 gene can be inhibited simultaneously by Gadd45, p21,

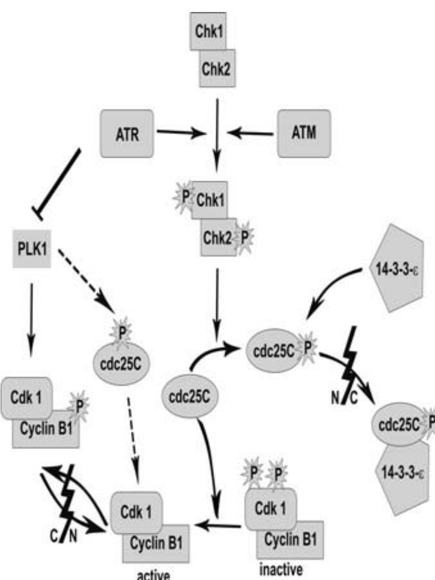


Figure 5. Molecular mechanisms of G2 arrest induced by genotoxic lesions. DNA damage leads to activation of checkpoint kinases Chl1/2 by ATM/ATR kinases. This prevents the activation of the master mitotic kinase Cdk1/cyclin B1, by nuclear/cytoplasmic extrusion of Cdk1-activating phosphatase, Cdk25C, or Plk-1-mediated inhibition of nuclear/cytoplasmic transport of the Cdk1−cyclin B1 complex. Plk-1 is also postulated to be involved in the decatenation checkpoint, where Plk-1 activity is inhibited by ATR/BRCA1, that leads to exclusion of activated Cdk1−cyclin B1 from the nucleus and inhibition of the G2 to M transition. N/C and C/N, nuclear-cytoplasmic and cytoplasmic-nuclear transport, respectively. Dashed arrows show the proposed mechanism of the “decatenation” checkpoint.

and 14-3-3σ, which are upregulated after DNA damage in a p53-dependent manner.[107,108] P53 by itself represses the expression of many mitotic regulators, including cyclin B1 and Cdk1, to help ensure that cells do not escape the initial G2 block and enter long-term G2 arrest.[109] High levels of p21 inhibit Cdk1 activity; however, the role of p21 in the G2 arrest induced by DNA damage by suppressing of Cdk1 activity has been debated for some time. P21 binds and strongly inhibits Cdk2/cyclin E complexes, but its affinity toward Cdk1/cyclin B1 and Cdk2/cyclinA is much lower.[110] It was shown that p21 does not interfere with the dephosphorylation of two inhibitory phosphorylation sites on Cdc2, Thr 14, and Tyr 15; however, it blocks the activating phosphorylation of Cdc2 on Thr 161, that is required for cyclin B1 binding.[110]

The G2 checkpoint must be eventually inactivated to allow entry into mitosis and resumption of cell proliferation; however, the molecular mechanism of checkpoint inactivation is not well characterized (for a recent, review see ref 111). Similarly to what was described for the intra-S checkpoint (see above), termination of the G2 DNA damage checkpoint is dependent on Plk1 activity and involves PP1 phosphatase-mediated Chk1 inactivation,[114] as shown in the yeast model,[115] and PP2A that dephosphorylates Chk2.[114] PP1 and PP2A phosphatases have been shown to mediate 14-3-3 release from Cdc25C and,[115,116] in this way, may be involved in G2 checkpoint inactivation and control of mitosis. Finally, the role of ARTEMIS, a phosphorylation target of ATM and ATR, in G2 checkpoint recovery has been documented.[117]

3.5. Decatenation Checkpoint

Inhibition of DNA topoisomerase II by its catalytic inhibitors does not produce direct DNA damage such as strand breaks (for a review, see ref 118). However, catalytic inhibitors of topoisomerase II, such as bisdioxopiperazine compounds, e.g., ICRF-193, have been proposed to activate a so-called "decatenation checkpoint" that delays G2 to M progression.[119−121] In this way, using this checkpoint mechanism, cells are able to monitor chromatid catenation status and inhibit progression into mitosis until the chromatids are correctly decatenated by topoisomerase II. Entanglements of sister chromatids may arise not only as a result of topoisomerase II inhibition by antitumor drugs but also as a consequence of DNA replication or nonreplicative catenations produced incidentally during the interphase.[122] Subsequent studies have shown that activation of the decatenation checkpoint is independent of ATM but relies on ATR/BRCA1 activity, inhibition of the Plk1, and nuclear exclusion of cyclin B1.[123,124] It is believed that the decatenation checkpoint is independent of the DNA damage checkpoint, that operates in G2, although both checkpoints share all their molecular components and regulators. Signal transduction leading to G2 arrest in response to ICRF-193 treatment was found to be sensitive to caffeine, 2-aminopurine, and phosphatase inhibitors such as okadaic acid.[119,125,126]

The idea of the decatenation checkpoint has been challenged by several independent studies. First, activation of the decatenation checkpoint has never been shown in normal human cells and all experimental evidence for its existence is based on the use of the ICRF-193 derivative. Other catalytic inhibitors such as merbarone and aclarubicin and even other ICRF compounds (ICRF-159 and ICRF-187) do not induce this checkpoint.[125,127] Second, it was shown that ICRF-193 is able to induce DNA damage[128−132] and activate the DNA damage checkpoint in G2, in an ATM/ATR- and Chk1-dependent manner.[133,134]

ICRF-193 and aclarubicin as well as inhibitors of histone deacetylase have been shown to activate a functionally similar checkpoint, called the chromatin deacetylation checkpoint, that responds to structural changes in DNA and delays entry into and exit from mitosis.[132] This chromatin deacetylation checkpoint is also independent of ATM activity but may be overcome by the inhibitor of p38 kinase. Interference with histone acetylation leads to production of structural and topological changes in DNA, as does inhibition of the catalytic activity of topoisomerase II; therefore, signals that activate the p38 MAPK checkpoint are similar to those of the decatenation checkpoint. Intriguingly, earlier studies with the same cellular system (rat kangaroo kidney Ptk1 cells) showed no abnormalities in the entry to mitosis after treatment with ICRF compounds.[127] Other studies revealed that, after treatment with histone deacetylase inhibitors, both normal and tumor cells arrested in G2 with high levels of p21, greatly decreased Cdc25C content, low Cdk2 activity, and no effect on Cdk1 phosphorylation.[135] The authors also postulated that this histone deacetylase inhibitor-sensitive checkpoint is distinct from G2/M checkpoints activated by genotoxic stress and may be the human homologue of the yeast G2 checkpoint, which responds to aberrant histone acetylation states.[136] Whether both these checkpoints, the decatenation checkpoint and the chromatin deacetylation checkpoint, are part of the same signaling pathway or completely independent requires further studies.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

3.6. DNA Damage during Mitosis and the Spindle Checkpoint

Until recently, it was not known whether DNA damage can arrest cell cycle progression, when it is produced during mitosis. In other words, it was not clear whether mitotic checkpoints can sense DNA breaks or changes in DNA structure and topology and arrest the G2-M transition. There are two checkpoints during mitosis which are activated in response to problems with microtubule attachment to kinetochores (the spindle assembly checkpoint, SAC) and interference in centrosome separation (regulated by ubiquitin ligase Chfr), both effects induced primarily by inhibitors of tubulin polymerization (for recent reviews, see refs 137 and 138). However, the spindle assembly checkpoint can also be induced by interference with the kinetochore assembly and impairment of microtubule motors (for example, dynein or CENP-E). The spindle assembly checkpoint is regulated by a number of regulatory proteins, including MAD1/2, Bub3, and BubR1 and many others. The Chfr-dependent checkpoint is activated in the prophase and senses if chromosomes are effectively separated, which is also dependent on the functionality of microtubules.

There are two conflicting reports on whether DNA damage induces mitotic arrest. First, it was shown that human cells, in which chromosomal damage was induced by laser microsurgery or topoisomerase II α inhibitors, arrested in the metaphase, but this effect was dependent on the dose and timing of DNA damage.[139] If low levels of DNA damage were generated during the prophase, they did not impede entry into the metaphase and cells completed mitosis on time. In contrast, more severe damage substantially delayed mitotic transit by metaphase arrest, after the formation of the bipolar spindle and destruction of cyclin A, which was independent of the p53 functionality and ATM kinase. Cells with damaged chromosomes and blocked in the metaphase contained Mad2-positive kinetochores. Consequently, the block was overridden by inactivation of Mad2.[139] These studies were supported by results obtained by another group where DNA damage during the spindle assembly checkpoint led to degradation of Cdc25A and inhibition of Cdk1 and consequently reversed cells to a G2-like state with 4N DNA content.[140] A different scenario was recently reported for cells which arrested in the prometaphase, after induction of double-stranded DNA breaks during entry into mitosis. DNA damage resulted in prolonged hyperphosphorylation of BubR1 and its association with kinetochores, a phenomenon observed during activation of SAC.[141] Downregulation of BubR1 by siRNA led to abrogation of the mitotic delay in response to chromosome damage, suggesting the involvement of SAC in the DNA damage response.

Other studies provided evidence that DNA strand breaks induced by γ-irradiation or doxorubicin do not delay mitotic transit in tumor cells.[142] A durable metaphase arrest was only induced if these cells were treated with ICRF-193 or high doses of etoposide. Since arrest after treatment with ICRF-193 was not accompanied by recruitment of Mad2 or Bub1 to kinetochores, nor by histone H2AX phosphorylaton, it was concluded that metaphase arrest was not due to activation of the spindle assembly checkpoint or response to DNA damage. The effect of very high doses of etoposide on the metaphase−anaphase transit could be explained by its possible inhibitory action toward microtubules.

It is not clear whether mitotic delay observed after induction of DNA damage is indeed associated with the

activation of SAC or rather reflects functional redundancy between the DNA damage checkpoint during G2 and mitotic spindle checkpoints induced in the prophase and metaphase. For example, Plk-1 kinase may be targeted by both DNA damage and spindle assembly checkpoints.[143−146] Plk-1 is also directly ubiquitinylated by Chfr, which leads to Plk-1 degradation and delays activation of Cdc25C and inactivation of Wee1 and arrests cells at the entry into mitosis.[147] It should also be pointed out that degradation of cyclin B1 before the metaphase to anaphase transition is regulated by the anaphase-promoting complex (APC/C) and activation of the spindle assembly checkpoint, which inhibits APC/C activity, delays degradation of cyclin B1, and blocks G2-M progression at the metaphase.[148]

3.7. The G1 Postmitotic Checkpoint (Tetraploidy Checkpoint)

In addition to the spindle assembly checkpoint, the fidelity of chromosome segregation during mitosis can be controlled by the G1 postmitotic checkpoint. The functional significance of the tetraploidy checkpoint is to detect and eliminate cells that have undergone aberrant chromosome segregation and are susceptible to entering the next replication round in the following S phase. Since cancer cells usually have an abnormal number of chromosomes, it has been postulated that aneuploidy stems from the inefficiency of checkpoint mechanisms during mitosis, which allows polyploidy. Additional events, such as loss of at least some extra chromosomes in subsequent mitotic divisions, lead to aneuploid cells, with genetically unstable genomes.

Tetraploidy may be stimulated by different agents and drugs such as the following: (i) inhibitors of microtubule polymerization and assembly; (ii) inhibitors of DNA topoisomerase II, especially catalytic inhibitors of this enzyme; (iii) abrogators that cause override of mitotic checkpoints; (iv) inhibitors of actin polymerization and cell division (cytokinesis), such as cytochalasins.[149] Tumor cells which have only partially functional DNA damage and spindle assembly checkpoints may respond to DNA damaging agents by arresting in G1 after abnormal mitosis without cytokinesis. Tetraploidy may also be induced during combination therapies when tumor cells are treated with both DNA damaging agents and microtubule inhibitors or checkpoint abrogators.

There is a controversy whether the tetraploidy checkpoint really exists. Initial studies have shown that nontransformed rat embryo fibroblasts, following defective cytokinesis, arrest indefinitely in tetraploid G1.[149] However, the authors themselves postulated that the spindle assembly checkpoint and the tetraploidy checkpoint are likely to be equivalent, since both involve inactivation of Cdk2 kinase, hypophosphorylation of retinoblastoma protein, and increased levels of p21(WAF1) and cyclin E, and both are p53-dependent. An important role in preventing endoreduplication is played by p21, which directly inhibits Cdk2/cyclin E and inhibits S phase entry of cells with 4N DNA.[150]

Further studies showed that both normal and immortalized human fibroblasts, exposed to low concentrations of cytochalasin D, became binuclear and did not arrest at 4NG1 but underwent mitosis and normal cytokinesis.[151] Similarly, fusion of two human foreskin fibroblasts produced a binuclear cell, that entered the S phase without any delay.[152] It was concluded that polyploidy *per se* does not activate the tetraploid G1 arrest nor do failed cytokinesis, binuclear cells, and numerical chromosomal and centrosomal changes. These observations provide a functional demonstration that the tetraploidy checkpoint does not exist in normal mammalian somatic cells and most probably in human tumor cells. The tetraploidy checkpoint has many features of p53-dependent arrest induced by DNA damage in diploid G1[153,154] and can be triggered by chromosomal damage produced during aberrant mitotic division.

4. Different Outcomes Initiated by DNA Damage Checkpoint Signaling

Tumor cells in which DNA checkpoints have been activated may undergo long-term proliferation arrest at the G1/S border and in G2 or slow down cell cycle progression during the S or M phase. During this long- or short-term arrest, cells activate different DNA repair pathways, which remove DNA lesions, which leads to deactivation of checkpoint signaling and recovery of cell proliferation. If repair of DNA lesions is not successfully completed due to, e.g., defects in checkpoint maintenance mechanisms or defective DNA repair systems, this activates different cell death pathways such as apoptosis, mitotic catastrophe, or necrosis. It is currently not clear whether a particular cell death pathway or other drug-induced cellular outcomes (discussed below) are associated with the activation of a specific DNA damage checkpoint.

Apart from cell death induced by antitumor drugs that damage DNA, they produce other cytostatic effects such as long-term growth arrest of tumor cells with a senescent phenotype, called drug-induced premature senescence. This process is induced when tumor cells with functional p53 have greatly suppressed cell death pathways by overexpression of apoptosis inhibitor proteins (IAPs) or hyperactivation of survival pathways such as PI3K/PKB/Akt.[155−158] It has been proposed that induction of premature senescence by antitumor treatment may constitute a new therapeutic strategy that leads to "proliferative death" of tumor cells, without actually killing them.[159,160] Several studies have shown that induction of premature senescence depends on DNA damage signaling and engages some of the DNA damage checkpoint regulators (for a review, see ref 161 and references therein).

Yet another effect that frequently follows DNA damage induced by anticancer treatment of tumor cells is induction of the epithelial-to-mesenchymal transition (EMT). The EMT is a process by which cells acquire molecular alterations, that facilitate cell motility and invasion by enhanced expression of EMT-related genes. This process was initially recognized to be associated with several critical stages of embryonic development but, more recently, has also been implicated in the switch from proliferating to a very invasive phenotype of tumor cells (for a recent review, see ref 162). Accordingly, the epithelial-to-mesenchymal transition has been associated with drug resistance of tumor cells exposed to DNA damaging agents, oxaliplatin, and other antitumor drugs.[163,164] One of the mechanisms that may be responsible for decreased drug sensitivity of tumor cells which underwent EMT could be decreased propensity to ensue apoptotic cell death following, e.g., UV irradiation.[165] Interestingly, the EM transition may also promote the generation of cancer stem cells (CSC) from more differentiated neoplastic cells, as has been shown for breast carcinomas both in cell culture and in mice.[166]

Another biological function for the DNA damage-activated signaling network could be regulation of cell differentiation. It has been shown that treatment of tumor cells with

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

topoisomerase I/II inhibiting drugs induces differentiation both in cell culture and in tumor xenografts.[167−169] Differentiation induced by topoisomerase II inhibitors such as doxorubicin was associated with lower sensitivity to apoptosis-inducing topoisomerase II-directed drugs (for a review, see refs 170 and 171).

5. Cross-talk between Cell Cycle Checkpoints DNA Repair and Other Intracellular Signaling Pathways

It is important to note that activation of DNA damage checkpoints may be cross-regulated by several stress and antiapoptotic signaling pathways, which operate during progression through the cell cycle, particularly during G2 and M.

An important component of stress signaling is related to the activity of mitogen-activated protein kinases (MAPKs). Antiapoptotic signaling, which promotes cell survival during checkpoint activation, is associated with the IAPs, such as survivin; another is regulated by phosphatidylinositol 3-kinases, including Akt/PKB.[172,173] A member of the family of dimeric transcription factors, NF-kB, is also involved in the regulation of DNA damage response and cell survival following treatment with genotoxic drugs. Finally, DNA repair pathways are implicated in the activation of DNA damage checkpoints during S and G2, and direct interaction between checkpoint and DNA repair regulators has been observed, especially those involved in mismatch repair (for a review, see ref 174).

5.1. MAPK Stress Signaling and DNA Damage Checkpoints

There are numerous reports that show involvement of MAPKs in the regulation of G1/S and G2/M transit in unstressed cells and following DNA damage. MAPKs is a family of serine/threonine kinases which regulate divergent processes, including proliferation, differentiation, stress adaptation, and apoptosis, through phosphorylation of a wide range of effector proteins, most notably transcription factors. The MAPK family is divided into three multimember subfamilies: the extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases (JNK), and the p38 kinases. All enzymes promote both cell death and survival following different types of cellular stress, including DNA damage.

