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G1 Tetraploidy Checkpoint and the Suppression of Tumorigenesis

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Abstract Checkpoints suppress improper cell cycle progression to ensure that cells maintain the integrity of their genome. During mitosis, a metaphase checkpoint requires the integration of all chromosomes into a metaphase array in the mitotic spindle prior to mitotic exit. Still, mitotic errors occur in mammalian cells with a relatively high frequency. Metaphase represents the last point of control in mitosis. Once the cell commits to anaphase there are no checkpoints to sense segregation defects. In this context, we will explore our recent finding that non-transformed mammalian cells have a checkpoint that acts subsequent to mitotic errors to block the proliferation of cells that have entered G1 with tetraploid status. This arrest is dependent upon both p53 and pRb, and may represent an important function of both p53 and pRb as tumor suppressors. Further, we discuss the possibility that this mechanism may similarly impose G1 arrest in cells that become aneuploid through errors in mitosis. J. Cell. Biochem. 88: 673–683, 2003.

Key words: aneuploidy; tetraploid checkpoint; p53; pRb; genomic instability

Recent research has identified checkpoints throughout the cell cycle that act to preserve the integrity of the genome. Checkpoints at G1 and G2 act to ensure repair of damaged DNA prior to progression to S-phase and mitosis, respectively [Kastan et al., 1992]. Other checkpoints ensure completion of DNA replication and DNA decatenation prior to mitotic entry [Clarke and Gimenez-Abian, 2000]. During mitosis, the metaphase checkpoint acts to ensure that all chromosomes are aligned prior to anaphase entry and that the mitotic machinery, including the mitotic spindle and kinetochores, is prepared for accurate chromosome segregation [Gorbsky, 2001]. Despite the action of these checkpoints, errors in mitosis occur at a relatively high frequency [Lengauer et al., 1997; Cimini et al., 2001]. Errors in mitosis can result either in aneuploidization, or tetraploidization through a cytokinetic failure produced by bridged chromosomes [Andreassen et al., 1996].

Aneuploidization and chromosomal instability (CIN) are hallmarks of human tumor development [Cahill et al., 1999], and tetraploidization is a frequent precursor of aneuploidy in tumor development [Shackney et al., 1989]. Recently, we have identified a checkpoint that arrests in G1 cells that become tetraploid due to errors in mitosis [Andreassen et al., 2001a; Borel et al., 2002]. This process appears to enhance the fidelity of cell proliferation by blocking the propagation of cells that become tetraploid despite the action of other cell cycle checkpoints. This result suggests that two tandem checkpoint mechanisms cooperate to ensure euploid cell progeny: one that delays completion of individual steps of division until the process can be completed accurately, and a second that arrests cells in which the first process has failed. Here we discuss mechanisms of tetraploidization and aneuploidization and mechanisms of the tetraploidy checkpoint. Further, we consider the consequences of the tetraploidy checkpoint for tumor chemotherapy and whether a similar checkpoint may impose G1 arrest in cells which become aneuploid through mitotic errors.

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ANEUPLOIDIZATION AND CANCER

Aneuploidy and CIN are characteristic of the great majority of human tumors [Cahill et al., 1999], and are linked to the progressive development of high-grade, invasive tumors [Sandberg, 1977; Rabinovitch et al., 1989]. Additionally, a high degree of aneuploidy is correlated with a poor patient prognosis [Schutte et al., 1987; Stephenson et al., 1987]. Recent evidence suggests that aneuploidy may be a necessary intermediate in the formation of many solid human tumors [Li et al., 2000].

Tetraploidy is frequently an intermediate in tumor progression toward aneuploid status. In many human carcinomas, cells with tetraploid DNA content arise as an early step in tumorigenesis and precede the formation of aneuploid cells [Shackney et al., 1989]. Examples of human tumors that develop in this fashion are esophageal adenocarcinoma [Reid et al., 1987; Rabinovitch et al., 1989] and cervical carcinoma [Heselmeyer et al., 1996]. Tetraploidization also occurs as an intermediate step prior to aneuploidization and tumor formation in rodent tumor model systems [Ornitz et al., 1987]. Thus, one pathway to tumor formation clearly involves tetraploidization, leading to the development of aneuploidy, from which clones with the capacity for tumor growth can develop [Nowell, 1976].

G1 TETRAPLOIDY CHECKPOINT

Our recent work has demonstrated that a surveillance mechanism reads tetraploid status in G1 in non-transformed mammalian cells, and blocks cell cycle progression in this circumstance [Andreassen et al., 2001a]. Further, the control that prevents cell cycle progression past G1 tetraploidy requires intact p53 and pRB function [Andreassen et al., 2001a; Borel et al., 2002]. p53 and pRB are the principal gatekeepers of the pathway of activation of S phase and entry into a new cell cycle. p53 dependent response is of paramount importance to cell cycle arrest following DNA damage, and it in turn controls pRB release of transcription factors of the E2F family that are required for S phase entry [reviewed in Weinberg, 1995; Harbour and Dean, 2000]. While p53 is induced in response to DNA damage [Kastan et al., 1991], it also can be induced by other G1 stresses, including alterations in the cell's cytoskeleton [Trielli et al., 1996] and nucleoside metabolism [Linke et al., 1996]. In comparison to the arrest of cells in response to DNA damage due to gamma irradiation, the capacity to arrest in response to tetraploid status can be more profound and more durable [Andreassen et al., 2001b].

It is striking that neither p53 null status nor pRB suppression leads to gross aneuploidy in the absence of other metabolic defects [Borel et al., 2002; Bunz et al., 2002], despite the fact that elements of these pathways are suppressed in almost all tumors [reviewed in Weinberg, 1995; Sherr, 1996] and despite the fact that aneuploidy is a hallmark of tumorigenesis.

