Chapter 22

The Relationship Between Checkpoint Adaptation and Mitotic Catastrophe in Genomic Changes in Cancer Cells

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Chapter Outline

1. CANCER AND ITS HALLMARKS

Cancer is a complex disease that was characterized by six hallmarks in 2000 [1]. These hallmarks are sustained proliferative signaling, resisting cell death, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, and inducing angiogenesis. Following a decade of research, these hallmarks were revisited in 2011 [1], and genomic instability remained as an overarching theme in cancer cell biology. Recent studies of checkpoint adaptation and DNA damage induced by mitosis suggest that these phenomena may perpetuate genome instability and in part contribute to the vast differences in genomes present in tumors.

Of the hallmarks of cancer, sustained proliferative signaling and resisting cell death are the most relevant to understanding the relationships between checkpoint adaptation and genomic instability. It is estimated that the majority of cancers have mutations in at least one of the genes that encode the tumor-suppressor retinoblastoma (Rb), p21 or p53 [2]. The Cancer Genome Atlas (TCGA) analyzed 3281 tumors from 12 cancer types (breast adenocarcinoma, lung adenocarcinoma, lung squamous cell carcinoma, uterine corpus endometrial carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, colorectal carcinoma, bladder urothelial carcinoma, kidney renal clear cell carcinoma, ovarian serous carcinoma, and acute myeloid leukemia) for point mutations and small insertions/deletions and found that TP53 was mutated in 42% of samples, making it the most frequently mutated gene in human cancers [3]. Because cancer cells can lose the ability to regulate the cell cycle and to induce cell death, this has implications for cancer treatments. Many cancer treatments aim to inhibit cell division and induce cell death by causing irreparable amounts of DNA damage. Recent evidence about checkpoint adaptation and reexamination of older literature about the regulation of the cell cycle and genotoxic agents suggest that checkpoint adaptation has a role in contributing to genomic changes in cancer cells.

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2. THE CELL CYCLE

To divide, a cell must pass through four phases of the cell cycle: G1 (Gap 1) when cells grow and prepare for DNA synthesis; S (DNA synthesis) when cells replicate their DNA; G2 (Gap 2) when cells continue to grow and prepare for mitosis; and M (mitosis) when cells separate copies of their DNA as chromosomes and then undergo cytokinesis. To maintain the integrity of the genome during the cell cycle, several events must occur: DNA replication must be accurate; chromosomes must be distributed correctly during mitosis and cytokinesis; and damaged DNA must be detected and repaired [4].

During mitosis cells adopt a rounded morphology, known as mitotic cell rounding. Mitotic cell rounding is evolutionarily conserved and is nearly universal in metazoan and eukaryotic cells that lack a cell wall. Mitotic cell rounding is required for chromosome capture, spindle formation, and spindle stability and therefore has an important role in cell division [5]. To exhibit mitotic rounding, cells must disassemble focal adhesion complexes to decrease adhesion to their substrate and reorganize the actin cytoskeleton. The rounded morphology of mitotic cells can be used to visually distinguish mitotic cells from interphase cells. It can also be utilized to separate and collect mitotic cell populations from interphase cell populations during tissue culture by mechanical shake-off [6,7]. In experiments testing for checkpoint adaptation, cell rounding is a convenient feature that permits one to identify cells undergoing checkpoint adaptation (Fig. 22.1) and is used in addition to the detection of other mitotic markers such as histone H3 phosphorylated on serine-10 [8].

The cell cycle is highly regulated to prevent cells from transmitting damaged DNA to daughter cells. Cyclin-dependent kinases (Cdks) are the main regulators of the cell cycle and are highly conserved catalytic subunits of a family of serine/threonine kinases [9]. The mitotic cyclins, of which cyclin B is the prototype, are synthesized during S and G2 phases of the cell cycle [10]. Cyclin B binds to Cdk1 creating a Cdk1–cyclin B dimer, and the activity of this complex controls the transition between G2 phase and mitosis. Once Cdk1 is bound to cyclin B, Cdk1 is phosphorylated on threonine 161 by Cdk-activating kinase (CAK). This phosphorylation stabilizes the Cdk1–cyclin B complex and induces conformational changes necessary for kinase activity [10,11]. However, despite being bound to cyclin B, Cdk1-cyclin B is held inactive by phosphorylation on threonine 14 and tyrosine 15. Threonine 14 is phosphorylated by Myt1 kinase and tyrosine 15 is phosphorylated by Wee1 kinase [12]. These phosphate groups prevent Cdk1 from binding to and hydrolyzing adenosine triphosphate (ATP) [10], preventing the transfer of a phosphate group from ATP onto a Cdk1 substrate. The phosphate groups on threonine 14 and tyrosine 15 are removed by Cdc25 phosphatases, promoting entry into mitosis [13]. Once active, Cdk1-cyclin B phosphorylates the Cdk1 inhibitor Wee1 and the Cdk1-activating Cdc25 phosphatases through two different feedback loops [14]. Interestingly, the overexpression of only cyclin B and Cdc25 phosphatase is necessary to cause cells in G2 phase to prematurely enter mitosis [15].