A number of independent studies have shown that the ERK signaling pathway is strongly activated by DNA damaging agents such as ionizing radiation (IR) (for a review, see ref 175). Exposure of MCF-7 cells to IR irradiation resulted in ERK1/2 activation and induction of G2/M arrest. Furthermore, inhibition of ERK1/2 signaling resulted in more than 85% attenuation of IR-induced G2/M arrest and concomitant diminution of IR-induced activation of ATR, Chk1, and Wee1 kinases as well as phosphorylation of Cdc25A-Thr506, Cdc25C-Ser216, and Cdc2-Tyr15. Inactivation of ATR, by either incubation of cells with caffeine, a well-known but unspecific ATR/ATM inhibitor, or transfection of cells with siRNA targeting ATR, abrogated IR-induced Chk1 phosphorylation and G2/M arrest but had no effect on IR-induced ERK1/2 activation. In contrast, inhibition of ERK1/2 signaling resulted in marked attenuation in IR-induced ATR activity with little, if any, effect on IR-induced ATM activation. These results implicate IR-induced ERK1/2 activation as an important regulator of the G2/M checkpoint response to IR in tumor cells.[176] It is interesting that pharmacological inhibition of Chk1 by UCN-01 leads to activation of ERK1/2.[177] Chk1 knockdown by itself reduced basal ERK1/2 activation and antagonized the ability of UCN-01 to activate ERK1/2, implying the existence of a still unrecognized link between Chk1 and ERK1/2 signaling.[178] When UCN-1 was combined with an MEK inhibitor, compound PD184352, UCN-01-induced MAPK activation was blocked and accompanied by marked mitochondrial damage and apoptosis.[179] More recent studies showed direct interaction between another component of DNA damage signaling, BRCA1, and ERK1/2 in both nonirradiated and irradiated cells, and BRCA1 was shown to be necessary for ERK1/2 activation.[180]

In addition to ERK1/2 signaling, two other mitogen-activated protein (MAP) kinase kinases, MEK1 and MEK2 kinases, are activated by DNA damage induced by IR. Blockage of this activation, through the use of dominant negative MEK2, increased the sensitivity of cells to ionizing radiation and decreased their ability to recover from G2 arrest.[181] There are also conflicting data concerning the role of MAPKAP2 (MK2) in checkpoint control following DNA damage. MK2 shares substrate specificity with Chk1 and Chk2 and has been proposed to be involoved in S-phase and G2 arrest induced by DNA damage.[182] However, downregulation of MK2 was unable to abrogate camptothecin-, SN38-, or 5-fluorouracil-induced cell cycle arrest in the S phase, as well as doxorubicin-induced G2-phase arrest.[183,184]

To add to the complexity of intracellular signaling induced by DNA damage, activation of the MAPK signaling pathway seems to play an important role in DNA repair by homologous recombination (HRR); however, different MAPKs have divergent effects on HRR. Accordingly, signaling through ERK1/2 and JNK was shown to be an important positive regulator of HRR, whereas the p38 MAPK pathway inhibited HRR.[185] Chemical inhibition of ATM blocked radiation-induced ERK1/2 phosphorylation, suggesting that ATM directly regulates ERK1/2 signaling. Conversely, inhibition of MAP/ERK kinase signaling resulted in severely reduced levels of DNA damage foci containing phosphorylated S1981-ATM but not γ-H2AX foci.[185] Collectively, these results show that ATM-dependent signaling through the RAF/MEK/ERK pathway is critical for radiation-induced ATM activation and efficient HRR, suggestive of a regulatory feedback loop between ERK and ATM kinases.

5.2. NF-kB and DNA Damage Checkpoints

Almost all chemotherapeutic DNA-damaging agents influence NF-kB signaling by either its activation or inhibition. Activation of NF-kB was reported for doxorubicin,[186−191] camptothecin,[192−196] melphalan,[197,198] nitrosourea,[199] and etoposide.[188,192,200,201] There are also reports which show that some DNA damaging agents, such as daunorubicin, doxorubicin, and camptothecin, may actually suppress NF-kB signaling.[202,203]

Two parallel cascades are necessary for NF-kB activation by DNA damage. The first one depends on ATM and is activated by DNA double-strand breaks induced by, e.g., topoisomerase inhibitors.[193,204,205] The second cascade is dependent on a p53-induced protein with a death domain (PIDD) and is activated by an unknown stress signal. The point of convergence of these two cascades is the NF-kB essential modulator (NEMO), that is phosphorylated by ATM.[206] Surprisingly, it was recently found that ATR also

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

interacts with NEMO but does not cause its phosphorylation. Consequently, ATR repressed NF-kB activation following replication stress, induced by hydroxyurea or aphidicolin, most probably by competing with ATM for NEMO association.[207] In agreement, analysis of gene expression profiles in cells treated with two different DNA damaging drugs showed two opposing effects and hydroxyurea induced mostly pro-apoptotic, whereas etoposide promoted expression of antiapoptotic genes, with different biological outcomes. Thus, ATM and ATR may regulate the balance between these opposing signals during activation and maintenance of different DNA damage checkpoints.

5.3. Survival Signaling during G2-M

An important survival signaling during G2 and mitosis is associated with survivin. It was evidenced that tumor cells exposed to DNA damage counteract cell death by releasing the antiapoptotic protein survivin from mitochondria, in a process that involves activated Chk2.[208] In addition, survivin acts in concert with Aurora B and Plk-1 during mitosis to block mitotic cell death.[209,210] This is especially important in the situation when DNA damage activates mitotic checkpoints or the G2 checkpoint is not fully functional and cells with damaged genomes progress from G2 into mitosis. Expression of survivin is regulated in a p53-dependent fashion,[211,212] and DNA damage downregulates survivin in cells with a functional p53 pathway and upregulates it when p53 is mutated.[213] More importantly, survivin expression is frequently deregulated in tumor cells,[172] that can greatly influence the sensitivity of these cells to DNA damaging agents.

Another important antiapoptotic signaling, that may influence both checkpoint response and cell survival during G2 and M, is regulated by the PI3K/PKB/Akt pathway. However, conflicting data have been reported concerning the role of PKB/Akt in the G2 checkpoint induced by DNA damaging agents. Akt has been shown to phosphorylate the Wee1 kinase, resulting in inhibitory phosphorylation of Cdk1, thus blocking G2/M progression.[214] In contrast, it has also been reported that activated Akt shortens the G2/M arrest induced by DNA damage.[215,216] The important function of the PI3K/Akt signaling is to protect cells during mitosis from cell death, since chemical inhibition of PI3K/Akt is accompanied by mitotic catastrophe.[217] This protective effect of PI3K/Akt operates also in tumor cells undergoing long-term G2 arrest following treatment with DNA damaging drugs such as cisplatin. It was shown that chemical inhibition of PI3K/Akt in G2-arrested myeloid leukemia cells by wortmannin or LY294002 strongly enhanced the cytotoxicity of cisplatin, without influencing the G2 checkpoint.[218]

There is also a direct link between the ATM/ATR and PI3K/Akt pathways, as it was shown that Chk1 is a substrate of Akt kinase.[219] When Chk1 protein is phosphorylated by Akt on serine 280, it does not enter into protein complexes after replication arrest and fails to undergo activating phosphorylation on serine 345 by ATM/ATR. Reciprocal regulation of Akt by the ATM/ATR pathway has also been observed, and full activation of this kinase following DNA damage is mediated by ATM.[220] Moreover, overexpression of ATM greatly enhanced phosphorylation of Akt at Ser473 but had little effect on the phosphorylation at Thr308. The ATM-induced Akt phosphorylation at Ser473 was blocked by the PI3K inhibitors LY-294002 and wortmannin as well as by siRNA for ATM.[217] A recent study showed that PKB/Akt could also be activated by DNA damage induced by Temozolomide in a ATR-dependent fashion.[221]

5.4. DNA Damage Checkpoints and DNA Repair Pathways

Results of numerous studies show that some of the checkpoint signaling regulators and proteins involved in DNA repair functionally interact with each other. For example, Chk1 binds and phosphorylates the essential recombination repair protein Rad51 and Chk1-depleted cells fail to activate the homologous recombination repair system.[222] Furthermore, Chk1 phosphorylates RPA at stalled replication forks that enable its dissociation from ssDNA and subsequent formation of Rad51 repair foci.[223]

Several reports show the important role of the mismatch repair system (MMR) pathway in the activation of S-phase and G2 cell cycle checkpoints. It was evidenced that cells defective in MMR show tolerance to DNA damage, the so-called radio-resistant DNA synthesis (RDS), suggestive of defects in S-phase checkpoint activation.[224] In cells with defective MMR, activation of ATM occurred normally but the activation of the checkpoint kinase Chk2 and subsequent degradation of Cdc25A was abrogated. Further studies showed that two MMR regulators, MSH2 and MLH1, bind to Chk2 and ATM, respectively. In this way, the MMR complex formed at the DNA damage sites facilitates activation of Chk2 by ATM,[224] and possibly also Chk1, in an ATR-dependent manner.[225] MLH1 and MSH2 have also been implicated in G2 arrest induced by IR or antimetabolites.[224,226] The two checkpoint kinases have different functions, and phospho Ser317-Chk1 is involved in a MMR-mediated G2 arrest induced by 6-thioguanine (6-TG), whereas phospho Thr68-Chk2 has been postulated to be involved in a subsequent tetraploid G1-S checkpoint.[227] The possible function of MLH1 could be either proofreading or sensing of DNA lesions[228] or may directly function in DNA damage-induced G2 arrest, by, e.g., influencing Cdk1 signaling during G2 through p38 kinase.[226,229]

The relationship between MMR status and DNA damage checkpoints is particularly important, since loss of DNA mismatch repair is observed in a variety of human cancers. It is also of concern, as it may directly or indirectly induce cellular resistance to chemotherapeutic agents. First, MMR defects may impair the ability of the cell to detect DNA lesions and activate apoptosis, or second, these MMR defects increase the mutation rate in the genome and thereby promote drug resistance (for a review, see ref 230). A classical example is platinum drugs, for which a functional MMR system is required to detect DNA adducts formed by, e.g., cisplatin and carboplatin. It leads to activation of the so-called futile DNA repair and eventually cell death.[231] When MMR is deficient, platinum adducts are rapidly recognized/repaired by other DNA repair systems and cells become drug resistant.[232] MMR proteins do not recognize the adducts formed by oxaliplatin, a newer platinum analogue, and the MMR repair pathway is not triggered.[233] Consequently, oxaliplatin preserves its activity toward cells with MMR defects, that are resistant to cisplatin and carboplatin.

It should be noted that MMR deficiency does not always lead to drugs resistance. For example, cells with defects in MMR are resistant to alkylating agents such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), 6-TG, or Temozolomide.[234,235] Other studies have shown that MMR nonfunctionality leads to an increased cytotoxic effect of camptoth-

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

ecin and etoposide;[236,237] however, treatment of MMR-deficient cells with high-dose IR produced a similar cytotoxic effect to that of MMR-proficient cells.[226] This apparent discrepancy between the effect of MMR deficiency and the cytotoxic effect of antitumor agents has recently been clarified, at least for drugs that induce DNA–DNA cross-links, such as psolaren. These studies showed that MSH2 was involved in error-free repair of DNA–DNA cross-links whereas MLH1 participates in Chk1-dependent G2 checkpoint activation following treatment with psolaren + UVA.[238] Consequently, MSH2-defective cells were more sensitive to DNA–DNA cross-links but MLH1-deficient cells were more resistant to these lesions.

Finally, DNA damage checkpoint proteins may directly interact with components of the BER repair pathway. It was shown that the BER scaffold protein XRCC1 forms complexes with and is phosphorylated by ATM-activated Chk2. This seems to increase interaction between XRCC1 and glycosylases, but not other BER proteins,[239] and may promote repair of DNA lesions by BER. Accordingly, XRCC1-deficient cells are hypersensitive to alkylating agent MMS.[240]

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

6. Defects in DNA Damage Checkpoints in Tumor Cells

There are many studies describing defects in checkpoint signaling in tumor cells *in vitro*; relatively much less attention has been devoted to the *in vivo* situation, especially changes in the expression/functionality of different checkpoint regulators in tumor cells from cancer patients. Even less is known about the correlation between the functionality of checkpoint signaling in clinical tumor samples and the efficacy of anticancer therapies using DNA damaging drugs. The situation is further complicated by the fact that many of the DNA damage checkpoint regulators are involved in both DNA damage signaling and DNA repair. For this reason, it is not always clear whether the changed response to anticancer treatment of human tumors in patients is related to defects in DNA damage checkpoint functionality in tumor cells or noneffective repair of DNA lesions induced by anticancer treatment. However, this fundamental aspect could be less important from the clinical perspective, where increased activity and selectivity of DNA damaging drugs toward tumor cells is expected, regardless of whether it is associated with defective cell cycle checkpoints or nonefficient DNA repair.

6.1. Defects in DNA Damage Sensing and Signaling Pathways of the Cell Cycle Checkpoint Response in Tumor Cells

Tumor cells have usually defective expression or activity of sensing kinases, in particular ATM and BRCA1/2. About 1% of the general human population is estimated to carry heterozygotic mutations in the *ATM* gene. Reduced or absent expression of ATM has been documented for breast cancer, where it occurs in about 30–50% of invasive breast carcinomas (for review, see ref 241). Moreover, the study of p53 status in some of these tumors has revealed that the ATM/p53 signaling pathway is frequently altered by either a very low ATM expression or mutation in the *P53* gene or both.[242] Reduced ATM expression observed, for example, in breast cancer is frequently due to epigenetic silencing by methylation of the gene promoter.[243] Low levels of ATM are sometimes observed in tumor cells with decreased DNA-PK expression. This might result from a cross-regulation of gene expression between ATM and the catalytic subunit of DNA-PK, since downregulation of the latter protein leads to low ATM levels.[244] In some tumors, such as malignant melanomas, increased expression of ATM is observed.[245]

No deleterious mutations were identified in the *ATR* gene in the human population, although haplotype analysis showed the *ATR* gene polymorphisms. In addition, none of the mutated variants of the *ATR* gene found in breast/ovarian families from different regions support a major role for ATR mutations in hereditary susceptibility to breast and ovarian cancer.[246,247] No information could be found in the literature whether ATR mutations are related to the susceptibility of tumors to anticancer therapy.

Other checkpoint signaling kinases BRCA1/2 are also frequently mutated in many different ethnic populations (for a review, see ref 248). Tumors that arise in individuals carrying BRCA1 or BRCA2 germline mutations usually show loss of the wild-type allele (loss of heterozygosity), and the remaining mutated gene encodes for proteins which are defective in checkpoint signaling and/or DNA repair. In about 5% of breast and ovarian cancers, germline mutations in *BRCA1* and *BRCA2* are observed (for a review, see ref 249). In parallel, somatic mutations in *BRCA1* or *BRCA2* genes are also infrequently detected in sporadic (nonfamilial) breast cancers.[250] Mutational analysis of BRCA1/2 is usually carried out to estimate the potential risk for breast and ovarian cancers but also to relate specific mutations with the clinicopathological characteristics of these tumors in cancer patients. However, much less is known about the potential impact of different mutant forms of BRCA1/2 on tumor sensitivity to DNA damaging agents in cancer patients.

Even in the absence of BRCA1 mutations, a significant fraction of breast and ovarian cancers expresses low levels of BRCA1 protein, mostly due to hypermethylation of BRCA1 promoter. It occurs with 10–30% frequency in various nonfamilial breast tumors[251] and 15–31% ovarian carcinomas.[252,253] Low expression levels of BRCA1 in ovarian cancer patients are often associated with loss of heterozygosity in this gene.[254] In contrast, BRCA2 is generally hypomethylated and overexpressed in breast and ovarian cancers.[255] BRCA 1/2 deficiency not only leads to defects in the functionality of the intra-S checkpoint but is also associated with nonefficient DSB repair by homologous recombination.

Regulatory proteins involved in DNA repair foci formation after DNA damage which interact with checkpoint signaling kinases are also inactivated in some tumors. For example, Fanconi anemia (FA) proteins are frequently nonfunctional in pancreatic cancers as well as carcinomas of the lung, ovary, and cervix.[256] Defects in the Fanconi anemia pathway (e.g., mutations of FANCC and FANCG proteins or silencing the *FANCF* gene) render tumor cells hypersensitive to antitumor drugs inducing DNA–DNA cross-links, such as mitomycin C, cisplatin, and nitrosoureas. Current efforts are aimed at identifying compounds that inhibit the FA pathway in tumor cells that could chemosensitize FA-competent tumor cells to commonly used DNA cross-linking drugs.[257] Similarly, functional deficiency of the MRN complex in tumor cells has been reported, for example in three of seven colon carcinoma cell lines of the NCI Anticancer Drug Screen.[258] Activation of Chk2 was also defective in three of four MRN-proficient colorectal cell lines because of low Chk2 levels.