To explain this phenomenon, we have proposed [Andreassen et al., 2001a,b; Borel et al., 2002] that the gross aneuploidy that often accompanies tumorigenesis arises through a two-step process. The first step involves an aberrant mitotic exit to either aneuploid or tetraploid status in G1, and the second step, the absence of a G1 surveillance mechanism that normally would prevent cell cycle progression of cells with an abnormal chromosome complement (Fig. 1). The first failure could arise from any of a number of checkpoint failures, or from abnormal mitosis or cell cleavage despite normal surveillance mechanisms. The second failure would arise from the suppression of either p53 or pRB dependent G1 surveillance mechanisms.

A major unresolved question concerning the tetraploidy arrest phenomenon involves the nature of the arrest signal. To arrest in G1 with an abnormal genome, does the cell recognize molecular cues that arise from abnormal chromosome numbers, or from abnormal centrosome numbers, or potentially from both? Although we have demonstrated G1 arrest in tetraploid cells, the capacity of aneuploidy to create G1 arrest remains to be established. A capacity of aneuploidy to arrest cells in G1, if true, would suggest that each chromosome (or at least one or more specific chromosomes) contains a checkpoint inducing mechanism when present in aneuploid numbers. Additionally, as tetraploidy following mitotic or cleavage failure is accompanied by the inheritance of double the normal centrosome number, centrosomes could also, in principle, transmit a signal indicating their presence in abnormal quantity. The finding that the absence of any centrosome induces G1 arrest [Hinchcliffe et al., 2001; Khodjakov and Rieder, 2001] demonstrates

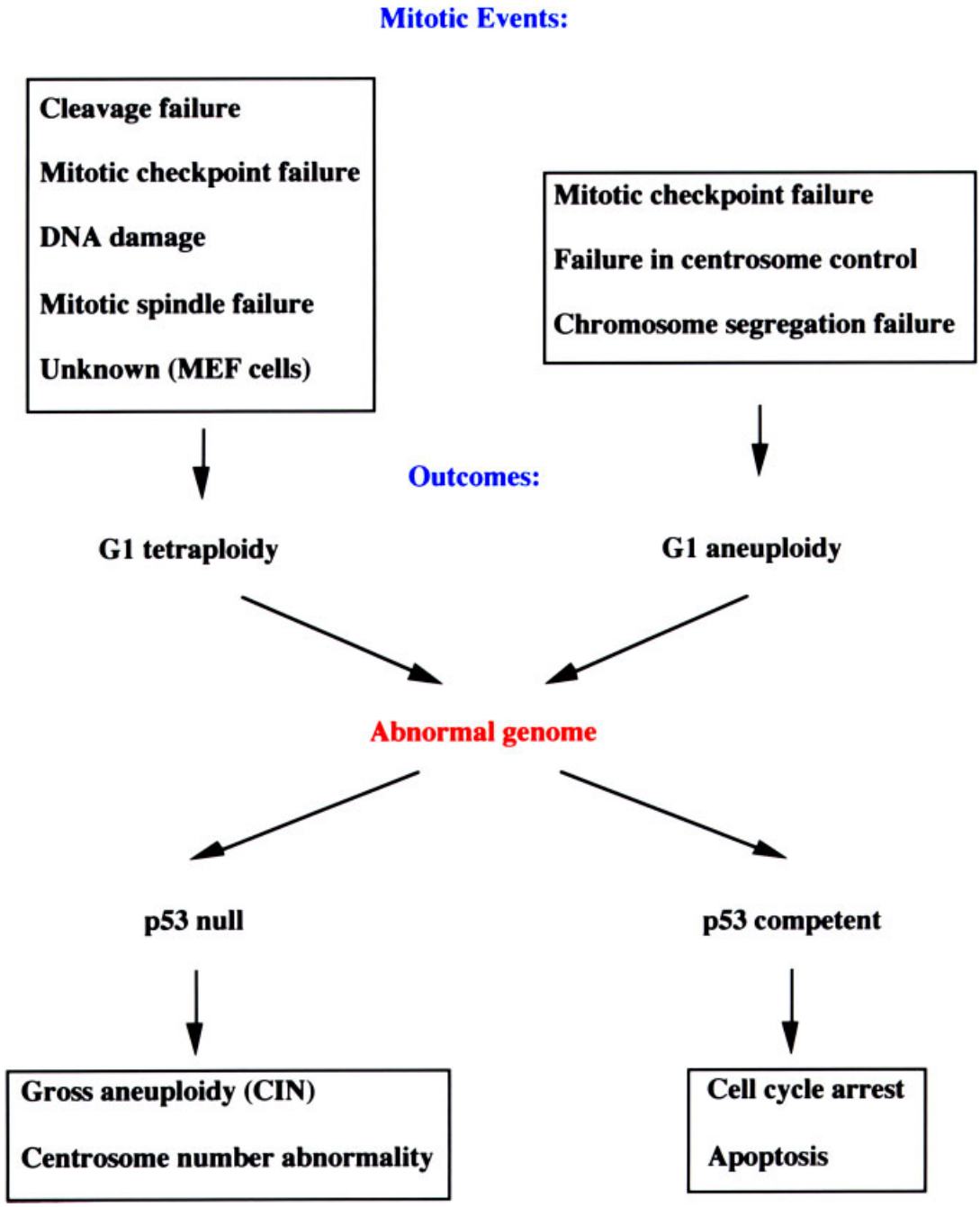

Fig. 1. Cartoon of a proposed two-step process for the generation of cells proliferating with gross aneuploidy. The first event is a failure in mitosis or cleavage generating tetraploidy or aneuploidy. The second event is failure of surveillance by p53 or pRb in tetraploid (or aneuploid) G1, permitting cell cycle progression. The G1 arrest of tetraploid cells following mitotic errors has been demonstrated [Andreassen et al., 2001a; Borel et al., 2002], while a similar mechanism in response to aneuploidy remains hypothetical. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the potential of centrosome number in signaling tetraploid arrest.

Generation of Tetraploidy

The means by which tetraploidy arises during tumorigenesis is unknown. However, there are various mechanisms by which it might arise. For example, tetraploidy can be induced by events that either disrupt chromosome segregation during mitosis, or disrupt the cell cleavage required for the physical formation of two daughter cells [Andreassen et al., 1996].