There are conflicting data about genetic and functional changes of checkpoint signaling kinases Chk1/2 in human tumors. Mutations in Chk1 are observed in both sporadic and hereditary colorectal cancers.[259] Another study reported that expression of Chk1 and Chk2 and their phosphorylated, i.e. active, forms (pChk1, pChk2) is downregulated in about 50% colon cancers,[260] whereas mutations of Chk2 are rare in sporadic osteosarcoma, breast, lung, and ovarian cancers.[261] In some tumors, there is a significant correlation between p53 status and Chk1/2 expression; for example, a majority (>70%) of gastric tumors with high levels of Chk1 and Chk2 expression also had mutated p53.[262] There are also the BRCA1-associated cancers with Chk2 mutations which, in addition, contain mutations in p53.[263]

Multiethnic studies revealed that Chk2 mutations occur with a low frequency of about 0.5% and are associated with a moderate risk factor for breast and prostate cancer.[264−267] Different variants of Chk2 mutants have been observed with large germline deletions (del5395) or truncating mutations (IVS2+1G>A and 1100delC) as well as missense variants (I157T and H143Y).[267,268] In vitro studies revealed that expression of some variants (e.g., R145W or H143Y) resulted in gross protein destabilization whereas others (e.g., 1100del, D347A, or D438Y) had greatly suppressed *in vitro* kinase activity.[266] At least some of these genetic Chk2 changes may directly influence the sensitivity of breast and prostate cancers in patients.

6.2. Deregulation of Checkpoint Maintenance Mechanisms in Tumor Cells

The most extensively studied component of the maintenance mechanism of G1/S and G2 checkpoints induced by DNA damage is p53. Mutation of p53 is the most frequently observed genetic lesion in human tumors (for most recent information see http://www-p53.iarc.fr). Mutations of p53 usually lead to inactivation of p53 function. However, specific p53 mutations could change the expression of checkpoint regulatory proteins, especially these that produce gain-of-function alterations in p53 protein. There is at least one example of mouse cells carrying common p53 cancer mutations (R248W and R273H) which have impaired the G2 checkpoint induced by DNA damage. Detailed studies on the molecular mechanism of this phenomenon revealed that these mutant p53 proteins interacted with Mre11 nuclease and suppressed the binding of the MRN complex to DNA double-stranded breaks, leading to impaired ATM activation.[266] Our own results confirm that human colon carcinoma HT-29 cells, expressing R273H mutant p53 protein, are able to activate the G2 checkpoint following DNA damage but are defective in maintaining the G2 arrest, progress into mitosis with damaged DNA, and undergo mitotic catastrophe.[269]

In addition, in human tumors in which the *P53* gene is not mutated, the p53 function may be disrupted by alterations in cellular proteins that modulate the expression, localization, and activity of the protein. For example, during normal cellular growth, Mdm2 binds p53 and targets it for ubiquitin-mediated degradation (for a review, see ref 270). The *Mdm2* gene has been shown to be up-regulated in human tumors and tumor cell lines by gene amplification, increased transcript levels, and enhanced translation. A detailed study using about 4000 samples from tumors or xenografts of 28 different tumor types showed that, in about 7% of human tumors, mostly with wild-type p53, the *Mdm2* gene was amplified, with the highest frequency observed in soft tissue tumors (20%), osteosarcomas (16%), and esophageal carcinomas (13%).[271] Alternatively, HPV-encoded E6 protein binds p53 and targets it for ubiquitin-mediated degradation, thus abrogating p53-dependent signaling.[272] This mechanism of p53 inactivation is characteristic for cervical carcinomas (for a recent review, see ref 273).

Inactivation of p53 functions leads to a general defect in the G1/S checkpoint response to DNA damage and partial inactivation of the G2 checkpoint, at least its p53-dependent branches. It is believed that these two defects in both G1/S and G2 checkpoint functionality explain higher sensitivity of tumors to DNA damaging agents. The situation is of course much more complex, as p53 is also involved in the activation of cell death pathways and, in many tumors, inactivation of p53 may lead to drug resistance. In this way, checkpoint functionality in tumor cells with inactive p53 contributes to a delicate balance between sensitivity and resistance to antitumor therapies based on genotoxic drugs.

6.3. Defects of Effector and Regulatory Proteins Involved in the DNA Damage Checkpoint Response

Deregulation of the intracellular protein levels of checkpoint effector and regulatory proteins is frequently observed in many different tumors. For example, expression levels of cyclin-dependent kinases cdks are elevated in many tumor cells (for a review, see ref 274). This may greatly influence the stringency of DNA damage checkpoints. Reduced background expression levels of cdk inhibitor p21 are also observed, e.g., in a majority of melanoma cell lines.[275] This could be associated with increased sensitivity of p21-deficient cells to DNA alkylators, as observed in *in vitro* tumor models.[276] The expression of 14-3-3σ is down-regulated by its gene promoter methylation in several types of human cancer, among them prostate, lung, breast, and several types of skin cancer (for a review, see ref 277). Alternatively, proteolytic inactivation of 14-3-3σ has been found in breast and prostate cancers. Interestingly, in breast cancer, the estrogen-responsive E3 ubiquitin ligase Efp specifically targets 14-3-3σ for degradation.[278] In agreement, cellular levels of 14-3-3σ increase with malignant tumor progression in endometrial carcinoma, and this is inversely correlated with estrogen receptor α and the progesterone receptor.[279] A similar situation may occur after antihormone therapy of hormone-dependent breast tumors, and this effect may lead not only to hormone independence of tumor cells but also to their resistance to DNA damaging drugs. Changes in the expression of different DNA damage checkpoint regulators are frequently correlated with tumor grade, e.g., of breast carcinomas.[280]

6.4. Other Specific Changes in the DNA Damage Checkpoint Response in Tumor Cells

Some specific defects in DNA damage checkpoint regulation in tumor cells have been reported. Deficiency of the decatenation checkpoint has been observed in small lung cancer cells *in vitro*, and it was independent of the impaired DNA damage G2 checkpoint. It was postulated that the nonfunctional decatenation G2 checkpoint is implicated in the development of human lung cancers but can also be responsible for selective killing of lung cancer cells with such defects by catalytic inhibitors of topoisomerase II.[281] Un-

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

fortunately, no specific molecular changes have been reported in relation to inactivation of the decatenation checkpoint in these cells. Other *in vitro* studies showed that transitional bladder carcinomas had severe defects in the decatenation checkpoint,[282] again with no molecular mechanism provided for these defects.

6.5. Cancer Stem Cells and DNA Damage Checkpoint Defects

There is growing evidence that tumors contain a small fraction of cells, with stem cell-like properties, called cancer stem cells (CSCs) or cancer initiating cells, that maintain the heterogeneity of the tumor and appear to be resistant to apoptosis induced by therapeutic agents (for a review, see ref 283). The existence of CSCs may explain why standard anticancer treatment, that frequently successfully eliminates differentiated cancer cells, does not lead to cancer cure.[284−286] The cancer stem cell hypothesis has recently created great interest but also many controversies,[287] mostly due to the fact that the biology of CSCs and their importance for tumor sensitivity to anticancer treatment are still largely unknown.

Interestingly, some of the DNA damage checkpoints are not functional in stem cells and possibly also in CSCs. For example, it has been reported that the decatenation checkpoint is not functional in stem cells,[288] with no data available so far on whether a similar effect is observed for CSCs. There are also reasons to believe that other components of the DNA damage response are changed in CSCs, due to deregulated induction of p53 and ATM/ATR pathways or defects in cell cycle checkpoint control, as reported for embryonic stem cells or teratocarcinomas.[289,290] After treatment with antitumor drugs, dysfunctional DNA damage checkpoints may enable cancer stem cells, to survive and produce cell progeny with modified/unstable genomes. This could lead either to increased cell killing or to genetic changes and drug resistance. If changes in the functionality of DNA damage checkpoints observed in stem cells are confirmed in CSCs, this could explain the resistance of CSCs to standard DNA damaging agents, such as cisplatin, that has been recently observed in a mouse mammary tumor model.[291]

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

7. DNA Damage Checkpoint Functionality and Sensitivity of Tumors to DNA Damaging Agents

Numerous studies have shown defects in the expression or activity of different DNA damage checkpoint regulators in tumor cells. However, the question remains if and how changed functionality and/or expression of checkpoint regulators translates into differential sensitivity of tumor cells to anticancer agents that induce DNA damage, especially in cancer patients.

Lack of normal ATM function in the inherited ataxia-telangiectasia (A-T) syndrome patients results in profound hypersensitivity to genotoxic agents. Much less is known about the drug sensitivity of ATM-deficient tumors in cancer patients. Additionally, increased levels and activity of ATM have been observed in some tumors, but it is not clear whether this leads to drug resistance.[245] The possible resistance phenotype of tumor cells overexpressing ATM can be inferred from the fact that in nonsmall lung cancers increased expression of ATM is associated with a notably shorter median survival than that in patients with low levels of these checkpoint regulators.[292] It should also be mentioned that the function of the ATM kinase declines significantly with age, at least in mice, that may then be responsible for the decline of the p53 response to γ-irradiation observed in aged subjects.[293] The importance of ATM functionality for the sensitivity of tumor cells to DNA damage is probably more complex, as recent studies have shown the existence of the redundant G2 checkpoint mechanism in cells with inactivated ATM, that is regulated by DNA-PK.[294] This suggests that very specific ATM inhibitors, with no effect on other PIKK kinases, may be, in fact, less efficient in sensitizing tumor cells to genotoxic drugs than the relatively less specific abrogator, that is able to suppress the activity of both ATM and DNA-PK.

BRCA-deficient breast and ovarian cancers are generally more sensitive to drugs that produce single- and double-stranded lesions in DNA, such as mitomycin C, cisplatin, topotecan, doxorubicin, etoposide, and others (for a review, see ref 295). This is particularly well-documented for cisplatin-based therapies in cancer patients. For example, retrospective analysis of clinical and histopatological data showed that BRCA heterozygotes had a better response to platinum chemotherapy, compared with women who had sporadic disease with no BRCA mutations.[296] Recent clinical data confirmed that cisplatin-based neoadjuvant therapy, followed by mastectomy and conventional chemotherapy, is effective in a higher proportion in patients with BRCA1-associated breast cancers.[297] Conversely, suppression of BRCA1 levels is associated with marked resistance of breast and ovarian tumors to the taxanes and navelbine (for a review, see ref 298). This observation supports the idea that BRCA1 plays an important role not only in the DNA damage checkpoint but also in the mitotic spindle assembly checkpoint.[299] Finally, the relationship between tumor sensitivity to DNA damaging drugs and BRCA1/2 functionality has been confirmed by recent studies on the molecular mechanism of acquired resistance to platinum analogues in tumors carrying frame-shift BRCA1/2 mutations. These studies have shown that cisplatin resistance in these tumors originates from restored BRCA1/2 expression, as a result of a secondary intragenic mutations that corrected the open reading frames of mutated BRCA1/2.[300−302] This mechanism may explain why the majority of BRCA1/2-deficient cancers become resistant to DNA damaging agents during anticancer treatment and patients frequently die from refractory diseases.

So far, no systematic studies have been performed which show that Chk1 and Chk2 mutations change tumor sensitivity to anticancer treatment with DNA damaging agents in human patients. All published data revealed a strong correlation between Chk1/2 mutations and predisposition to different cancers (discussed in section 6.1). However, the same genetic data could be analyzed retrospectively to find out if cancer patients with specific Chk1/2 mutations respond differentially to DNA damaging drugs.

8. Chemical Inhibition and Modulation of the DNA Damage Checkpoint Signaling and Sensitization of Tumor Cells to Anticancer Drugs

As mentioned previously, experimental data, mostly from the *in vitro* studies, showed that tumor cells with defects in checkpoint pathways have frequently increased sensitivity to antitumor agents, which induce DNA damage. Moreover, genetic studies suggested that drug resistance may be associated with an increased ability to arrest cell cycle progression after genotoxic stress, especially in the G2 phase,

that promotes more efficient repair of drug-induced DNA lesions.[303] Accordingly, it was postulated that, by developing strategies to attenuate or disable checkpoint functionality in tumor cells, one could sensitize these cells to antitumor agents. This, of course, was only possible after a sufficient knowledge had been accumulated about molecular mechanisms, which regulate the induction and maintenance of DNA damage checkpoints. In this way, using chemical inhibitors of checkpoint regulators, in combination with DNA damaging agents, we could mimic inherent defects in checkpoint control observed in many tumor cells and increase the efficacy of anticancer therapies based on genotoxic drugs.

Generally, two basic approaches have been proposed to abrogate or modulate DNA damage checkpoints to sensitize tumor cells to DNA damaging agents. The first is based on suppressing the p53-independent checkpoint mechanisms by chemical inhibition of different checkpoint kinases; the other could target its p53-dependent components. In addition to that, inhibition of different enzymes involved in the regulation of cell cycle machinery is also considered, mainly because the intracellular levels and activity of these proteins are frequently deregulated in tumor cells. Targeting of the DNA damage checkpoint pathway by chemical inhibitors involves three strategies, which are aimed at the following:

(1) inactivation of DNA damage sensing kinases, currently limited to chemical inhibitors of ATM and to some extent DNA-PK;
(2) inhibition of signaling kinases, mostly Chk1, Chk2, and Plk1;
(3) inhibition of effector and regulatory proteins such as different cyclin-dependent kinases Cdks, phosphatases (cdc25A/B/C), or p53.

The rationale for DNA damage checkpoint abrogation as a way to sensitize tumor cells to anticancer treatment is that these cells have frequently inactivated the DNA damage checkpoint in G1, largely due to a deficiency of p53 or pRB. Thus, these tumor cells solely rely on maintaining S or G2 arrest in response to DNA damage, whereas normal cells have a functional G1 arrest mechanism conferred by the p53 pathway. Consequently, inhibition of the S- and G2-checkpoints in p53-deficient tumor cells would drive them to cell death by, e.g., mitotic catastrophe, but normal cells would be less affected due to the existence of p53-mediated G1 arrest or more stringent arrest in G2. Many different *in vitro* studies have indeed shown that tumor cells treated with a combination of DNA damaging drugs and checkpoint abrogators, such as caffeine or UCN-01, are sensitized to a greater extent if they have an inactivated p53 pathway.[304,305] However, this intellectually elegant concept has never been conclusively proved in the clinical setting. Moreover, other studies did not confirm an increased cell killing effect of DNA damaging drugs by checkpoint abrogation in p53-deficient cells, and even the whole concept has been challenged.[306,307]

8.1. Chemical Inhibitors and Modulators of the DNA Damage Checkpoint Regulatory Proteins—Activity and Specificity

Several checkpoint abrogators and modulators, including broadly unspecific inhibitors of ATM (pentoxyfylline) as well as checkpoint kinases Chk1/2, Cdks, and Plk1, have entered clinical trials (see Figures 6 and 7 for chemical structures and Table 1 for details). Many more compounds are in preclinical studies (for chemical structures of exemplary inhibitors, see Figures 6 and 8). It is interesting to note that a wide diversity of chemical structures serves as a basis for the development of new checkpoint abrogators and modulators. These include analogues of natural compounds such as staurosporin (e.g., UCN-01),[308] methylxanthines (pentoxyfylline),[309] flavones (flavopiridol),[310] or purines (roscovitine),[311] but also synthetic derivatives such as modified peptides (CBP501),[312] diazapinoindolones (PF-477736),[313] and macrocyclic urea analogues,[314] to name just a few exemplary compounds. It is also worth mentioning that searching for inhibitors of checkpoint kinases or Cdks leads to the development of very unspecific compounds, with inhibitory activity toward many different substrates, very often not even related to checkpoint regulation. For example, roscovitine, that was developed as a "specific inhibitor of cyclin-dependent kinases", showed inhibitory activity toward more than 150 different enzymes.[315] UCN-01 can be another example of a checkpoint abrogator that was reported to be a relatively selective inhibitor of Chk1 and cTak1 kinases.[316,317] However, initial studies identified UCN-01 as a protein kinase C inhibitor, and further studies showed that it targets also Cdks and PDK1 kinase.[318] It should be noted that, despite many possible targets of UCN-01, the only documented mechanism that is responsible for the abrogation of the DNA damage checkpoint is its inhibitory activity toward Chk1.

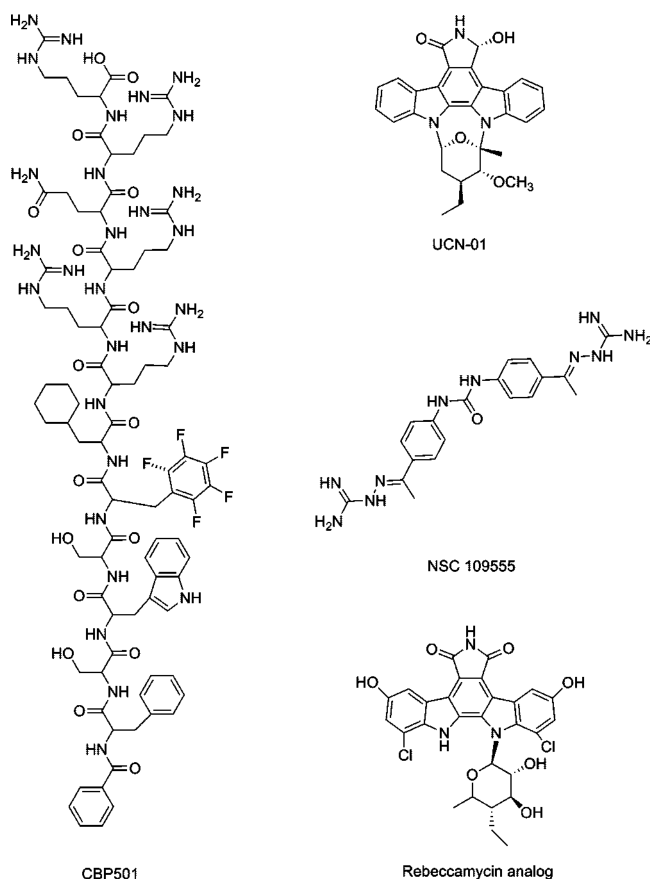


Figure 6. Chemical structures of inhibitors of checkpoint signaling kinases, which are currently under preclinical development or undergoing clinical trials (see Table 1 and www.ClinicalTrials.gov for details).

Finally, for some inhibitors it is their general toxicity, and not checkpoint abrogation potential, that limits their clinical

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

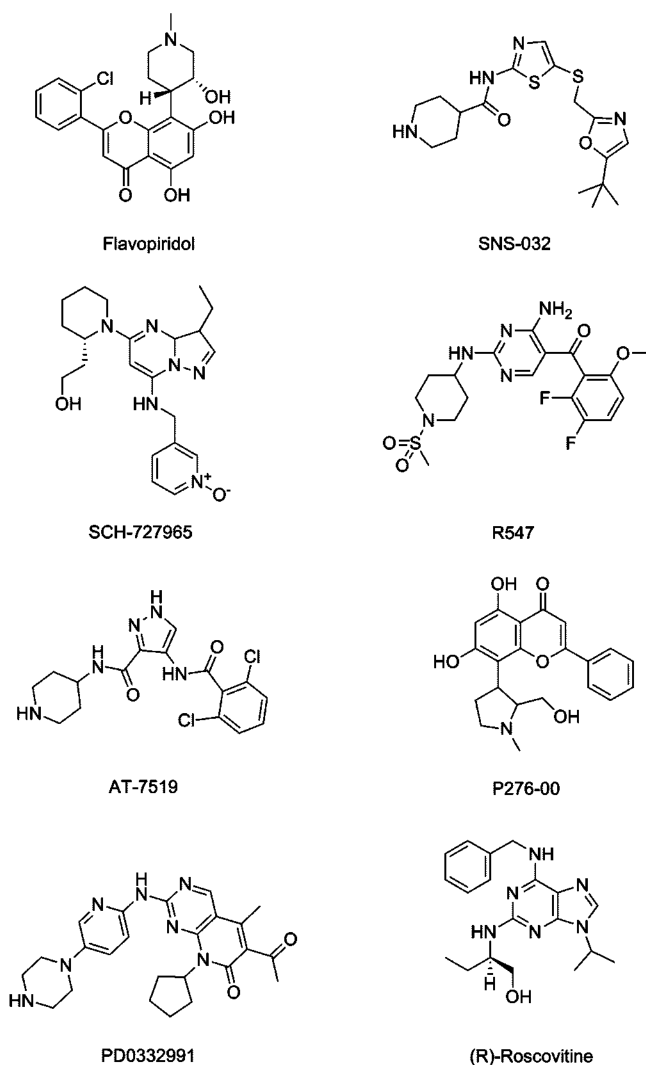


Figure 7. Chemical structures of inhibitors of cyclin-dependent kinases, which are currently undergoing clinical trials (see Table 1 for details).

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

application. Pentoxyfylline could serve here as an example of a less toxic caffeine analogue, that is currently undergoing clinical trials in combination with DNA damaging agents (radiation, mitoxantrone, and cytarabine), despite the fact that it has many different cellular targets, from adenosine receptors to PIKK kinases (for a recent review, see ref 319).

8.2. Checkpoint Inhibitors and Modulators—Current Developments and Perspectives

Several checkpoint abrogators and modulators are active alone (see Table 1 and www.ClinicalTrials.gov for details); for example, flavopiridol showed high clinical activity toward refractory chronic lymphocytic leukemia.[320] Most checkpoint inhibitors are also being tested in combination with many different DNA damaging drugs, including antimetabolites (5-fluorouracil, cytarabine, gemcitabine), topoisomerase inhibitors (doxorubicin, topotecan, and irinotecan), or platinum drugs (cisplatin and carboplatin). Although many clinical trials with different drugs and checkpoint modulators are still under way, some disclosed results are rather disappointing. A phase II trial with topotecan and UCN-01 used in combination toward advanced ovarian carcinomas showed no significant antitumor activity.[321] Similarly, alvocidib (flavopiridol) and seliciclib (roscovitine) are in late-stage clinical trials; however, so far both checkpoint modulators have demonstrated only modest activity (for a recent review, see ref 322). One possibility could be that the synergistic effect of checkpoint modulators used in combination with DNA damaging drugs is obscured by their relatively high toxicity. Many second-generation inhibitors of Chk2 kinase or Cdc25 phosphatases, such as compounds BN82002 and NSC109555[323,324] (see Figures 6 and 8 for the chemical structures), are in preclinical studies and will hopefully show a better therapeutic effect than their forerunners.

Chemical inhibitors of ATM and other PIKK kinases could lead to an increased antitumor effect of DNA damaging drugs toward tumor cells. The recent advent of more specific ATM inhibitors, CGK733, KU55933, and compound CP466722 (see Figure 8 for chemical structures), made it possible to show that even transient inhibition of ATM is sufficient to potentiate the effect of ionizing radiation.[325−327] These results show that specific ATM inhibitors, as part of combination regimens with DNA damaging agents, may be used in clinical practice. In addition, cells with defects in PARP-1 activity, an enzyme involved in DNA damage signaling and repair,[328] are hypersensitive to ATM inhibitors.[325] Conversely, cells with nonfunctional ATM or BRCA1/2 are extremely sensitive to PARP-1 inhibitors.[325,329,330] Based on these studies, a very promising therapeutic approach has been proposed where recombination repair deficient cancers may be selectively eradicated by chemical inhibitors of PARP-1.

Finally, a novel direction has recently emerged, where in the same chemical entity one could combine DNA damaging activity and inhibitory action toward checkpoint kinases, as exemplified by new derivatives of a structural analogue of staurosporine, rebeccamycin. Rebeccamycin is an inhibitor of DNA topoisomerase I; however, its new analogues have been developed which showed a potent inhibitory activity toward Chk1. Interestingly, some of these compounds retained a genotoxic activity either through intercalation into the DNA and/or by topoisomerase I-mediated DNA cleavage (see Figure 6 for the chemical structure of the rebeccamycin analogue).[331] Therefore, rebeccamycin derivatives may represent a novel class of potential antitumor agents, that have a dual effect; that is, they induce DNA damage and inhibit Chk1 DNA damage checkpoint kinase. Rebeccamycin analogues recently entered clinical trials, and it will be interesting to see whether such compounds show comparable or higher antitumor effects as combinations of DNA damaging agents and checkpoint abrogators.

9. Concluding Remarks and Perspectives

Many cancer cells have defects in checkpoint control mechanisms induced by DNA damage. These defects include not only changed expression or activity of checkpoint regulators but also inactivation of the p53 pathway. This leads to two opposing effects, that is, reduced DNA repair capacity due to inability to sustain DNA damage-induced arrest in S and G2 phases but at the same time to attenuation of G1/S and G2 checkpoints as well as to lower propensity to undergo cell death, e.g., by apoptosis. Defects in checkpoint response initiated by DNA damage are also responsible, at least in some situations, for higher efficiency of antitumor drugs toward tumor cells.

During the past decade, an enormous amount of data was accumulated about the basic mechanisms responsible for

Table 1. Chemical Checkpoint Abrogators and Modulators Undergoing Clinical Trials

checkpoint mechanism	compound	proposed target(s)	clinical trials	ref
DNA damage sensing kinases	pentoxyfylline	ATM/ATR/DNA-PK	in combination, phase I	309
signaling kinases	UCN-01	Chk1	in combination, phase I/II	308
	CBP501	MK2/cTak1/Chk1	alone and in combination, phase I/II	312
	XL844	Chk1/Chk2	alone and in combination, phase I	341
	AZD7762	Chk1	in combination, phase I	342
	PF477736	Chk1	in combination, phase I	313
	BI 2536	Plk1	alone, phase I/II	343
	ON01910	Plk1	alone and in combination, phase I/II	344
	GSK461364	Plk1	alone, phase I	undisclosed
effector proteins	alvocidib (flavopiridol)	Cdks	alone and in combination, phase I/II	310
	seliciclib (roscovitine)	Cdks	alone, phase II	311
	PD-0332991	Cdks	alone and in combination, phase I/II	345
	P1446A-05	Cdks	alone, phase I	undisclosed
	P276−00	Cdks	alone, phase I/II	346
	R547	Cdks	alone, phase I	347
	BMS-387032/SNS-032	Cdks	alone, phase I	348
	SCH-727965	Cdks	alone and in combination, phase I/II	349
	AT7519	Cdks	alone, phase I	350

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

induction and maintenance of DNA damage checkpoints. With this knowledge, new therapeutic strategies were developed which exploit defects in cell cycle checkpoint responses to enhance the efficacy of antitumor DNA damaging agents. Two approaches are currently explored: (i) specific targeting of tumor cells with enhanced sensitivity to DNA damaging drugs by selection of patients with defects in checkpoint control; (ii) applying new therapeutic regimens, where specific chemical abrogators or modulators of checkpoint control are used in combination with standard DNA damaging drugs.

9.1. Possible Adverse Effects of DNA Damage Checkpoint-Targeted Chemotherapy

There are some concerns about the use of checkpoint abrogating compounds in the treatment of cancer patients. One possible problem could be that checkpoint regulators play an important role in DNA repair in nonstressed cells, for example during DNA replication. The fundamental role of Chk1 kinase during the normal S phase is to avoid aberrantly increased initiation of DNA replication and DNA breakage, even in the absence of genotoxic stress, that was confirmed by the early embryonic lethality of Chk1−/− mice.[332] Consequently, inhibition of Chk1 kinase during anticancer treatment, which is *per definition* not specific to tumor cells, may lead to accumulation of DNA lesions in normal cells and, in this way, promote secondary tumors. In contrast, Chk2-deficient cells from Chk2−/− mice have an intact DNA damage response and, surprisingly, are resistant to ionizing irradiation.[333] This suggests that selective inhibition of Chk2 during, e.g., radiotherapy may be used to protect normal cells in sensitive tissues from the toxic effects of DNA damage induced by irradiation and possibly other DNA damaging agents. Another effect that could be envisaged as a result of combined treatment with DNA damaging agents and checkpoint abrogators is possible potentiation of the mutagenic effects of antitumor drugs that induce genotoxic stress.

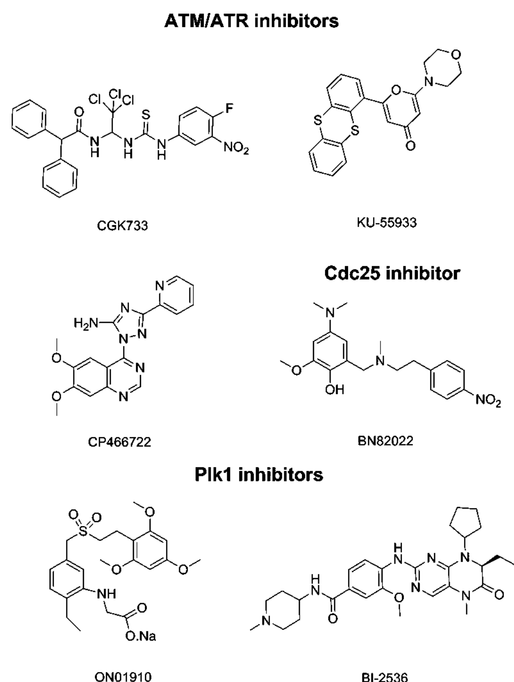


Figure 8. Chemical structures of several inhibitors of DNA damage signaling pathways, which are currently under preclinical development (inhibitors of ATM, ATR, and Cdc25 phosphatases) or are undergoing clinical trials (inhibitors of Plk1 kinase).

9.2. Future Directions in DNA Damage Checkpoint-Targeted Chemotherapy

There are important questions which still remain largely unanswered and concern rational drug design of new DNA damaging agents as well as new and more specific DNA damage checkpoint abrogators or modulators, which include the following:

(1) Are there any DNA lesions which are more effective in activating specific types of checkpoint response in tumor cells compared to others?

(2) Is inhibition of particular DNA damage checkpoint pathways more specific and toxic to certain tumor cells compared to others?

(3) Is a particular combination of a DNA damaging agent/drug, which induces specific DNA lesions, and a given checkpoint abrogator more efficient in inducing cell death of tumor cells?

If answers to these questions were known, they would provide important directions for medicinal chemists of which of very many types of DNA damaging compounds should be developed and which DNA damaging drug-checkpoint abrogator combinations are more effective and specific in killing tumor cells in patients.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

Several other aspects of the checkpoint response activated by DNA damage still await further clarification. For example, a considerable amount of data has been accumulated about different mechanisms involved in the DNA damage checkpoint signaling and maintenance. In contrast, still relatively little is known about checkpoint termination signals (for a recent review, see ref 111). It could be envisaged that, similarly to sensitization of tumor cells to genotoxic therapies by inhibition of checkpoint activation, another approach could be equally effective, that would lead to premature termination of the DNA damage checkpoint signaling. This should also increase the sensitivity of tumor cells to DNA damaging agents by shortening the time required for repair of DNA lesions.

An alternative approach to sensitize tumor cells to DNA damage may be to use rationally designed small molecules, which specifically bind to important regulatory domains of checkpoint regulators or protein−protein interface regions. Such molecules may both inhibit checkpoint activity or correct its defects. Currently, this approach is limited to inhibition of Chk1/2 kinases[334] and reactivation of p53 functions, mostly by small peptides and synthetic low molecular weight compounds (for a recent review, see ref 335). However, it is not clear whether reactivation of p53 leads to re-establishing important functions of p53 in checkpoint maintenance or, rather, increased cytotoxic activity of chemotherapeutics is only related to its pro-apoptotic action. A peptidic inhibitor of checkpoint kinases Chk1 and Chk2, compound CBP501, was approved for clinical trials in combination with gemcitabine;[336] clinical applications of p53 reactivating compounds to treat human tumors in patients are still only planned.

Another direction is to apply the knowledge about possible differences in DNA damage checkpoint functions to target cancer stem cells by DNA damaging agents. As mentioned above (see section 6.5), data available in the literature suggest that normal stem cells and possibly also cancer stem cells have nonfunctional decatenation checkpoints[288] and may have only partially active p53-dependent checkpoint signaling.[289,290] However, our knowledge in this area is still too rudimentary to determine whether these differences can be exploited in selective targeting of these cancer-producing cells.

The problem of possible interactions between different cell cycle checkpoints is, in our opinion, still underestimated. Most, if not all, anticancer treatment regimens are based on multiple antitumor agents, where drugs activating different checkpoints are used in combination, for example antimitotics and DNA damaging agents. As was mentioned above, at least in some situations, treatment of tumor cells with genotoxic drugs not only induces DNA damage checkpoints in G1, S, and G2 but also activates the spindle checkpoint and arrest cell cycle progression in mitosis.[137] It is important to establish whether abrogation of DNA damage checkpoints in tumor cells with an inactive or partially active spindle checkpoint mechanism leads to increased cytotoxicity of DNA damaging drugs.

It should be borne in mind that, in some situations, nonfunctional G2 and mitotic checkpoints may allow cells with damaged genomes to progress through mitosis, divide, and survive. This is possible, for example, when survival signaling during the G2/M transition is hyperactivated by overexpression of antiapoptotic proteins (IAPs), such as survivin,[337] or increased acitvity of PI3/PKB kinases observed in many tumor types.[338] In the original model, it is expected that mitotic checkpoints, including those initiated in response to DNA damage, prevent mitotic exit of tumor cells with damaged DNA. If this condition is not met, relaxed mitotic checkpoint control will lead to aberrant mitotic division and result in tumor cells with modified genomes, with possibly increased proliferative potential or resistance to anticancer drugs.[339] As mentioned above, at least one component of the DNA damage response, BRCA1 kinase, which is frequently inactivated in human tumors, is also involved in the spindle checkpoint activation.[299] To avoid this problem, a multikinase targeting approach has been proposed,[340] where both cell cycle checkpoints and survival signaling pathways are inhibited. This may also lead to increased activity of DNA damaging agents, especially in cells with strong antiapoptotic signaling. Again, the situation may be more complex, since abrogation of G2 arrest induced by cisplatin in acute myeloid leukemia cells, combined with inhibition of the survival signaling associated with PI3 kinases, was actually less efficient in sensitizing tumor cells to cisplatin than inhibition of PI3K alone.[337] From this perspective, it is even more important to perceive the functioning of DNA damage checkpoints in relation to other cell cycle checkpoints, in particular those activated in G2 and M.