Also, failure of checkpoint control during mitosis can lead to the induction of tetraploidy [Andreassen et al., 1996, 2001a; Lanni and Jacks, 1998].

Mitosis in eukaryotic cells is universally controlled by the cdc2 kinase [reviewed by Nurse, 1990]. Various mitotic checkpoints [reviewed by Gorbsky, 2001] act at prophase and metaphase to ensure that the inactivation of cdc2 and the onset of anaphase occur only after all chromosomes are aligned at the metaphase plate. Mitotic arrest ensues if the mitotic spindle cannot form [Andreassen and Margolis, 1994; Lanni and Jacks, 1998] or if kinetochores cannot interact normally with spindle microtubules [Rieder et al., 1994; Tomkiel et al., 1994; Nicklas et al., 1995; Skoufias et al., 2001]. These mitotic checkpoints are under the control of several centromere associated proteins, including bub1, mad2, and bubR1 [Li and Benezra, 1996; Taylor and McKeon, 1997; Cahill et al., 1998; Gorbsky et al., 1998], that interact as a complex [Sharp-Baker and Chen, 2001; Sudakin et al., 2001] and regulate progression from metaphase into anaphase by reading spindle attachment and/or tension at the kinetochore. Repressed mad2 function can induce mitotic exit without chromosome segregation [Gorbsky et al., 1998], thus creating tetraploid G1 cells. Compromised bub1 function also leads to tetraploidy in drug arrested mitotic cells [Taylor and McKeon, 1997]. The bub1 dominant negative mutant not only generates tetraploidy in this way, but also inhibits apoptosis that frequently results from spindle malfunction [Taylor and McKeon, 1997]. It is of substantial interest, in this respect, that bubR1 mutation is reported to be frequent in colorectal cancer cells exhibiting a CIN phenotype [Cahill et al., 1998]. Also, a human T cell leukemia virus-1 oncoprotein, Tax, interferes with mad1 function, and, therefore, with the function of the mad1 binding partner mad2 in HTLV-1 transformed cells [Jin et al., 1998].

Tetraploid cells may also be generated through failure to repair DNA damage in G2. DNA damage typically creates a p53 dependent pause or arrest in the G1 and G2 phases of the cell cycle [Harper et al., 1993; Bunz et al., 1998]. However, failure of DNA repair, combined with cell cycle progression in the absence of absolute G2 arrest, will create massive chromosome bridging in anaphase of mitosis, resulting in tetraploid G1 cells [Andreassen et al., 2001b].

After the onset of anaphase, cleavage furrow formation coordinates with chromosome segregation and is completed at about the same time that chromosomes decondense and nuclei reform [Chaudhary and Courvalin, 1993]. During anaphase, DNA decatenation permits separation of the daughter chromatids. If chromatid arm separation fails because decatenation has not been completed, the resulting chromatin bridge prevents successful cleavage [Downes et al., 1991] and produces tetraploidy. Tetraploidy can also arise through cleavage failure after successful completion of anaphase [Andreassen et al., 2001a]. There are potentially many ways in which cleavage failure can arise by compromise of any of a large number of proteins known to play a specific role in cell cleavage.

It is of substantial interest that defects or absence of any of the class of passenger proteins that are required for cell cleavage has a pleiotrophic effect on mitotic exit. Proteins in this class include aurora B, INCENP, TD-60, ORC6, and survivin [Adams et al., 2001; Prasanth et al., 2002]. In addition to the induction of cleavage failure that follows on compromised function, suppression of function in these proteins also causes chromosome lagging, and consequent aneuploidy upon mitotic exit. Thus, progeny cells must be prepared to respond to induction of both aneuploidy and tetraploidy following passenger protein suppression.

Direct Induction of Aneuploidy in Tumor Cells

As exemplified above, aneuploidy can certainly arise without a tetraploid intermediate. Aneuploidy can be induced either through passenger protein compromise, malfunction of proteins that release chromatid cohesion in anaphase [Jallepalli et al., 2001], or through mitotic checkpoint control suppression. Following failure of a metaphase checkpoint, chromosomes not at the metaphase plate when anaphase ensues can be randomly inherited, yielding an aneuploid condition. A low level of aneuploid mitotic exit, with lagging chromosomes, is evident in normal culture cells [Cimini et al., 2001], and is exacerbated by transient mitotic arrest with nocodazole [Cimini et al., 2001]. Very low dosage taxol treatment has been reported to induce aneuploid mitotic exit in

HeLa cells [Torres and Horwitz, 1998]. Adenomatous polyposis coli (APC), a protein that associates with both microtubules and kinetochores [Nakamura et al., 2001], is frequently truncated in colon carcinomas. Expression of the truncated form induces aneuploidy with some frequency, apparently through exit from mitosis in the presence of multiple spindle defects [Fodde et al., 2001; Kaplan et al., 2001].

Failure of metaphase checkpoint control is commonly followed by anaphase progression despite the presence of several lagging or nonsegregated chromosomes [Gorbsky et al., 1998; Skoufias et al., 2001]. Such chromosome lagging has been seen in cells with compromised function of the kinetochore associated checkpoint proteins mad2 [Gorbsky et al., 1998; Waters et al., 1998] and bub1 [Taylor and McKeon, 1997]. As the outcome of such abnormalities in anaphase will be aneuploid progeny cells, a surveillance mechanism that protects against both aneuploidy and tetraploidy would ideally function in the event of aneuploid chromosome inheritance.

Aneuploidy can also arise, in principle, directly as a result of failure of centrosome control during interphase. The duplication of the centrosome normally occurs during S phase through a cdk2 dependent mechanism [reviewed by Hinchcliffe and Sluder, 2002], and is under a system of constraint that ensures there is one and only one duplication event during interphase. The nature of such precise control is not clear.

As a result of fidelity in duplication, a nontransformed cell has two centrosomes at mitosis, which dictate the formation of two spindle poles. If more than one duplication event has occurred in interphase, a multipolar spindle will result, and the genome will be segregated in an aneuploid manner.