Finally, one of the strategies to specifically target tumor cells by DNA damaging drugs is based on the selection of patients with defects in DNA damage checkpoint control. However, analysis of the available literature data points to the importance of using functional assays with tumor samples from patients, to screen for potential defects in the functionality of checkpoint kinases. These functional assays may complement mutational and mRNA level analysis of genes coding for checkpoint signaling proteins, such as ATM, components of the MRN complex, BRCA1/2, or Chk1/2. Combined results from the two approaches could then be used in selection of patients, with specific defects in DNA damage response, who will most likely benefit from chemotherapy based on genotoxic drugs. A recent report shows that this type of analysis of the functionality of the homologous recombination pathway can be successfully used in clinical samples.[298]

10. References

(1) Elledge, S. J. *Science* **1996**, *274*, 1664.
(2) Chabner, B. A. *Cancer Chemotherapy: Principle and Practise*; JB Lippincott Co.: Philadelphia, 1990; p 341.
(3) Barker, S.; Weinfeld, M.; Murray, D. *Mutat. Res.* **2005**, *589*, 111.
(4) Cao, P. R.; McHugh, M. M.; Melendy, T.; Beerman, T. *Mol. Cancer Ther.* **2003**, *2*, 651.
(5) Larsen, A. K.; Escargueil, A. E.; Skladanowski, A. *Pharmacol. Ther.* **2003**, *99*, 167.
(6) Bonilla, P. J.; Freytag, S. O.; Lutter, L. C. *Nucleic Acids Res.* **1991**, *19*, 3965.
(7) Mondal, N.; Parvin, J. D. *Nature* **2001**, *413*, 435.
(8) Losada, A.; Hirano, T. *Curr. Biol.* **2001**, *11*, 268.
(9) Losada, A. *Biochim. Biophys. Acta* **2008**, *1786*, 41.
(10) Duvic, M.; Vu, J. *Expert Opin. Invest. Drugs* **2007**, *16*, 1111.
(11) Glaser, K. B. *Biochem. Pharmacol.* **2007**, *74*, 659.
(12) Garcia-Manero, G. *Curr. Opin. Oncol.* **2008**, *20*, 705.
(13) Mikhailov, A.; Shinohara, M.; Rieder, C. L. *J. Cell Biol.* **2004**, *166*, 517.
(14) Kaneko, Y. S.; Watanabe, N.; Morisaki, H.; Akita, H.; Fujimoto, A.; Tominaga, K.; Terasawa, M.; Tachibana, A.; Ikeda, K.; Nakanishi, M. *Oncogene* **1999**, *18*, 3673.
(15) Lukas, C.; Bartkova, J.; Latella, L.; Falck, J.; Mailand, N.; Schroeder, T.; Sehested, M.; Lukas, J.; Bartek, J. *Cancer Res.* **2001**, *61*, 4990.
(16) Jazayeri, A.; Falck, J.; Lukas, C.; Bartek, J.; Smith, G. C.; Lukas, J.; Jackson, S. P. *Nat. Cell Biol.* **2006**, *8*, 37.
(17) Brown, K. D.; Ziv, Y.; Sadanandan, S. N.; Chessa, L.; Collins, F. S.; Shiloh, Y.; Tagle, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1840.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

(18) Lakin, N. D.; Weber, P.; Stankovic, T.; Rottinghaus, S. T.; Taylor, A. M. R.; Jackson, P. *Oncogene* **1996**, *13*, 2707.
(19) Cliby, W. A.; Roberts, C. J.; Cimprich, K. A.; Stringer, C. M.; Lamb, J. R.; Schreiber, S. L.; Friend, S. H. *EMBO J.* **1998**, *17*, 159.
(20) Tibbetts, R. S.; Brumbaugh, K. M.; Williams, J. M.; Sarkaria, J. N.; Cliby, W. A.; Shieh, S. Y.; Taya, Y.; Prives, C.; Abraham, R. T. *Genes Dev.* **1999**, *13*, 152.
(21) Carson, C. T.; Schwartz, R. A.; Stracker, T. H.; Lilley, C. E.; Lee, D. V.; Weitzman, M. D. *EMBO J.* **2003**, *22*, 6610.
(22) Mochan, A.; Venere, M.; DiTullio, R. A., Jr.; Halazonetis, T. D. *Cancer Res.* **2003**, *63*, 8586.
(23) Uziel, T.; Lerenthal, Y.; Moyal, L.; Andegeko, Y.; Mittelman, L.; Shiloh, Y. *EMBO J.* **2003**, *22*, 5612.
(24) Costanzo, V.; Paull, T.; Gottesman, M.; Gautier, J. *PLoS Biol.* **2004**, *2*, E110.
(25) Horejsi, Z.; Falck, J.; Bakkenist, C. J.; Kastan, M. B.; Lukas, J.; Bartek, J. *Oncogene* **2004**, *23*, 3122.
(26) You, Z.; Chahwan, C.; Bailis, J.; Hunter, T.; Russell, P. *Mol. Cell. Biol.* **2005**, *25*, 5363.
(27) Dupre, A.; Boyer-Chatenet, L.; Gautier, J. *Nat. Struct. Mol. Biol.* **2006**, *13*, 451.
(28) Shechter, D.; Costanzo, V.; Gautier, J. *DNA Repair (Amsterdam)* **2004**, *3*, 901.
(29) Cortez, D.; Guntuku, S.; Qin, J.; Elledge, S. J. *Science* **2001**, *294*, 1713.
(30) Zou, L.; Elledge, S. J. *Science* **2003**, *300*, 1542.
(31) Zou, Y.; Liu, Y.; Wu, X.; Shell, S. M. *J. Cell Physiol.* **2006**, *208*, 267.
(32) Kumagai, A.; Dunphy, W. G. *Mol. Cell* **2000**, *6*, 839.
(33) Lee, J.; Kumagai, A.; Dunphy, W. G. *Mol. Cell* **2003**, *11*, 329.
(34) Uziel, T.; Lerenthal, Y.; Moyal, L.; Andegeko, Y.; Mittelman, L.; Shiloh, Y. *EMBO J.* **2003**, *22*, 5612.
(35) Bakkenist, C. J.; Kastan, M. B. *Nature* **2003**, *421*, 499.
(36) Kozlov, S. V.; Graham, M. E.; Peng, C.; Chen, P.; Robinson, P. J.; Lavin, M. F. *EMBO J.* **2006**, *25*, 3504.
(37) Lee, J. H.; Paull, T. T. *Science* **2005**, *308*, 551.
(38) Pellegrini, M.; Celeste, A.; Difilippantonio, S.; Guo, R.; Wang, W.; Feigenbaum, L.; Nussenzweig, A. *Nature* **2006**, *443*, 222.
(39) Tibbetts, R. S.; Cortez, D.; Brumbaugh, M.; Scully, R.; Livingston, D. M.; Elledge, S. J.; Abraham, R. T. *Genes Dev.* **2000**, *14*, 2989.
(40) Brown, E. J.; Baltimore, D. *Genes Dev.* **2000**, *14*, 397.
(41) Watters, D.; Khanna, K. K.; Beamish, H.; Birrell, G.; Spring, K.; Kedar, P.; Gatei, M.; Stenzel, D.; Hobson, K.; Kozlov, S.; Zhang, N.; Farrell, A.; Ramsay, J.; Gatti, R.; Lavin, M. *Oncogene* **1997**, *14*, 1911.
(42) Kastan, M. B.; Lim, D. S. *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 175.
(43) de Klein, A.; Muijtjens, M.; van Os, R.; Verhoeven, Y.; Smit, B.; Carr, A. M.; Lehmann, A. R.; Hoeijmakers, J. H. *Curr. Biol.* **2000**, *10*, 479.
(44) Doroucher, D.; Jackson, S. P. *Curr. Opin. Cell Biol.* **2001**, *13*, 225.
(45) Falck, J.; Coates, J.; Jackson, S. P. *Nature* **2005**, *434*, 605.
(46) Shao, R. G.; Cao, C. X.; Zhang, H.; Kohn, K. W.; Wold, M. S.; Pommier, Y. *EMBO J.* **1999**, *18*, 1397.
(47) Li, J.; Stern, D. F. *J. Biol. Chem.* **2005**, *280*, 12041.
(48) Stiff, T.; O'Driscoll, M.; Rief, N.; Iwabuchi, K.; Löbrich, M.; Jeggo, P. A. *Cancer Res.* **2004**, *64*, 2390.
(49) Molinari, M.; Mercurio, C.; Dominguez, J.; Goubin, F.; Draetta, G. F. *EMBO Rep.* **2000**, *1*, 71.
(50) Falck, J.; Mailand, N.; Syljuåsen, R. G.; Bartek, J.; Lukas, J. *Nature* **2001**, *410*, 842.
(51) Boutros, R.; Dozier, C.; Ducommun, B. *Curr. Opin. Cell Biol.* **2006**, *18*, 185.
(52) Bartek, J.; Lukas, J. *Curr. Opin. Cell Biol.* **2001**, *13*, 738.
(53) Bartek, J.; Lukas, J. *FEBS Lett.* **2001**, *490*, 117.
(54) Dumaz, N.; Meek, D. W. *EMBO J.* **1999**, *18*, 7002.
(55) Dornan, D.; Hupp, T. R. *EMBO Rep.* **2001**, *2*, 139.
(56) Shieh, S. Y.; Ahn, J.; Tamai, K.; Taya, Y.; Prives, C. *Genes Dev.* **2000**, *14*, 289.
(57) Hirao, A.; Kong, Y. Y.; Matsuoka, S.; Wakeham, A.; Ruland, J.; Yoshida, H.; Liu, D.; Elledge, S. J.; Mak, T. W. *Science* **2000**, *287*, 1824.
(58) Waterman, M. J.; Stavridi, E. S.; Waterman, J. L.; Halazonetis, T. D. *Nat. Genet.* **1998**, *19*, 175.
(59) Shafman, T.; Khanna, K. K.; Kedar, P.; Spring, K.; Kozlov, S.; Yen, T.; Hobson, K.; Gatei, M.; Zhang, N.; Watters, D.; Egerton, M.; Shiloh, Y.; Kharbanda, S.; Kufe, D.; Lavin, M. F. *Nature* **1997**, *387*, 520.
(60) Baskaran, R.; Wood, L. D.; Whitaker, L. L.; Canman, C. E.; Morgan, S. E.; Xu, Y.; Barlow, C.; Baltimore, D.; Wynshaw-Boris, A.; Kastan, M. B.; Wang, J. Y. *Nature* **1997**, *387*, 516.
(61) Khosravi, R.; Maya, R.; Gottlieb, T.; Oren, M.; Shiloh, Y.; Shkedy, D. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 14973.
(62) Maya, R.; Balass, M.; Kim, S. T.; Shkedy, D.; Leal, J. F.; Shifman, O.; Moas, M.; Buschmann, T.; Ronai, Z.; Shiloh, Y.; Kastan, M. B.; Katzir, E.; Oren, M. *Genes Dev.* **2001**, *15*, 1067.
(63) Goldberg, Z.; Vogt Sionov, R.; Berger, M.; Zwang, Y.; Perets, R.; Van Etten, R. A.; Oren, M.; Taya, Y.; Haupt, Y. *EMBO J.* **2002**, *21*, 3715.
(64) Pereg, Y.; Shkedy, D.; de Graaf, P.; Meulmeester, E.; Edelson-Averbukh, M.; Salek, M.; Biton, S.; Teunisse, A. F.; Lehmann, W. D.; Jochemsen, A. G.; Shiloh, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 5056.
(65) Li, M.; Chen, D.; Shiloh, A.; Luo, J.; Nikolaev, A. Y.; Qin, J.; Gu, W. *Nature* **2002**, *416*, 648.
(66) Li, M.; Brooks, C. L.; Kon, N.; Gu, W. *Mol. Cell* **2004**, *13*, 879.
(67) Meulmeester, E.; Maurice, M. M.; Boutell, C.; Teunisse, A. F.; Ovaa, H.; Abraham, T. E.; Dirks, R. W.; Jochemsen, A. G. *Mol. Cell* **2005**, *18*, 565.
(68) Zhang, Y. P.; Xiong, A. *Science* **2001**, *292*, 1910.
(69) Kohn, K. W.; Pommier, Y. *Biochem. Biophys. Res. Commun.* **2005**, *331*, 816.
(70) Harper, J. W.; Adami, G. R.; Wie, N.; Keyomarsi, K.; Elledge, S. J. *Cell* **1993**, *75*, 805.
(71) Sheaff, R. J.; Groudine, M.; Gordon, M.; Roberts, J. M.; Clurman, B. E. *Genes Dev.* **1997**, *11*, 1464.
(72) Vlach, J.; Hennecke, S.; Amati, B. *EMBO J.* **1997**, *16*, 5334.
(73) Stewart, Z. A.; Pietenpol, J. A. *Chem. Res. Toxicol.* **2001**, *14*, 243.
(74) Botz, J.; Zerfass-Thome, K.; Spitkovsky, D.; Delius, H.; Vogt, B.; Eilers, M.; Hatzigeorgiou, A.; Jansen-Dürr, P. *Mol. Cell. Biol.* **1996**, *16*, 3401.
(75) Geng, Y.; Eaton, E. N.; Picón, M.; Roberts, J. M.; Lundberg, A. S.; Gifford, A.; Sardet, C.; Weinberg, R. A. *Oncogene* **1996**, *12*, 1173.
(76) Lundberg, A. S.; Weinberg, R. A. *Mol. Cell. Biol.* **1998**, *18*, 753.
(77) Harbour, J. W.; Dean, D. C. *Genes Dev.* **2000**, *14*, 2393.
(78) Lin, W. C.; Lin, F. T.; Nevins, J. R. *Genes Dev.* **2001**, *15*, 1833.
(79) Hara, E.; Smith, R.; Parry, D.; Tahara, H.; Stone, S.; Peters, G. *Mol. Cell. Biol.* **1996**, *16*, 859.
(80) Falck, J.; Petrini, J. H.; Williams, B. R.; Lukas, J.; Bartek, J. *Nat. Genet.* **2002**, *30*, 290.
(81) Heffernan, T. P.; Simpson, D. A.; Frank, A. R.; Heinloth, A. N.; Paules, R. S.; Cordeiro-Stone, M.; Kaufmann, W. K. *Mol. Cell. Biol.* **2002**, *22*, 8552.
(82) Gottifredi, V.; Prives, C. *Semin. Cell Dev. Biol.* **2005**, *16*, 355.
(83) Zhou, L.; Elledge, S. J. *Science* **2003**, *300*, 1542.
(84) Mordes, D. A.; Glick, G. G.; Zhao, R.; Cortez, D. *Genes Dev.* **2008**, *22*, 1478.
(85) Lin, S. Y.; Li, K.; Stewart, G. S.; Elledge, S. J. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 6484.
(86) Losada, A. *Biochim. Biophys. Acta* **2008**, *1786*, 41.
(87) Yazdi, P. T.; Wang, Y.; Zhao, S.; Patel, N.; Lee, E. Y.; Qin, J. *Genes Dev.* **2002**, *16*, 571.
(88) Kim, S. T.; Xu, B.; Kastan, M. B. *Genes Dev.* **2002**, *16*, 560.
(89) Luo, H.; Li, Y.; Mu, J. J.; Zhang, J.; Tonaka, T.; Hamamori, Y.; Jung, S. Y.; Wang, Y.; Qin, J. *J. Biol. Chem.* **2008**, *283*, 19176.
(90) Bartek, J.; Lucas, C.; Lucas, J. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 792.
(91) Goldwasser, F.; Shimizu, T.; Jackman, J.; Hoki, Y.; O'Connor, P. M.; Kohn, K. W.; Pommier, Y. *Cancer Res.* **1996**, *56*, 4430.
(92) Levesque, A. A.; Kohn, E. A.; Bresnick, E.; Eastman, A. *Oncogene* **2005**, *24*, 3786.
(93) Yoo, H. Y.; Kumagai, A.; Shevchenko, A.; Shevchenko, A.; Dunphy, W. G. *Cell* **2004**, *117*, 575.
(94) Ababou, M.; Dutertre, S.; Lécluse, Y.; Onclercq, R.; Chatton, B.; Amor-Guéret, M. *Oncogene* **2000**, *19*, 5955.
(95) Davies, S. L.; North, P. S.; Dart, A.; Lakin, N. D.; Hickson, I. D. *Mol. Cell. Biol.* **2004**, *24*, 1279.
(96) Dutertre, S.; Ababou, M.; Onclercq, R.; Delic, J.; Chatton, B.; Jaulin, C.; Amor-Guéret, M. *Oncogene* **2000**, *19*, 2731.
(97) Pichierri, P.; Rosselli, F.; Franchitto, A. *Oncogene* **2003**, *22*, 1491.
(98) Constantinou, A.; Tarsounas, M.; Karow, J. K.; Brosh, R. M.; Bohr, V. A.; Hickson, I. D. *EMBO Rep.* **2000**, *1*, 80.
(99) Sakamoto, S.; Nishikawa, K.; Heo, S. J.; Goto, M.; Furuichi, Y.; Shimamoto, A. *Genes Cells* **2001**, *6*, 421.
(100) Akkari, Y. M.; Bateman, R. L.; Reifsteck, C. A.; Olson, S. B.; Grompe, M. *Mol. Cell. Biol.* **2000**, *20*, 8283.
(101) Deming, P. B.; Cistulli, C. A.; Zhao, H.; Graves, P. R.; Piwnica-Worms, H.; Paules, R. S.; Downes, C. S.; Kaufmann, W. K. *Proc. Natl. Acad. Sci. U. S. A* **2001**, *98*, 12044.
(102) Peng, C. Y.; Graves, P. R.; Thoma, R. S.; Wu, Z.; Shaw, A. S.; Piwnica-Worms, H. *Science* **1997**, *277*, 1501.
(103) Toyoshima-Morimoto, F.; Taniguchi, E.; Shinya, N.; Iwamatsu, A.; Nishida, E. *Nature* **2001**, *410*, 215.
(104) Charrier-Savournin, F. B.; Château, M. T.; Gire, V.; Sedivy, J.; Piette, J.; Dulic, V. *Mol. Biol. Cell* **2004**, *15*, 3965.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

(105) O'Connell, M. J.; Raleigh, J. M.; Verkade, H. M.; Nurse, P. *EMBO J.* **1997**, *16*, 545.
(106) Liu, F.; Stanton, J. J.; Wu, Z.; Piwnica-Worms, H. *Mol. Cell. Biol.* **1997**, *17*, 571.
(107) Wang, X. W.; Zhan, Q.; Coursen, J. D.; Khan, M. A.; Kontny, H. U.; Yu, L.; Hollander, M. C.; O'Connor, P. M.; Fornace, A. J.; Harris, C. C. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 3706.
(108) Taylor, W. R.; Stark, G. R. *Oncogene* **2001**, *20*, 1803.
(109) Imbriano, C.; Gurtner, A.; Cocchiarella, F.; Di Agostino, S.; Basile, V.; Gostissa, M.; Dobbelstein, M.; Del Sal, G.; Piaggio, G.; Mantovani, R. *Mol. Cell. Biol.* **2005**, *25*, 3737.
(110) Smits, V. A.; Klompmaker, R.; Vallenius, T.; Rijksen, G.; Mäkela, T. P.; Medema, R. H. *J. Biol. Chem.* **2000**, *275*, 30638.
(111) Calonge, T. M.; O'Connell, M. J. *DNA Repair (Amsterdam)* **2008**, *7*, 136.
(112) van Vugt, M. A.; Brás, A.; Medema, R. H. *Mol. Cell* **2004**, *15*, 799.
(113) den Elzen, N. R.; O'Connell, M. J. *EMBO J.* **2004**, *23*, 908.
(114) Liang, X.; Reed, E.; Yu, J. J. *Int. J. Mol. Med.* **2006**, *17*, 703.
(115) Margolis, S. S.; Walsh, S.; Weiser, D. C.; Yoshida, M.; Shenolikar, S.; Kornbluth, S. *EMBO J.* **2003**, *22*, 5734.
(116) Margolis, S. S.; Perry, J. A.; Forester, C. M.; Nutt, L. K.; Guo, Y.; Jardim, M. J.; Thomenius, M. J.; Freel, C. D.; Darbandi, R.; Ahn, J. H.; Arroyo, J. D.; Wang, X. F.; Shenolikar, S.; Nairn, A. C.; Dunphy, W. G.; Hahn, W. C.; Virshup, D. M.; Kornbluth, S. *Cell* **2006**, *127*, 759.
(117) Geng, L.; Zhang, X.; Zheng, S.; Legerski, R. J. *Mol. Cell. Biol.* **2007**, *27*, 2625.
(118) Larsen, A. K.; Escargueil, A. E.; Skladanowski, A. *Pharmacol. Ther.* **2003**, *99*, 167.
(119) Downes, C. S.; Clarke, D. J.; Mullinger, A. M.; Giménez-Abián, J. F.; Creighton, A. M.; Johnson, R. T. *Nature* **1994**, *372*, 467.
(120) Nakagawa, T.; Hayashita, Y.; Maeno, K.; Masuda, A.; Sugito, N.; Osada, H.; Yanagisawa, K.; Ebi, H.; Shimokata, K.; Takahashi, T. *Cancer Res.* **2004**, *64*, 4826.
(121) Damelin, M.; Bestor, T. H. *Br. J. Cancer.* **2007**, *96*, 201.
(122) Giménez-Abián, J. F.; Clarke, D. J.; Devlin, J.; Giménez-Abián, M. I.; De la Torre, C.; Johnson, R. T.; Mullinger, A. M.; Downes, C. S. *Chromosoma* **2000**, *109*, 235.
(123) Deming, P. B.; Cistulli, C. A.; Zhao, H.; Graves, P. R.; Piwnica-Worms, H. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 12044.
(124) Deming, P. B.; Flores, K. G.; Downes, C. S.; Paules, R. S.; Kaufmann, W. K. *J. Biol. Chem.* **2002**, *277*, 36832.
(125) Anderson, H.; Roberge, M. *Cell Growth Differ.* **1996**, *7*, 83.
(126) Andreassen, P. R.; Martineau, S. N.; Margolis, R. L. *Mutat. Res.* **1996**, *372*, 181.
(127) Gorbsky, G. J. *Cancer Res.* **1994**, *54*, 1042.
(128) Boos, G.; Stopper, H. *Toxicol. Lett.* **2000**, *116*, 7.
(129) Huang, K. C.; Gao, H.; Yamasaki, E. F.; Grabowski, D. R.; Liu, S.; Shen, L. L.; Chan, K. K.; Ganapathi, R.; Snapka, R. M. *J. Biol. Chem.* **2001**, *276*, 44488.
(130) Pastor, N.; Dominguez, I.; Mateos, S.; Cortes, F. *Mutat. Res.* **2002**, *515*, 171.
(131) Hajji, N.; Pastor, N.; Mateos, S.; Dominguez, I.; Cortes, F. *Mutat. Res.* **2003**, *530*, 35.
(132) Mikhailov, A.; Shinohara, M.; Rieder, C. L. *J. Cell Biol.* **2004**, *166*, 517.
(133) Park, I.; Avraham, H. K. *Exp. Cell Res.* **2006**, *312*, 1996.
(134) Robinson, H. M.; Bratlie-Thoresen, S.; Brown, R.; Gillespie, D. A. *Cell Cycle* **2007**, *6*, 1265.
(135) Qiu, L.; Burgess, A.; Fairlie, D. P.; Leonard, H.; Parsons, P. G.; Gabrielli, B. G. *Mol. Biol. Cell* **2000**, *11*, 2069.
(136) Ekwall, K.; Olsson, T.; Turner, B. M.; Cranston, G.; Allshire, R. C. *Cell* **1997**, *91*, 1021.
(137) Musacchio, A.; Salmon, E. D. *Nat. Rev. Mol. Cell. Biol.* **2007**, *8*, 379.
(138) Privette, L. M.; Petty, E. M. *Transl. Oncol.* **2008**, *1*, 57.
(139) Mikhailov, A.; Cole, R. W.; Rieder, C. L. *Curr. Biol.* **2002**, *12*, 1797.
(140) Chow, J. P.; Siu, W. Y.; Fung, T. K.; Chan, W. M.; Lau, A.; Arooz, T.; Ng, C. P.; Yamashita, K.; Poon, R. Y. *Mol. Biol. Cell* **2003**, *14*, 3989.
(141) Choi, E.; Lee, H. *FEBS Lett.* **2008**, *582*, 1700.
(142) Skoufias, D. A.; Lacroix, F. B.; Andreassen, P. R.; Wilson, L.; Margolis, R. L. *Mol. Cell* **2004**, *15*, 977.
(143) Smits, V. A.; Klompmaker, R.; Arnaud, L.; Rijksen, G.; Nigg, E. A.; Medema, R. H. *Nat. Cell Biol.* **2000**, *2*, 672.
(144) Toyoshima-Morimoto, F.; Taniguchi, E.; Shinya, N.; Iwamatsu, A.; Nishida, E. *Nature* **2001**, *410*, 215.
(145) van Vugt, M. A.; Smits, V. A.; Klompmaker, R.; Medema, R. H. *J. Biol. Chem.* **2001**, *276*, 41656.
(146) Tsvetkov, L.; Stern, D. F. *Cell Cycle* **2005**, *4*, 166.
(147) Kang, D.; Chen, J.; Wong, J.; Fang, G. *J. Cell Biol.* **2002**, *156*, 249.
(148) Geley, S.; Kramer, E.; Gieffers, C.; Gannon, J.; Peters, J. M.; Hunt, T. *J. Cell Biol.* **2001**, *153*, 137.
(149) Andreassen, P. R.; Lohez, O. D.; Lacroix, F. B.; Margolis, R. L. *Mol. Biol. Cell* **2001**, *12*, 1315.
(150) Stewart, Z. A.; Leach, S. D.; Pietenpol, J. A. *Mol. Cell. Biol.* **1999**, *19*, 205.
(151) Uetake, Y.; Sluder, G. *J. Cell Biol.* **2004**, *165*, 609.
(152) Wong, C.; Stearns, T. *BMC Cell Biol.* **2005**, *6*, 6.
(153) Minn, A. J.; Boise, L. H.; Thompson, C. B. *Genes Dev.* **1996**, *10*, 2621.
(154) Lanni, J. S.; Jacks, T. *Mol. Cell. Biol.* **1998**, *18*, 1055.
(155) Xu, Q.; Simpson, S. E.; Scialla, T. J.; Bagg, A.; Carroll, M. *Blood* **2003**, *102*, 972.
(156) David, O.; Jett, J.; LeBeau, H.; Dy, G.; Hughes, J.; Friedman, M.; Brody, A. R. *Clin. Cancer Res.* **2004**, *10*, 6865.
(157) Dai, D. L.; Martinka, M.; Li, G. *J. Clin. Oncol.* **2005**, *23*, 1473.
(158) Pelloski, C. E.; Lin, E.; Zhang, L.; Yung, W. K.; Colman, H.; Liu, J. L.; Woo, S. Y.; Heimberger, A. B.; Suki, D.; Prados, M.; Chang, S.; Barker, F. G.; Fuller, G. N.; Aldape, K. D. *Clin. Cancer Res.* **2006**, *12*, 3935.
(159) Schmitt, C. A.; Fridman, J. S.; Yang, M.; Lee, S.; Baranov, E.; Hoffman, R. M.; Lowe, S. W. *Cell* **2002**, *109*, 335.
(160) te Poele, R. H.; Okorokov, A. L.; Jardine, L.; Cummings, J.; Joel, S. P. *Cancer Res.* **2002**, *62*, 1876.
(161) Bartek, J.; Bartkova, J.; Lukas, J. *Oncogene* **2007**, *26*, 7773.
(162) Yang, J.; Weinberg, R. A. *Dev. Cell* **2008**, *14*, 818.
(163) Hiscox, S.; Jiang, W. G.; Obermeier, K.; Taylor, K.; Morgan, L.; Burmi, R.; Barrow, D.; Nicholson, R. I. *Int. J. Cancer* **2006**, *118*, 290.
(164) Yoshino, I.; Kometani, T.; Shoji, F.; Osoegawa, A.; Ohba, T.; Kouso, H.; Takenaka, T.; Yohena, T.; Maehara, Y. *Cancer* **2007**, *110*, 369.
(165) Robson, E. J.; Khaled, W. T.; Abell, K.; Watson, C. J. *Differentiation* **2006**, *74*, 254.
(166) Mani, S. A.; Guo, W.; Liao, M. J.; Eaton, E. N.; Ayyanan, A.; Zhou, A. Y.; Brooks, M.; Reinhard, F.; Zhang, C. C.; Shipitsin, M.; Campbell, L. L.; Polyak, K.; Brisken, C.; Yang, J.; Weinberg, R. A. *Cell* **2008**, *133*, 704.
(167) Pérez, C.; Vilaboa, N. E.; Garcı´a-Bermejo, L.; de Blas, E.; Creighton, A. M.; Aller, P. *J. Cell. Sci.* **1997**, *110*, 337.
(168) Hasinoff, B. B.; Abram, M. E.; Barnabé, N.; Khélifa, T.; Allan, W. P.; Yalowich, J. C. *Mol. Pharmacol.* **2001**, *59*, 453.
(169) Santos, A.; Calvet, L.; Terrier-Lacombe, M. J.; Larsen, A.; Bénard, J.; Pondarré, C.; Aubert, G.; Morizet, J.; Lavelle, F.; Vassal, G. *Cancer Res.* **2004**, *64*, 3223.
(170) Larsen, A. K. *Ann. Oncol.* **1994**, *5*, 679.
(171) Pantazis, P.; Dejesus, A.; Early, J.; Rodriguez, R.; Chatterjee, D.; Han, Z.; Wyche, J.; Giovanella, B. *Anticancer Res.* **1995**, *15*, 1873.
(172) Altieri, D. C. *Adv. Cancer Res.* **2003**, *88*, 31.
(173) Bellacosa, A.; Kumar, C. C.; Di Cristofano, A.; Testa, J. R. *Adv. Cancer Res.* **2005**, *94*, 29.
(174) O'Brien, V.; Brown, R. *Carcinogenesis* **2006**, *27*, 682.
(175) Dent, P.; Yacoub, A.; Fisher, P. B.; Hagan, M. P.; Grant, S. *Oncogene* **2003**, *22*, 5885.
(176) Yan, Y.; Black, C. P.; Cowan, K. H. *Oncogene* **2007**, *26*, 4689.
(177) Dai, Y.; Yu, C.; Singh, V.; Tang, L.; Wang, Z.; McInistry, R.; Dent, P.; Grant, S. *Cancer Res.* **2001**, *61*, 5106.
(178) Pei, X. Y.; Li, W.; Dai, Y.; Dent, P.; Grant, S. *Mol. Pharmacol.* **2006**, *70*, 1965.
(179) Dai, Y.; Chen, S.; Pei, X. Y.; Almenara, J. A.; Kramer, L. B.; Venditti, C. A.; Dent, P.; Grant, S. *Blood* **2008**, *112*, 2439.
(180) Yan, Y.; Black, C. P.; Cao, P. T.; Haferbier, J. L.; Kolb, R. H.; Spieker, R. S.; Ristow, A. M.; Cowan, K. H. *Cancer Res.* **2008**, *68*, 5113.
(181) Abbott, D. W.; Holt, J. T. *J. Biol. Chem.* **1999**, *274*, 2732.
(182) Reinhardt, H. C.; Aslanian, A. S.; Leesm, J. A.; Yaffe, M. B. *Cancer Cell* **2007**, *11*, 175.
(183) Xiao, Z.; Xue, J.; Sowin, T. J.; Zhang, H. *Mol. Cancer Ther.* **2006**, *5*, 1935.
(184) Zhang, W. H.; Poh, A.; Fanous, A. A.; Eastman, A. *Cell Cycle* **2008**, *7*, 1668.
(185) Golding, S. E.; Rosenberg, E.; Neill, S.; Dent, P.; Povirk, L. F.; Valerie, K. *Cancer Res.* **2007**, *67*, 1046.
(186) Wang, C.; Mayo, M. W.; Baldwin, A. S. *Science* **1996**, *274*, 784.
(187) Boland, M. P.; Foster, S. J.; O'Neill, L. A. J. *J. Biol. Chem.* **1997**, *272*, 12952.
(188) Arlt, A.; Vorndamm, J.; Breitenbroich, M.; Folsch, U. R.; Kalthoff, H.; Schmidt, W. E.; Schafer, H. *Oncogene* **2001**, *20*, 859.
(189) Lavon, I.; Pikarsky, E.; Gutkovich, E.; Goldberg, I.; Bar, J.; Oren, M.; Ben-Neriah, Y. *Cancer Res.* **2003**, *63*, 25–30.
(190) Spalding, A. C.; Jotte, R. M.; Scheinman, R. I.; Geraci, M. W.; Clarke, P.; Tyler, K. L.; Johnson, G. L. *Oncogene* **2002**, *21*, 260.
(191) Ashikawa, K.; Shishodia, S.; Fokt, I.; Priebe, W.; Aggarwal, B. B. *Biochem. Pharmacol.* **2004**, *67*, 353.
(192) Piret, B.; Piette, J. *Nucleic Acids Res.* **1996**, *24*, 4242.
(193) Piret, B.; Schoonbroodt, S.; Piette, J. *Oncogene* **1999**, *18*, 2261.