Recent evidence has suggested that the protein kinase Mps1 may directly regulate centrosome duplication and its fidelity during S phase [Fisk and Winey, 2001]. Although such a role for Mps1 has obvious potential significance for maintenance of the integrity of the genome, the potential role for Mps1 in such centrosome control in mammalian cells is controversial [Stucke et al., 2002]. Indeed, the Mps1 protein kinase has a clear role in mitotic checkpoint control at kinetochores [Abrieu et al., 2001; Stucke et al., 2002], and compromise of its function can thus indirectly lead to aneuploidy and abnormal centrosome inheritance. In light of arguments suggesting a potential role of Mps1 in generating aneuploidy, it is worth noting that there is, to date, no evidence that Mps1 malfunction occurs in tumorigenesis.

The story of Mps1 may be a cautionary tale. In addition to Mps1, it has been reported that p53 [Fukasawa et al., 1996] and aurora A/STK15 [Zhou et al., 1998] are involved in the regulation of centrosome duplication during interphase, as centrosome number abnormalities can result from disruption of either protein's function. However, neither p53 nor aurora A has convincingly been shown to influence centrosome duplication during a single cell cycle. Indeed, both we [Borel et al., 2002] and others [Meraldi et al., 2002] have recently shown that neither p53 nor aurora A directly controls centrosome duplication. In fact, the pathway by which supernumerary centrosomes are created appears to arise principally through the creation of a tetraploid G1 cell, which then proceeds to the next mitosis in the absence of p53 function. It remains to be seen if Mps1 dependent induction of centrosome number abnormalities can be direct under some circumstances, or if supernumerary centrosomes always arise as the indirect products of mitotic checkpoint failure. What is intriguing, and perhaps misleading, is that p53 [Tarapore et al., 2001], aurora A [Zhou et al., 1998], and Mps1 [Fisk and Winey, 2001] have all been reported to associate with centrosomes and thus would potentially be in a position to locally influence centrosome duplication.

Going From Tetraploidy to Aneuploidy

There are two fundamental questions with respect to the problem of the development of aneuploidy in tumor cells. Above we have addressed how the tetraploid or aneuploid status may arise. A further essential question relates to the controls that prevent replication after the generation of tetraploidy or aneuploidy, and how the tumor cell can evade such controls. The various mechanisms of directly inducing tetraploidy or aneuploidy are summarized in Figure 1.

As described above, models for generation of aneuploidy include uncontrolled centrosome duplication and failure of mitotic checkpoints, both of which will generate abnormal chromosome distribution when the cell exits mitosis. As for evasion of controls, there is evidence that

non-transformed cells have p53 dependent controls that prevent cell cycle progression past G1 if there is a failure of spindle function [Minn et al., 1996; Lanni and Jacks, 1998]. This mechanism, called a "spindle assembly" checkpoint, does not influence mitotic arrest but instead mediates either apoptosis or arrest in G1 following evasion of mitotic arrest by inhibitors of microtubule assembly [Minn et al., 1996; Lanni and Jacks, 1998; Andreassen et al., 2001a].

In recent work [Andreassen et al., 2001a,b; Borel et al., 2002], we have demonstrated that the spindle assembly checkpoint appears to be a part of a general p53 dependent checkpoint control that acts in G1 to recognize tetraploid cells and induce their arrest. Although we have demonstrated that a tetraploidy checkpoint exists, there is a real possibility that low-level aneuploidy in G1 may be read equally well by p53 surveillance. In this light, it is of great interest to note that APC-deficient tumors progressively accumulate mutations in p53 [Fukasawa et al., 1996], and that APC induces persistent aneuploidy in ES cells which have defects in the p53 pathway [Fodde et al., 2001].

Recently, it has become clear that tetraploid arrest depends on either intact p53 or RB pocket protein controls [Borel et al., 2002]. Tumor aneuploidy can thus arise from any failure in mitotic function followed by failure of p53 or RB pocket protein surveillance in G1. Further, it is clear from this recent work that tetraploidy can spontaneously arise in normal primary fibroblasts in culture in the absence of any specifically induced mitotic failure, and that loss of p53 or pRB pocket protein family function, alone, can thus sometimes lead to gross aneuploidy [Borel et al., 2002].

The spontaneous arrest of MEF cells that arrive in tetraploid G1 [Borel et al., 2002] raises an important issue. It is noteworthy that tetraploidy (and presumably aneuploidy) arrest is highly durable in non-transformed cells with intact p53 and pRB pocket protein function. In keeping with this result, we have previously shown that the tetraploidy arrest persists for at least 72 h after the drug that caused tetraploidy (either nocodazole or dihydrocytochalasin B (DCB)) has been removed from culture medium. This ruled out the possibility that arrest in G1 was a direct consequence of the drug acting on the cytoskeleton [Andreassen et al., 2001a]. Similarly, whereas taxol induces G1 arrest in diploid non-transformed cells, it is readily reversed when the drug is removed. But tetraploid G1 cells generated by mitotic exit in taxol remain arrested indefinitely after drug removal [Trielli et al., 1996].

p53 and the RB Pocket Proteins Both Prevent Aneuploidy

As described above, both our work [Borel et al., 2002] and that of others [Meraldi et al., 2002] has recently ruled out the possibility that p53 directly regulates centrosome duplication. These results have established the central importance of the tetraploidy checkpoint, and potentially an aneuploidy checkpoint, in maintaining correct centrosome number as well as euploidy.

Importantly, our work also established that suppression of the RB pocket proteins has an effect equivalent to that of loss of p53 on bypass of the tetraploidy checkpoint and induction of aneuploidy and abnormal centrosome numbers [Borel et al., 2002]. Our work thus demonstrates that the p53 control of tetraploidy arrest and centrosome duplication operates through the circuitry of control of the phosphorylation status of the RB proteins, and the consequent induction of S phase through activation of the E2F family of transcription factors [reviewed in Weinberg, 1995; Harbour and Dean, 2000].

Such G1 arrest signals are thus parallel, in part, to those imposed by DNA damage. Like the tetraploidy checkpoint, the p53/ $p21^{WAF1}$ DNA damage response pathway converges on the RB pocket proteins [Dannenberg et al., 2000; Sage et al., 2000]. There is, however, an important difference. Unlike DNA damage arrest, tetraploidy arrest is durable, which can be understood in the context of the fact that tetraploidy or aneuploidy is an insult to the genome that cannot be repaired, and that continuation in the cell cycle will lead to catastrophic aneuploidization of progeny cells.

An important question that remains to be addressed is how two proteins, p53 and $p21^{WAF1}$, that normally turn over at a relatively high rate [Haupt et al., 1997; Kubbutat et al., 1997], can persist in a stable state in arrested cells. Focus of inquiry would naturally first fall on the role of p14ARF/p19ARF (human and mouse proteins, respectively), a G1 control protein that has been associated with durable G1 arrest through p53 stabilization [Kamijo et al., 1997; Weber et al.,

1999]. p14ARF/p19ARF is induced by senescence in MEF cells [Kamijo et al., 1997, 1999], and can be induced by spindle failure and entry into tetraploid G1 [Khan et al., 2000], but is not activated by DNA damage [Kamijo et al., 1997, 1999; Stott et al., 1998]. The p16INK4A/p14ARF/p19ARF locus is inactivated by mutation with high frequency in tumors, suggesting an essential role in such a genome stabilizing event as G1 arrest following on induction of aneuploidy or tetraploidy.

The argument has indeed been made that retention of ARF in tumor cell lines necessitates the loss of p53 for the line to be immortal [Stott et al., 1998]. The mechanism is clearly not so simple, however, as induced ARF expression arrests p53/MDM2/ARF triple knockout MEF cells in a replicative senescent G1 [Weber et al., 2000], and thus can function in cell cycle arrest independent of p53 status. Further, in some cells p16INK4A is tightly linked to p53 dependent senescence whereas ARF is not [Schmitt et al., 2002].

It is of great interest that there is a discrepancy between human and mouse cells with respect to the correlation of ARF loss with replicative senescence [Drayton and Peters, 2002]. Mouse cells must lose ARF to bypass senescence, while human cells do not have this requirement. We have demonstrated that the mouse non-transformed model, MEF cells, undergoes an unusual early senescence accompanied by augmentation of tetraploid G1 status [Borel et al., 2002]. Similar tetraploid senescence is not seen in human cell lines. It will be of substantial interest to determine if the discrepancy observed relates to the specific requirement for ARF induction in response to tetraploidy in G1.

Consequences for DNA Damage Induced Arrest

A central function of p53 and $p21^{WAF1}$ control is to prevent cell cycle progression following DNA damage [Kastan et al., 1991; Harper et al., 1993]. p53 and $p21^{WAF1}$ competent cells normally arrest in G1 and G2 phases of the cell cycle to permit repair of DNA strand breaks [Harper et al., 1993; Bunz et al., 1998]. Interestingly, we have recently found [Andreassen et al., 2001b] that cell cycle arrest in the human colon carcinoma HCT116 model system is transient in both G1 and G2 following DNA damage, and that it is not principally dependent on the function of p53 and $p21^{WAF1}$. Nor is the time of delay necessarily sufficient to permit repair before the cell cycle recommences. As a consequence, cells with inadequate repair proceed to a mitotic catastrophe, in which massive bridging prevents chromosome segregation. The result is that the progeny cell is tetraploid in G1. This cell arrests durably with a tetraploid status, and this tetraploid arrest is fully dependent on the presence of functional p53 and $p21^{WAF1}$ [Andreassen et al., 2001b].

If confirmed to be a widespread phenomenon, this result suggests that the function of p53 and $p21^{WAF1}$ is more important to preventing progression past tetraploidy in G1 than it is in preventing progression past DNA damage. If true, the rationale for this phenomenon is not difficult to imagine. DNA damage does not inevitably affect the integrity of the genome, whereas tetraploidy or aneuploidy induction permanently alters the genomic complement of DNA.

Consequences for Tumor Chemotherapy

Can the fact that cells with intact p53/$p21^{WAF1}$ and pRB pathways arrest in tetraploid G1, whereas cells with defects in these pathways do not, be exploited for tumor chemotherapy? As mentioned above, virtually all tumors are defective in either p53/$p21^{WAF1}$ or in RB pocket protein controls (reviewed in Weinberg, 1995; Sherr, 1996). We have recently found [Lohez et al., manuscript submitted] that fibroblasts exposed to inhibitors of actin assembly arrest transiently in G1 if they have intact RB pocket protein function, but will not arrest if the RB proteins are suppressed. As a consequence, non-transformed cells block in euploid G1 and in tetraploid G1 in the presence of a drug such as cytochalasin, whereas RB pocket protein suppressed cells do not arrest in either state. The RB pocket protein suppressed cells progress toward massive death, as most aneuploid progeny are non-viable, while non-transformed cells recover from the drug arrest in euploid G1, and proceed to proliferate normally. The 'trick' of this highly selective death is that the non-transformed cells that become tetraploid are incapable of proliferating, so only the euploid G1 cells become the progenitors of the recovering population (Fig. 2).

This result suggests that the tetraploidy checkpoint may well be exploited, in a similar manner, to induce cell death that is highly selective for tumor cells with compromised p53