(194) Huang, T. T.; Wuerzberger-Davis, S. M.; Seufzer, B. J.; Shumway, S. D.; Kurama, T.; Boothman, D. A.; Miyamoto, S. *J. Biol. Chem.* **2000**, *275*, 9501.
(195) Habraken, Y.; Piret, B.; Piette, J. *Biochem. Pharmacol.* **2001**, *62*, 603.
(196) Aleyasin, H.; Cregan, S. P.; Iyirhiaro, G.; O'Hare, M. J.; Callaghan, S. M.; Slack, R. S.; Park, D. S. *J. Neurosci.* **2004**, *24*, 2963.
(197) Donepudi, M.; Raychaudhuri, P.; Bluestone, J. A.; Mokyr, M. B. *J. Immunol.* **2001**, *166*, 6491.
(198) Donepudi, M.; Jovasevic, V. M.; Raychaudhuri, P.; Mokyr, M. B. *Cancer Immunol. Immunother.* **2003**, *52*, 162.
(199) Galter, D.; Mihm, S.; Droge, W. *Eur. J. Biochem.* **1994**, *221*, 639.
(200) Kasibhatla, S.; Brunner, T.; Genestier, L.; Echeverri, F.; Mahboubi, A.; Green, D. R. *Mol. Cell* **1998**, *1*, 543.
(201) Arlt, A.; Gehrz, A.; Muerkoster, S.; Vorndamm, J.; Kruse, M. L.; Fölsch, U. R.; Schäfer, H. *Oncogene* **2003**, *22*, 3243.
(202) Culmsee, C.; Siewe, J.; Junker, V.; Retiounskaia, M.; Schwarz, S.; Camandola, S.; El-Metainy, S.; Behnke, H.; Mattson, M. P.; Krieglstein, J. *J. Neurosci.* **2003**, *23*, 8586.
(203) Campbell, K. J.; O'Shea, J. M.; Perkins, N. D. *BMC Cancer* **2006**, *6*, 101.
(204) Lee, S. J.; Dimtchev, A.; Lavin, M. F.; Dritschilo, A.; Jung, M. *Oncogene* **1998**, *17*, 1821.
(205) Li, N.; Banin, S.; Ouyang, H.; Li, G. C.; Courtois, G.; Shiloh, Y.; Karin, M.; Rotman, G. *J. Biol. Chem.* **2001**, *276*, 8898.
(206) Wu, Z. H.; Shi, Y.; Tibbetts, R. S.; Miyamoto, S. *Science* **2006**, *311*, 1141.
(207) Wu, Z. H.; Miyamoto, S. *EMBO J.* **2008**, *27*, 1963–73.
(208) Ghosh, J. C.; Dohi, T.; Raskett, C. M.; Kowalik, T. F.; Altieri, D. C. *Cancer Res.* **2006**, *66*, 11576.
(209) Vogel, C.; Hager, C.; Bastians, H. *Cancer Res.* **2007**, *67*, 339.
(210) Feng, Y. B.; Lin, D. C.; Shi, Z. Z.; Wang, X. C.; Shen, X. M.; Zhang, Y.; Du, X. L.; Luo, M. L.; Xu, X.; Han, Y. L.; Cai, Y.; Zhang, Z. Q.; Zhan, Q. M.; Wang, M. R. *Int. J. Cancer* **2008**, *124*, 578.
(211) Mirza, A.; McGuirk, M.; Hockenberry, T. N.; Wu, Q.; Ashar, H.; Black, S.; Wen, S. F.; Wang, L.; Kirschmeier, P.; Bishop, W. R.; Nielsen, L. L.; Pickett, C. B.; Liu, S. *Oncogene* **2002**, *21*, 2613.
(212) Hoffman, W. H.; Biade, S.; Zilfou, J. T.; Chen, J.; Murphym, M. *J. Biol. Chem.* **2002**, *277*, 3247.
(213) Zhou, M.; Gu, L.; Li, F.; Zhu, Y.; Woods, W. G.; Findley, H. W. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 124.
(214) Katayama, K.; Fujita, N.; Tsuruo, T. *Mol. Cell. Biol.* **2005**, *25*, 5725.
(215) Henry, M. K.; Lynch, J. T.; Eapen, A. K.; Quelle, F. W. *Blood* **2001**, *98*, 34.
(216) Shtivelman, E. *Mol. Cancer Res.* **2003**, *1*, 959.
(217) Hemstrom, T. H.; Sandstrom, M.; Zhivotovsky, B. *Int. J. Cancer* **2006**, *119*, 1028.
(218) Skladanowski, A.; Bozko, P.; Sabisz, M.; Larsen, A. K. *Cell Cycle* **2007**, *6*, 2268.
(219) King, F. W.; Skeen, J.; Hay, N.; Shtivelman, E. *Cell Cycle* **2004**, *3*, 634.
(220) Viniegra, J. G.; Martı´nez, N.; Modirassari, P.; Losa, J. H.; Parada Cobo, C.; Lobo, V. J.; Luquero, C. I.; Alvarez-Vallina, L.; Ramón y Cajal, S.; Rojas, J. M.; Sánchez-Prieto, R. *J. Biol. Chem.* **2005**, *280*, 4029.
(221) Caporali, S.; Levati, L.; Starace, G.; Ragone, G.; Bonmassar, E.; Alvino, E.; D'Atri, S. *Mol. Pharmacol.* **2008**, *74*, 173.
(222) Sørensen, C. S.; Hansen, L. T.; Dziegielewski, J.; Syljuåsen, R. G.; Lundin, C.; Bartek, J.; Helleday, T. *Nat. Cell Biol.* **2005**, *7*, 195.
(223) Sleeth, K. M.; Sørensen, C. S.; Issaeva, N.; Dziegielewski, J.; Bartek, J.; Helleday, T. *J. Mol. Biol.* **2007**, *373*, 38.
(224) Brown, K. D.; Rathi, A.; Kamath, R.; Beardsley, D. I.; Zhan, Q.; Mannino, J. L.; Baskaran, R. *Nat. Genet.* **2003**, *33*, 80.
(225) Wang, Y.; Qin, J. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 15387.
(226) Yan, T.; Schupp, J. E.; Hwang, H. S.; Wagner, M. W.; Berry, S. E.; Strickfaden, S.; Veigl, M. L.; Sedwick, W. D.; Boothman, D. A.; Kinsella, T. J. *Cancer Res.* **2001**, *61*, 8290.
(227) Yan, T.; Desai, A. B.; Jacobberger, J. W.; Sramkoski, R. M.; Loh, T.; Kinsella, T. J. *Mol. Cancer Ther.* **2004**, *3*, 1147.
(228) Liu, A.; Yoshioka, K.; Salerno, V.; Hsieh, P. *J. Cell. Biochem.* **2008**, *105*, 245.
(229) Hirose, Y.; Katayama, M.; Stokoe, D.; Haas-Kogan, D. A.; Berger, M. S.; Pieper, R. O. *Mol. Cell. Biol.* **2003**, *23*, 8306.
(230) Fink, D.; Aebi, S.; Howell, S. B. *Clin. Cancer Res.* **1998**, *4*, 1.
(231) Aebi, S.; Kurdi-Haidar, B.; Gordon, R.; Cenni, B.; Zheng, H.; Fink, D.; Christen, R. D.; Boland, C. R.; Koi, M.; Fishel, R.; Howell, S. B. *Cancer Res.* **1996**, *56*, 3087.
(232) Plumb, J. A.; Strathdee, G.; Sludden, J.; Kaye, S. B.; Brown, R. *Cancer Res.* **2000**, *60*, 6039.
(233) Fink, D.; Nebel, S.; Aebi, S.; Zheng, H.; Cenni, B.; Nehmé, A.; Christen, R. D.; Howell, S. B. *Cancer Res.* **1996**, *56*, 4881.
(234) Hawn, M. T.; Umar, A.; Carethers, J. M.; Marra, G.; Kunkel, T. A.; Boland, C. R.; Koi, M. *Cancer Res.* **1995**, *55*, 3721.
(235) Caporali, S.; Falcinelli, S.; Starace, G.; Russo, M. T.; Bonmassar, E.; Jiricny, J.; D'Atri, S. *Mol. Pharmacol.* **2004**, *66*, 478.
(236) Pichierri, P.; Franchitto, A.; Piergentili, R.; Colussi, C.; Palitti, F. *Carcinogenesis* **2001**, *22*, 1781.
(237) Jacob, S.; Aguado, M.; Fallik, D.; Praz, F. *Cancer Res.* **2001**, *61*, 6555.
(238) Wu, Q.; Vasquez, K. M. *PLoS Genet.* **2008**, *4*, e1000189.
(239) Chou, W. C.; Wang, H. C.; Wong, F. H.; Ding, S. L.; Wu, P. E.; Shieh, S. Y.; Shen, C. Y. *EMBO J.* **2008**, *27*, 3140.
(240) Brem, R.; Fernet, M.; Chapot, B.; Hall, J. *DNA Repair (Amsterdam)* **2008**, *7*, 849.
(241) Angèle, S.; Tanière, P.; Hall, J. *Bull. Cancer* **2001**, *88*, 671.
(242) Angèle, S.; Treilleux, I.; Tanière, P.; Martel-Planche, G.; Vuillaume, M.; Bailly, C.; Brémond, A.; Montesano, R.; Hall, J. *Clin. Cancer Res.* **2000**, *6*, 3536.
(243) Treilleux, I.; Chapot, B.; Goddard, S.; Pisani, P.; Angèle, S.; Hall, J. *Histopathology* **2007**, *51*, 63.
(244) Peng, Y.; Woods, R. G.; Beamish, H.; Ye, R.; Lees-Miller, S. P.; Lavin, M. F.; Bedford, J. S. *Cancer Res.* **2005**, *65*, 1670.
(245) Roesch, A.; Becker, B.; Bentink, S.; Spang, R.; Vogl, A.; Hagen, I.; Landthaler, M.; Vogt, T. *Cancer Epidemiol., Biomarkers Prev.* **2007**, *16*, 2486.
(246) Heikkinen, K.; Mansikka, V.; Karppinen, S. M.; Rapakko, K.; Winqvist, R. *Breast Cancer Res.* **2005**, *7*, R495.
(247) Durocher, F.; Labrie, Y.; Soucy, P.; Sinilnikova, O.; Labuda, D.; Bessette, P.; Chiquette, J.; Laframboise, R.; Lépine, J.; Lespérance, B.; Ouellette, G.; Pichette, R.; Plante, M.; Tavtigian, S. V.; Simard, J. *BMC Cancer* **2006**, *6*, 230.
(248) Ferla, R.; Calò, V.; Cascio, S.; Rinaldi, G.; Badalamenti, G.; Carreca, I.; Surmacz, E.; Colucci, G.; Bazan, V.; Russo, A. *Ann. Oncol.* **2007**, *18* (Suppl 6), vi93.
(249) Nathanson, K. L.; Wooster, R.; Weber, B. L. *Nat. Med.* **2001**, *7*, 552.
(250) Rahman, N.; Stratton, M. R. *Annu. Rev. Genet.* **1998**, *32*, 95.
(251) Matros, E.; Wang, Z. C.; Lodeiro, G.; Miron, A.; Iglehart, J. D.; Richardson, A. L. *Breast Cancer Res. Treat.* **2005**, *91*, 179.
(252) Baldwin, R. L.; Nemeth, E.; Tran, H.; Shvartsman, H.; Cass, I.; Narod, S.; Karlan, B. Y. *Cancer Res.* **2000**, *60*, 5329.
(253) Thrall, M.; Gallion, H. H.; Kryscio, R.; Kapali, M.; Armstrong, D. K.; DeLoia, J. A. *Int. J. Gynecol. Cancer* **2006**, *16* (Suppl 1), 166.
(254) Russell, P. A.; Pharoah, P. D.; De Foy, K.; Ramus, S. J.; Symmonds, I.; Wilson, A.; Scott, I.; Ponder, B. A.; Gayther, S. A. *Int. J. Cancer* **2000**, *87*, 317.
(255) Collins, N.; Wooster, R.; Stratton, M. R. *Br. J. Cancer* **1997**, *76*, 1150.
(256) Gallmeier, E.; Kern, S. E. *Clin. Cancer Res.* **2007**, *13*, 4.
(257) Landais, I.; Sobeck, A.; Stone, S.; LaChapelle, A.; Hoatlin, M. E. *Int. J. Cancer* **2009**, *124*, 783.
(258) Takemura, H.; Rao, V. A.; Sordet, O.; Furuta, T.; Miao, Z. H.; Meng, L.; Zhang, H.; Pommier, Y. *J. Biol. Chem.* **2006**, *281*, 30814.
(259) Kim, C. J.; Lee, J. H.; Song, J. W.; Cho, Y. G.; Kim, S. Y.; Nam, S. W.; Yoo, N. J.; Park, W. S.; Lee, J. Y. *Eur. J. Surg. Oncol.* **2007**, *33*, 580.
(260) Stawinska, M.; Cygankiewicz, A.; Trzcinski, R.; Mik, M.; Dziki, A.; Krajewska, W. M. *Int. J. Colorectal Dis.* **2008**, *23*, 1243.
(261) Miller, C. W.; Ikezoe, T.; Krug, U.; Hofmann, W. K.; Tavor, S.; Vegesna, V.; Tsukasaki, K.; Takeuchi, S.; Koeffler, H. P. *Genes Chromosomes Cancer* **2002**, *33*, 17.
(262) Shigeishi, H.; Yokozaki, H.; Oue, N.; Kuniyasu, H.; Kondo, T.; Ishikawa, T.; Yasui, W. *Int. J. Cancer* **2002**, *99*, 58.
(263) Sullivan, A.; Yuille, M.; Repellin, C.; Reddy, A.; Reelfs, O.; Bell, A.; Dunne, B.; Gusterson, B. A.; Osin, P.; Farrell, P. J.; Yulug, I.; Evans, A.; Ozcelik, T.; Gasco, M.; Crook, T. *Oncogene* **2002**, *21*, 1316.
(264) Shiri-Sverdlov, R.; Oefner, P.; Green, L.; Baruch, R. G.; Wagner, T.; Kruglikova, A.; Haitchick, S.; Hofstra, R. M.; Papa, M. Z.; Mulder, I.; Rizel, S.; Bar Sade, R. B.; Dagan, E.; Abdeen, Z.; Goldman, B.; Friedman, E. *Hum. Mutat.* **2000**, *16*, 491.
(265) Cybulski, C.; Wokołorczyk, D.; Huzarski, T.; Byrski, T.; Gronwald, J.; Górski, B.; Debniak, T.; Masojć, B.; Jakubowska, A.; Gliniewicz, B.; Sikorski, A.; Stawicka, M.; Godlewski, D.; Kwias, Z.; Antczak, A.; Krajka, K.; Lauer, W.; Sosnowski, M.; Sikorska-Radek, P.; Bar, K.; Klijer, R.; Zdrojowy, R.; Małkiewicz, B.; Borkowski, A.; Borkowski, T.; Szwiec, M.; Narod, S. A.; Lubiński, J. *J. Med. Genet.* **2006**, *43*, 863.
(266) Bell, D. W.; Kim, S. H.; Godwin, A. K.; Schiripo, T. A.; Harris, P. L.; Haserlat, S. M.; Wahrer, D. C.; Haiman, C. A.; Daly, M. B.; Niendorf, K. B.; Smith, M. R.; Sgroi, D. C.; Garber, J. E.; Olopade, O. I.; Le Marchand, L.; Henderson, B. E.; Altshuler, D.; Haber, D. A.; Freedman, M. L. *Int. J. Cancer* **2007**, *121*, 2661.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