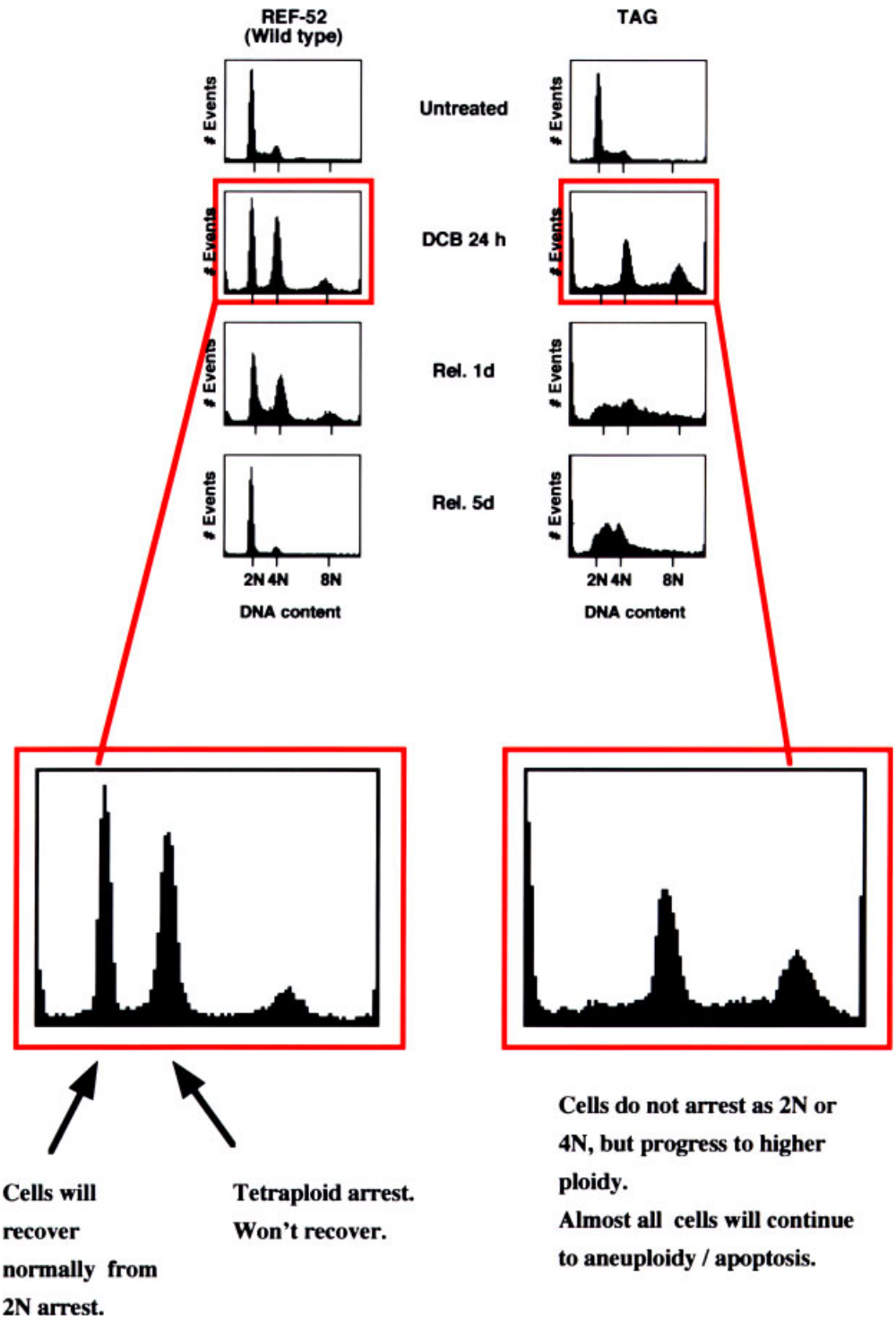

Fig. 2. Outcome of exposure of non-transformed fibroblasts (REF-52) or their T-antigen transformed variants (TAG) to dihydrocytochalasin B (DCB). Asynchronous REF-52 and TAG cells were exposed to 5 μM DCB for 24 h. Flow cytometric analysis of DNA content at the indicated times shows that TAG cells (**right**) rapidly become aneuploid upon release from DCB, as indicated by cells with a broadly distributed DNA content, ranging between 2N and 4N (see Rel. 1d, 5d), whereas REF-52 cells (**left**) recover from DCB arrest and maintain euploidy. Further analysis has revealed that the TAG cells rapidly die following entry into an aneuploid state, while the 2N arrested REF-52 remain viable. The 4N REF-52 remain indefinitely arrested, due to the tetraploidy checkpoint [Andreassen et al., 2001a] and thus do not contribute to the recovery population. In contrast, mutation of p53 or pRB permits progression to aneuploidy and cell death upon recovery from DCB arrest [Andreassen et al., 2001a; Borel et al., 2002]. The gray boxes indicate figure parts and their enlargements, in which we describe the outcome for different cell populations following DCB treatment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

or RB pocket protein function. An example of this is the demonstration by Waldman et al. [1996] that human colon carcinoma cells deficient for $p21^{WAF1}$ are selectively killed by DNA damaging agents. Using these same cells, we have shown that the selective killing of $p21^{WAF1}$-deficient cells is associated with a failure of the G1 tetraploidy checkpoint [Andreassen et al., 2001b].

RELATIONSHIP BETWEEN MECHANISM OF THE TETRAPLOIDY CHECKPOINT AND THE EXISTENCE OF AN ANEUPLOIDY CHECKPOINT

In this review, we have addressed several important unresolved questions related to the role that the G1 tetraploidy checkpoint plays in tumor suppression. Chief among these is whether a similar mechanism normally prevents the further replication of aneuploid cells. Given the predominant aneuploid status among tumors, such a mechanism would be vital to tumor suppression. Also unresolved is the nature of the stimulus that induces G1 tetraploidy arrest. Possibilities include chromosome counting or centrosome counting mechanisms. The mechanism that underlies the tetraploidy checkpoint has important implications for whether a G1 aneuploidy checkpoint exists. If an aneuploidy checkpoint exists, it will be fascinating to determine its threshold. If this checkpoint is capable of recognizing even small changes in the genome, then p53 or RB would have to be suppressed in order for a cell with microsatellite instability to proliferate. It will be of great interest to know whether microsatellite instability is thus associated with suppression of the p53 and RB pathways.

REFERENCES

Abrieu A, Magnaghi-Jaulin L, Kahana JA, Peter M, Castro A, Vigneron S, Lorca T, Cleveland DW, Labbe JC. 2001. Mps1 is a kinetochore-associated kinase essential for the vertebrate mitotic checkpoint. Cell 106: 83–93.

Adams RR, Carmena M, Earnshaw WC. 2001. Chromosomal passengers and the (aurora) ABCs of mitosis. Trends Cell Biol 11:49–54.

Andreassen PR, Margolis RL. 1994. Microtubule dependency of $p34^{cdc2}$ inactivation and mitotic exit in mammalian cells. J Cell Biol 127:789–802.

Andreassen PR, Martineau SN, Margolis RL. 1996. Chemical induction of mitotic checkpoint override in mammalian cells results in aneuploidy following a transient tetraploid state. Mutat Res 372:181–194.

Andreassen PR, Lohez OD, Lacroix FB, Margolis RL. 2001a. Tetraploid state induces p53-dependent arrest of nontransformed mammalian cells in G1. Mol Biol Cell 12:1315–1328.

Andreassen PR, Lacroix FB, Lohez OD, Margolis RL. 2001b. Neither $p21^{WAF1}$ nor 14-3-3sigma prevents G2 progression to mitotic catastrophe in human colon carcinoma cells after DNA damage, but $p21^{WAF1}$ induces stable G1 arrest in resulting tetraploid cells. Cancer Res 61:7660–7668.

Borel F, Lohez OD, Lacroix FB, Margolis RL. 2002. Multiple centrosomes arise from tetraploidy checkpoint failure and mitotic centrosome clusters in p53 and RB pocket protein-compromised cells. Proc Natl Acad Sci USA 99:9819–9824.

Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. 1998. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282:1497–1501.

Bunz F, Fauth C, Speicher MR, Dutriaux A, Sedivy JM, Kinzler KW, Vogelstein B, Lengauer C. 2002. Targeted inactivation of p53 in human cells does not result in aneuploidy. Cancer Res 62:1129–1133.

Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. 1998. Mutations of mitotic checkpoint genes in human cancers. Nature 392:300–303.

Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. 1999. Genetic instability and Darwinian selection in tumours. Trends Cell Biol 9:M57–M60.

Chaudhary N, Courvalin J-C. 1993. Stepwise reassembly of the nuclear envelope at the end of mitosis. J Cell Biol 122:295–306.

Cimini D, Howell B, Maddox P, Khodjakov A, Degrassi F, Salmon ED. 2001. Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. J Cell Biol 153:517–527.

Clarke DJ, Gimenez-Abian JF. 2000. Checkpoints controlling mitosis. Bioessays 22:351–363.

Dannenberg JH, van Rossum A, Schuijff L, te Riele H. 2000. Ablation of the retinoblastoma gene family deregulates G(1) control causing immortalization and increased cell turnover under growth-restricting conditions. Genes Dev 14:3051–3064.

Downes CS, Mullinger AM, Johnson RT. 1991. Inhibitors of DNA topoisomerase II prevent chromatid separation in mammalian cells but do not prevent exit from mitosis. Proc Natl Acad Sci USA 88:8895–8899.

Drayton S, Peters G. 2002. Immortalisation and transformation revisited. Curr Opin Genet Dev 12:98–104.

Fisk HA, Winey M. 2001. The mouse Mps1p-like kinase regulates centrosome duplication. Cell 106:95–104.

Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, van Es JH, Breukel C, Wiegant J, Giles RH, Clevers H. 2001. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat Cell Biol 3: 433–438.

Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF. 1996. Abnormal centrosome amplification in the absence of p53. Science 271:1744–1747.

Gorbsky GJ. 2001. The mitotic spindle checkpoint. Curr Biol 11:R1001–R1004.

Gorbsky GJ, Chen RH, Murray AW. 1998. Microinjection of antibody to Mad2 protein into mammalian cells in

mitosis induces premature anaphase. J Cell Biol 141: 1193–1205.
Harbour JF, Dean DC. 2000. The Rb/E2F pathway: Expanding roles and emerging paradigms. Genes Dev 14:2393–2409.
Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805–816.
Haupt Y, Maya R, Kazaz A, Oren M. 1997. Mdm2 promotes the rapid degradation of p53. Nature 387:296–299.
Heselmeyer K, Schrock E, Du Manoir S, Blegen H, Shah K, Steinbeck R, Auer G, Reid T. 1996. Gain of chromosome 3q defines the transition from cervical dysplasia to invasive carcinoma of the uterine cervix. Proc Natl Acad Sci USA 93:479–484.
Hinchcliffe EH, Sluder G. 2002. Two for two: Cdk2 and its role in centrosome doubling. Oncogene 21:6154–6160.
Hinchcliffe EH, Miller FJ, Cham M, Khodjakov A, Sluder G. 2001. Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. Science 291: 1547–1550.
Jallepalli PV, Waizenegger IC, Bunz F, Langer S, Speicher MR, Peters JM, Kinzler KW, Vogelstein B, Lengauer C. 2001. Securin is required for chromosomal stability in human cells. Cell 105:445–457.
Jin DY, Spencer F, Jeang KT. 1998. Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. Cell 93:81–91.
Kamijo T, Zindy F, Roussel MF, Quelle DE, Downing JR, Ashmun RA, Grosveld G, Sherr CJ. 1997. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell 91:649–659.
Kamijo T, van de Kamp E, Chong MJ, Zindy F, Diehl JA, Sherr CJ, McKinnon PJ. 1999. Loss of the ARF tumor suppressor reverses premature replicative arrest but not radiation hypersensitivity arising from disabled atm function. Cancer Res 59:2464–2469.
Kaplan KB, Burds AA, Swedlow JR, Bekir SS, Sorger PK, Nathke IS. 2001. A role for the adenomatous polyposis coli protein in chromosome segregation. Nat Cell Biol 3: 429–432.
Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51:6304–6311.
Kastan MB, Zhan Q, el-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ, Jr. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71:587–597.
Khan SH, Moritsugu J, Wahl GM. 2000. Differential requirement for p19ARF in the p53-dependent arrest induced by DNA damage, microtubule disruption, and ribonucleotide depletion. Proc Natl Acad Sci USA 97: 3266–3271.
Khodjakov A, Rieder CL. 2001. Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. J Cell Biol 153:237–242.
Kubbutat MH, Jones SN, Vousden KH. 1997. Regulation of p53 stability by Mdm2. Nature 387:299–303.
Lanni JS, Jacks T. 1998. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. Mol Cell Biol 18:1055–1064.
Lengauer C, Kinzler KW, Vogelstein B. 1997. Genetic instability in colorectal cancers. Nature 386:623–627.
Li Y, Benezra R. 1996. Identification of a human mitotic checkpoint gene: hsMAD2. Science 274:246–248.
Li R, Sonik A, Stindl R, Rasnick D, Duesberg P. 2000. Aneuploidy vs. gene mutation hypothesis of cancer: Recent study claims mutation but is found to support aneuploidy. Proc Natl Acad Sci USA 97:3236–3241.
Linke SP, Clarkin KC, Di Leonardo A, Tsou A, Wahl GM. 1996. A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev 10:934–947.
Meraldi P, Honda R, Nigg EA. 2002. Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53−/− cells. EMBO J 21: 483–492.
Minn AJ, Boise LH, Thompson CB. 1996. Expression of Bcl-xl and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. Genes Dev 10:2621–2631.
Nakamura M, Zhou XZ, Lu KP. 2001. Critical role for the EB1 and APC interaction in the regulation of microtubule polymerization. Curr Biol 11:1062–1067.
Nicklas RB, Ward SC, Gorbsky GJ. 1995. Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. J Cell Biol 130:929–939.
Nowell PC. 1976. The clonal evolution of tumor cell populations. Science 194:23–28.
Nurse P. 1990. Universal control regulating onset of M-phase. Nature 344:503–508.
Ornitz DM, Hammer RE, Messing A, Palmiter RD, Brinster RL. 1987. Pancreatic neoplasia induced by SV40 T-antigen expression in acinar cells of transgenic mice. Science 238:188–193.
Prasanth SG, Prasanth KV, Stillman B. 2002. Orc6 involved in DNA replication, chromosome segregation, and cytokinesis. Science 297:1026–1031.
Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. 1989. Progression to cancer in Barrett's esophagus is associated with genomic instability. Lab Invest 60:65–71.
Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS. 1987. Barrett's esophagus: Correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. Gastroenterology 93:1–11.
Rieder CL, Schultz A, Cole R, Sluder G. 1994. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. J Cell Biol 127:1301–1310.
Sage J, Mulligan GJ, Attardi LD, Miller A, Chen S, Williams B, Theodorou E, Jacks T. 2000. Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. Genes Dev 14:3037–3050.
Sandberg AA. 1977. Chromosome markers and progression in bladder cancer. Cancer Res 37:2950–2956.
Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, Lowe SW. 2002. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 109:335–346.