(267) Cybulski, C.; Wokołorczyk, D.; Kładny, J.; Kurzawski, G.; Suchy, J.; Grabowska, E.; Gronwald, J.; Huzarski, T.; Byrski, T.; Górski, B.; Debniak, T.; Narod, S. A.; Lubiński, J. *Eur. J. Hum. Genet.* **2007**, *15*, 237.
(268) Song, H.; Hollstein, M.; Xu, Y. *Nat. Cell Biol.* **2007**, *9*, 573.
(269) Hyzy, M.; Bozko, P.; Konopa, J.; Skladanowski, A. *Biochem. Pharmacol.* **2005**, *69*, 801.
(270) Freedman, D. A.; Wu, L.; Levine, A. J. *Cell. Mol. Life Sci.* **1999**, *55*, 96.
(271) Momand, J.; Jung, D.; Wilczynski, S.; Niland, J. *Nucleic Acids Res.* **1998**, *26*, 3453.
(272) Scheffner, M. *Pharmacol. Ther.* **1998**, *78*, 129.
(273) Paavonen, J. *Int. J. Infect. Dis.* **2007**, *11* (Suppl 2), S3.
(274) de Cárcer, G.; Pérez de Castro, I.; Malumbres, M. *Curr. Med. Chem.* **2007**, *14*, 969.
(275) Kaufmann, W. K.; Nevis, K. R.; Qu, P.; Ibrahim, J. G.; Zhou, T.; Zhou, Y.; Simpson, D. A.; Helms-Deaton, J.; Cordeiro-Stone, M.; Moore, D. T.; Thomas, N. E.; Hao, H.; Liu, Z.; Shields, J. M.; Scott, G. A.; Sharpless, N. E. *J. Invest. Dermatol.* **2008**, *128*, 175.
(276) Fan, S.; Chang, J. K.; Smith, M. L.; Duba, D.; Fornace, A. J., Jr.; O'Connor, P. M. *Oncogene* **1997**, *14*, 2127.
(277) Lodygin, D.; Hermeking, H. *Semin. Cancer Biol.* **2006**, *16*, 214.
(278) Urano, T.; Saito, T.; Tsukui, T.; Fujita, M.; Hosoi, T.; Muramatsu, M.; Ouchi, Y.; Inoue, S. *Nature* **2002**, *417*, 871.
(279) Nakayama, H.; Sano, T.; Motegi, A.; Oyama, T.; Nakajima, T. *Pathol. Int.* **2005**, *55*, 707.
(280) Ding, S. L.; Sheu, L. F.; Yu, J. C.; Yang, T. L.; Chen, B. F.; Leu, F. J.; Shen, C. Y. *Br. J. Cancer.* **2004**, *90*, 1995.
(281) Nakagawa, T.; Hayashita, Y.; Maeno, K.; Masuda, A.; Sugito, N.; Osada, H.; Yanagisawa, K.; Ebi, H.; Shimokata, K.; Takahashi, T. *Cancer Res.* **2004**, *64*, 4826.
(282) Doherty, S. C.; McKeown, S. R.; McKelvey-Martin, V.; Downes, C. S.; Atala, A.; Yoo, J. J.; Simpson, D. A.; Kaufmann, W. K. *J. Natl. Cancer Inst.* **2003**, *95*, 1859.
(283) Clarke, M. F.; Fuller, M. *Cell* **2006**, *124*, 1111.
(284) Blagosklonny, M. V. *Leukemia* **2006**, *20*, 385.
(285) Barnes, D. J.; Melo, J. V. *Cell Cycle* **2005**, *5*, 2862.
(286) Huff, C. A.; Matsui, W.; Smith, B. D.; Jones, R. J. *Blood* **2006**, *107*, 431.
(287) Kelly, P. N.; Dakic, A.; Adams, J. M.; Nutt, S. L.; Strasser, A. *Science* **2007**, *317*, 337.
(288) Damelin, M.; Sun, Y. E.; Sodja, V. B.; Bestor, T. H. *Cancer Cell* **2005**, *8*, 479.
(289) Aladjem, M. I.; Spike, B. T.; Rodewald, L. W.; Hope, T. J.; Klemm, M.; Jaenisch, R.; Wahl, G. M. *Curr. Biol.* **1998**, *8*, 145.
(290) Oren, M.; Reich, N. C.; Levine, A. J. *Mol. Cell. Biol.* **1982**, *2*, 443.
(291) Shafee, N.; Smith, C. R.; Wei, S.; Kim, Y.; Mills, G. B.; Hortobagyi, G. N.; Stanbridge, E. J.; Lee, E. Y. *Cancer Res.* **2008**, *68*, 3243.
(292) Xing, J.; Wu, X.; Vaporciyan, A. A.; Spitz, M. R.; Gu, J. *Cancer* **2008**, *112*, 2756.
(293) Feng, Z.; Hu, W.; Teresky, A. K.; Hernando, E.; Cordon-Cardo, C.; Levine, A. J. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 16633.
(294) Arlander, S. J.; Greene, B. T.; Innes, C. L.; Paules, R. S. *Cancer Res.* **2008**, *68*, 89.
(295) Kennedy, R. D.; Quinn, J. E.; Mullan, P. B.; Johnston, P. G.; Harkin, D. P. *J. Natl. Cancer Inst.* **2004**, *96*, 1659.
(296) Cass, I.; Baldwin, R. L.; Varkey, T.; Moslehi, R.; Narod, S. A.; Karlan, B. Y. *Cancer* **2003**, *97*, 2187.
(297) Byrski, T.; Huzarski, T.; Dent, R.; Gronwald, J.; Zuziak, D.; Cybulski, C.; Kladny, J.; Gorski, B.; Lubinski, J.; Narod, S. A. Breast Cancer Res. Treat. 2008, doi:0.1007/s10549-008-0128-9.
(298) Powell, S. N.; Kachnic, L. A. *Anticancer Agents Med. Chem.* **2008**, *8*, 448.
(299) Lafarge, S.; Sylvain, V.; Ferrara, M.; Bignon, Y. J. *Oncogene* **2001**, *20*, 6597.
(300) Sakai, W.; Swisher, E. M.; Karlan, B. Y.; Agarwal, M. K.; Higgins, J.; Friedman, C.; Villegas, E.; Jacquemont, C.; Farrugia, D. J.; Couch, F. J.; Urban, N.; Taniguchi, T. *Nature* **2008**, *451*, 1116.
(301) Swisher, E. M.; Sakai, W.; Karlan, B. Y.; Wurz, K.; Urban, N.; Taniguchi, T. *Cancer Res.* **2008**, *68*, 2581.
(302) Edwards, S. L.; Brough, R.; Lord, C. J.; Natrajan, R.; Vatcheva, R.; Levine, D. A.; Boyd, J.; Reis-Filho, J. S.; Ashworth, A. *Nature* **2008**, *451*, 1111.
(303) Weinert, T. *Cell* **1998**, *94*, 555.
(304) Powell, S. N.; DeFrank, J. S.; Connell, P.; Eogan, M.; Preffer, F.; Dombkowski, D.; Tang, W.; Friend, S. *Cancer Res.* **1995**, *55*, 1643.
(305) Wang, Q.; Fan, S.; Eastman, A.; Worland, P. J.; Sausville, E. A.; O'Connor, P. M. *J. Natl. Cancer Inst.* **1996**, *88*, 956.
(306) DeFrank, J. S.; Tang, W.; Powell, S. N. *Cancer Res.* **1996**, *56*, 5365.
(307) Deplanque, G.; Céraline, J.; Mah-Becherel, M. C.; Cazenave, J. P.; Bergerat, J. P.; Klein-Soyer, C. *Int. J. Cancer* **2001**, *94*, 363.
(308) Fuse, E.; Kuwabara, T.; Sparreboom, A.; Sausville, E. A.; Figg, W. D. *J. Clin. Pharmacol.* **2005**, *45*, 394.
(309) Fan, S.; Smith, M. L.; Rivet, D. J., 2nd; Duba, D.; Zhan, Q.; Kohn, K. W.; Fornace, A. J., Jr.; O'Connor, P. M. *Cancer Res.* **1995**, *55*, 1649.
(310) Shapiro, G. I. *Clin. Cancer Res.* **2004**, *10*, 4270s.
(311) Guzi, T. *Curr. Opin. Invest. Drugs* **2004**, *5*, 1311.
(312) Sha, S. K.; Sato, T.; Kobayashi, H.; Ishigaki, M.; Yamamoto, S.; Sato, H.; Takada, A.; Nakajyo, S.; Mochizuki, Y.; Friedman, J. M.; Cheng, F. C.; Okura, T.; Kimura, R.; Kufe, D. W.; Vonhoff, D. D.; Kawabe, T. *Mol. Cancer Ther.* **2007**, *6*, 147.
(313) Blasina, A.; Hallin, J.; Chen, E.; Arango, M. E.; Kraynov, E.; Register, J.; Grant, S.; Ninkovic, S.; Chen, P.; Nichols, T.; O'Connor, P.; Anderes, K. *Mol. Cancer Ther.* **2008**, *7*, 2394.
(314) Tao, Z. F.; Wang, L.; Stewart, K. D.; Chen, Z.; Gu, W.; Bui, M. H.; Merta, P.; Zhang, H.; Kovar, P.; Johnson, E.; Park, C.; Judge, R.; Rosenberg, S.; Sowin, T.; Lin, N. H. *J. Med. Chem.* **2007**, *50*, 1514.
(315) Bach, S.; Knockaert, M.; Reinhardt, J.; Lozach, O.; Schmitt, S.; Baratte, B.; Koken, M.; Coburn, S. P.; Tang, L.; Jiang, T.; Liang, D. C.; Galons, H.; Dierick, J. F.; Pinna, L. A.; Meggio, F.; Totzke, F.; Schächtele, C.; Lerman, A. S.; Carnero, A.; Wan, Y.; Gray, N.; Meijer, L. *J. Biol. Chem.* **2005**, *280*, 31208.
(316) Busby, E. C.; Leistritz, D. F.; Abraham, R. T.; Karnitz, L. M.; Sarkaria, J. N.; Kawakami, K.; Futami, H.; Takahara, J.; Yamaguchi, K. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 778.
(317) Graves, P. R.; Yu, L.; Schwarz, J. K.; Gales, J.; Sausville, E. A.; O'Connor, P. M.; Piwnica-Worms, H. *J. Biol. Chem.* **2000**, *275*, 5600.
(318) Senderowicz, A. M. *Curr. Opin. Cell Biol.* **2004**, *16*, 670.
(319) Sabisz, M.; Skladanowski, A. *Curr. Pharm. Biotechnol.* **2008**, *9*, 325.
(320) Byrd, J. C.; Lin, T. S.; Dalton, J. T.; Wu, D.; Phelps, M. A.; Fischer, B.; Moran, M.; Blum, K. A.; Rovin, B.; Brooker-McEldowney, M.; Broering, S.; Schaaf, L. J.; Johnson, A. J.; Lucas, D. M.; Heerema, N. A.; Lozanski, G.; Young, D. C.; Suarez, J. R.; Colevas, A. D.; Grever, M. R. *Blood* **2007**, *109*, 399.
(321) Welch, S.; Hirte, H. W.; Carey, M. S.; Hotte, S. J.; Tsao, M. S.; Brown, S.; Pond, G. R.; Dancey, J. E.; Oza, A. M. *Gynecol. Oncol.* **2007**, *106*, 305.
(322) Malumbres, M.; Pevarello, P.; Barbacid, M.; Bischoff, J. R. *Trends Pharmacol. Sci.* **2008**, *29*, 16.
(323) Brezak, M. C.; Quaranta, M.; Mondésert, O.; Galcera, M. O.; Lavergne, O.; Alby, F.; Cazales, M.; Baldin, V.; Thurieau, C.; Harnett, J.; Lanco, C.; Kasprzyk, P. G.; Prevost, G. P.; Ducommun, B. *Cancer Res.* **2004**, *64*, 3320.
(324) Jobson, A. G.; Cardellina, J. H., 2nd; Scudiero, D.; Kondapaka, S.; Zhang, H.; Kim, H.; Shoemaker, R.; Pommier, Y. *Mol. Pharmacol.* **2007**, *2*, 876.
(325) Bryant, H. E.; Helleday, T. *Nucleic Acids Res.* **2006**, *34*, 1685.
(326) Won, J.; Kim, M.; Kim, N.; Ahn, J. H.; Lee, W. G.; Kim, S. S.; Chang, K. Y.; Yi, Y. W.; Kim, T. K. *Nat. Chem. Biol.* **2006**, *2*, 369.
(327) Rainey, M. D.; Charlton, M. E.; Stanton, R. V.; Kastan, M. B. *Cancer Res.* **2008**, *68*, 7466.
(328) Schreiber, V.; Dantzer, F.; Ame, J. C.; de Murcia, G. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7*, 517.
(329) Bryant, H. E.; Schultz, N.; Thomas, H. D.; Parker, K. M.; Flower, D.; Lopez, E.; Kyle, S.; Meuth, M.; Curtin, N. J.; Helleday, T. *Nature* **2005**, *434*, 913.
(330) Farmer, H.; McCabe, N.; Lord, C. J.; Tutt, A. N.; Johnson, D. A.; Richardson, T. B.; Santarosa, M.; Dillon, K. J.; Hickson, I.; Knights, C.; Martin, N. M.; Jackson, S. P.; Smith, G. C.; Ashworth, A. *Nature* **2005**, *434*, 917.
(331) Marminon, C.; Anizon, F.; Moreau, P.; Pfeiffer, B.; Pierré, A.; Golsteyn, R. M.; Peixoto, P.; Hildebrand, M. P.; David-Cordonnier, M. H.; Lozach, O.; Meijer, L.; Prudhomme, M. *Mol. Pharmacol.* **2008**, *74*, 1620.
(332) Syljuåsen, R. G.; Sørensen, C. S.; Hansen, L. T.; Fugger, K.; Lundin, C.; Johansson, F.; Helleday, T.; Sehested, M.; Lukas, J.; Bartek, *J. Mol. Cell. Biol.* **2005**, *25*, 3553.
(333) Takai, H.; Naka, K.; Okada, Y.; Watanabe, M.; Harada, N.; Saito, S.; Anderson, C. W.; Appella, E.; Nakanishi, M.; Suzuki, H.; Nagashima, K.; Sawa, H.; Ikeda, K.; Motoyama, N. *EMBO J.* **2002**, *21*, 5195.
(334) Matthews, D. J.; Yakes, F. M.; Chen, J.; Tadano, M.; Bornheim, L.; Clary, D. O.; Tai, A.; Wagner, J. M.; Miller, N.; Kim, Y. D.; Robertson, S.; Murray, L.; Karnitz, L. M. *Cell Cycle* **2007**, *6*, 104.
(335) Wang, W.; El-Deiry, W. S. *Curr. Opin. Oncol.* **2008**, *20*, 90.
(336) Garber, K. *J. Natl. Cancer Inst.* **2005**, *97*, 1026.
(337) Bozko, P.; Sabisz, M.; Larsen, A. K.; Skladanowski, A. *Mol. Cancer Ther.* **2005**, *4*, 2016.
(338) Carnero, A.; Blanco-Aparicio, C.; Renner, O.; Link, W.; Leal, J. F. *Curr. Cancer Drug Targets* **2008**, *8*, 187.
(339) Erenpreisa, J.; Cragg, M. S. *Cancer Cell Int.* **2001**, *1*, 1.
(340) Faivre, S.; Djelloul, S.; Raymond, E. *Semin. Oncol.* **2006**, *33*, 407.
(341) Matthews, D. J.; Yakes, F. M.; Chen, J.; Tadano, M.; Bornheim, L.; Clary, D. O.; Tai, A.; Wagner, J. M.; Miller, N.; Kim, Y. D.; Robertson, S.; Murray, L.; Karnitz, L. M. *Cell Cycle* **2007**, *6*, 104.

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u

(342) Zabludoff, S.; Deng, C.; Grondine, M. R.; Sheehy, A. M.; Ashwell, S.; Caleb, B. L.; Green, S.; Haye, H. R.; Horn, C. L.; Janetka, J. W.; Liu, D.; Mouchet, E.; Ready, S.; Rosenthal, J. L.; Queva, C.; Schwartz, G. K.; Taylor, K. J.; Tse, A. N.; Walker, G. E.; White, A. M. *Mol. Cancer Ther.* **2008**, *7*, 2955.

(343) Tillement, V.; Haren, L.; Roullet, N.; Etievant, C.; Merdes, A. *Mol. Cancer* **2009**, *8*, 10.

(344) Gumireddy, K.; Reddy, M. V.; Cosenza, S. C.; Boominathan, R.; Baker, S. J.; Papathi, N.; Jiang, J.; Holland, J.; Reddy, E. P. *Cancer Cell* **2005**, *7*, 275.

(345) Toogood, P. L.; Harvey, P. J.; Repine, J. T.; Sheehan, D. J.; VanderWel, S. N.; Zhou, H.; Keller, P. R.; McNamara, D. J.; Sherry, D.; Zhu, T.; Brodfuehrer, J.; Choi, C.; Barvian, M. R.; Fry, D. W. *J. Med. Chem.* **2005**, *48*, 2388.

(346) Joshi, K. S.; Rathos, M. J.; Joshi, R. D.; Sivakumar, M.; Mascarenhas, M.; Kamble, S.; Lal, B.; Sharma, S. *Mol. Cancer Ther.* **2007**, *6*, 918.

(347) Chu, X. J.; DePinto, W.; Bartkovitz, D.; So, S. S.; Vu, B. T.; Packman, K.; Lukacs, C.; Ding, Q.; Jiang, N.; Wang, K.; Goelzer, P.; Yin, X.; Smith, M. A.; Higgins, B. X.; Chen, Y.; Xiang, Q.; Moliterni, J.; Kaplan, G.; Graves, B.; Lovey, A.; Fotouhi, N. *J. Med. Chem.* **2006**, *49*, 6549.

(348) Misra, R. N.; Xiao, H. Y.; Kim, K. S.; Lu, S.; Han, W. C.; Barbosa, S. A.; Hunt, J. T.; Rawlins, D. B.; Shan, W.; Ahmed, S. Z.; Qian, L.; Chen, B. C.; Zhao, R.; Bednarz, M. S.; Kellar, K. A.; Mulheron, J. G.; Batorsky, R.; Roongta, U.; Kamath, A.; Marathe, P.; Ranadive, S. A.; Sack, J. S.; Tokarski, J. S.; Pavletich, N. P.; Lee, F. Y.; Webster, K. R.; Kimball, S. D. *J. Med. Chem.* **2004**, *47*, 1719.

(349) Dickson, M. A.; Schwartz, G. K. *Curr. Oncol.* **2009**, *16*, 36.

(350) Wyatt, P. G.; Woodhead, A. J.; Berdini, V.; Boulstridge, J. A.; Carr, M. G.; Cross, D. M.; Davis, D. J.; Devine, L. A.; Early, T. R.; Feltell, R. E.; Lewis, E. J.; McMenamin, R. L.; Navarro, E. F.; O'Brien, M. A.; O'Reilly, M.; Reule, M.; Saxty, G.; Seavers, L. C.; Smith, D. M.; Squires, M. S.; Trewartha, G.; Walker, M. T.; Woolford, A. J. *J. Med. Chem.* **2008**, *51*, 4986.

CR900026U

Downloaded by UNIV MAASTRICHT on August 28, 2009 | http://pubs.acs.org
Publication Date (Web): June 12, 2009 | doi: 10.1021/cr900026u