Schutte B, Reynders MM, Wiggers T, Arends JW, Volovics L, Bosman FT, Blijham GH. 1987. Retrospective analysis of the prognostic significance of DNA content and proliferative activity in large bowel carcinoma. Cancer Res 47:5494–5496.

Shackney SE, Smith CA, Miller BW, Burholt DR, Murtha K, Giles HR, Ketterer DM, Pollice AA. 1989. Model for the genetic evolution of human solid tumors. Cancer Res 49:3344–3354.

Sharp-Baker H, Chen RH. 2001. Spindle checkpoint protein Bub1 is required for kinetochore localization of Mad1, Mad2, Bub3, and CENP-E, independently of its kinase activity. J Cell Biol 153:1239–1250.

Sherr CJ. 1996. Cancer cell cycles. Science 274:1672–1677.

Skoufias DA, Andreassen PR, Lacroix FB, Wilson L, Margolis RL. 2001. Mammalian Mad2 and Bub1/BubR1 recognize distinct spindle attachment and kinetochore tension checkpoints. Proc Natl Acad Sci USA 98:4492–4497.

Stephenson RA, James BC, Gay H, Fair WR, Whitmore WF, Jr., Melamed MR. 1987. Flow cytometry of prostate cancer: Relationship of DNA content to survival. Cancer Res 47:2504–2507.

Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, Palmero I, Ryan K, Hara E, Vousden KH, Peters G. 1998. The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J 17:5001–5014.

Stucke VM, Sillje HH, Arnaud L, Nigg EA. 2002. Human Mps1 kinase is required for the spindle assembly checkpoint but not for centrosome duplication. EMBO J 21:1723–1732.

Sudakin V, Chan GK, Yen TJ. 2001. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. J Cell Biol 154:925–936.

Tarapore P, Tokuyama Y, Horn HF, Fukasawa K. 2001. Difference in the centrosome duplication regulatory activity among p53 'hot spot' mutants: Potential role of Ser 315 phosphorylation-dependent centrosome binding of p53. Oncogene 20:6851–6863.

Taylor SS, McKeon F. 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell 89:727–735.

Tomkiel J, Cooke CA, Saitoh H, Bernat RL, Earnshaw WC. 1994. CENP-C is required for maintaining proper kinetochore size and for a timely transition to anaphase. J Cell Biol 125:531–545.

Torres K, Horwitz SB. 1998. Mechanisms of Taxol-induced cell death are concentration dependent. Cancer Res 58: 3620–3626.

Trielli MO, Andreassen PR, Lacroix FB, Margolis RL. 1996. Differential Taxol-dependent arrest of transformed and nontransformed cells in the G1 phase of the cell cycle, and specific-related mortality of transformed cells. J Cell Biol 135:689–700.

Waldman T, Lengauer C, Kinzler KW, Vogelstein B. 1996. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. Nature 38:713–716.

Waters JC, Chen RH, Murray AW, Salmon ED. 1998. Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. J Cell Biol 141:1181–1191.

Weber JD, Taylor LJ, Roussel MF, Sherr CJ, Bar-Sagi D. 1999. Nucleolar Arf sequesters Mdm2 and activates p53. Nat Cell Biol 1:20–26.

Weber JD, Jeffers JR, Rehg JE, Randle DH, Lozano G, Roussel MF, Sherr CJ, Zambetti GP. 2000. p53-independent functions of the p19(ARF) tumor suppressor. Genes Dev 14:2358–2365.

Weinberg RA. 1995. The retinoblastoma protein and cell cycle control. Cell 81:323–330.

Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR, Sen S. 1998. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy, and transformation. Nat Genet 20:189–193.