The oscillating nature of cyclin B levels can be used to detect cells that are capable of entering mitosis. This is because cyclin B is synthesized through S and G2 phases of the cell cycle and then degraded at the metaphase–anaphase transition of mitosis [16]. Cdk1 phosphorylation is also a good measure of whether cells have active Cdk1 or not; if cells have high levels of tyrosine 15–phosphorylated Cdk1, then they do not contain enough active Cdk1 to be in mitosis [7,10]. Additionally, to detect Cdk1 activity, a specific Cdk1 activity assay can be performed. In 2013, Lewis et al. published a protocol to detect Cdk1 activity by using western blotting to quantify the amount of phosphorylation of threonine 320 on an artificial Cdk1 substrate consisting of glutathione *S*-transferase (GST) and amino acids 316–324 from the PP1-alpha catalytic subunit (PP1Cα) [17].

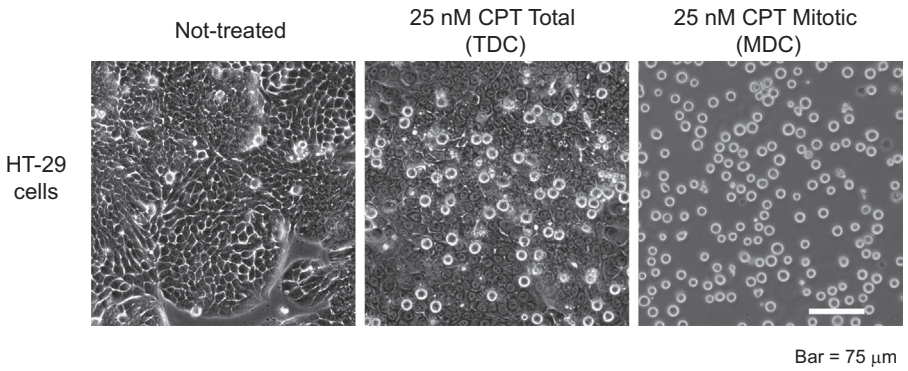

FIGURE 22.1 Human cancer cells undergo checkpoint adaptation. HT-29 human colon cancer cells were either not treated (left panel) or treated by 25 nM camptothecin (CPT) for 48 h (TDC; total and DNA-damaged cells, middle panel); 25 nM CPT is a cytotoxic concentration in which the cells die by 96 h. In a separate experiment, the rounded, mitotic cells (MDC; mitotic and DNA-damaged cells) from a TDC can be isolated by a mechanical shake-off, and investigated for survival properties and genomic changes. Scale bar represents 75 μm.

3. CELL-CYCLE CHECKPOINTS

Cell-cycle checkpoints enable a cell to ensure that important processes, such as DNA replication, are complete [18]. Cell-cycle checkpoints prevent the transmission of genetic errors to daughter cells. There exist three major cell-cycle checkpoints; the G1/S checkpoint, the G2/M checkpoint, and the spindle assembly checkpoint (SAC). The SAC ensures that chromosome segregation occurs correctly and is activated at the metaphase to anaphase transition in mitosis, in response to microtubule defects [19] or an erroneous kinetochore attachment [20]. Cells also arrest at the SAC when they enter mitosis with damaged DNA [21]. Inactivation of the SAC can lead to chromosome mis-segregation and aneuploidy [22].

The G1/S and G2/M checkpoints are initiated in response to DNA damage to prevent the transmission of damaged or incomplete chromosomes to daughter cells. The DNA-damage checkpoints provide cells with time to repair damaged DNA. If the DNA damage is irreparable, cells may initiate senescence (growth arrest) or cell death. The G1/S checkpoint prevents cells from replicating damaged DNA, whereas the G2/M checkpoint prevents cells from dividing with damaged DNA [18]. The G1/S checkpoint does not function when p53 or p21 are either absent or not functional [23]. This checkpoint is often defective in cancer cells because many of them have mutations in the genes that encode either p53, pRb, or p21 [2,19]. This means that the only DNA-damage checkpoint available to these cancer cells is the G2/M checkpoint [18].

The kinases ataxia telangiectasia mutated (ATM) and ATM and Rad3 related (ATR) are both involved in the initiation of the G2/M checkpoint, however ATR is the main effector kinase associated with G2/M arrest [24]. When single-stranded DNA (ssDNA) is present, it is bound by replication protein A (RPA) [25]. RPA recruits the ATR-interacting protein (ATRIP) in complex with ATR and the Rad9–Rad1–Hus1 (9-1-1) complex to ssDNA [26]. The 9-1-1 complex then recruits DNA topoisomerase-binding protein 1 (TOPBP1) which triggers the ATR-mediated phosphorylation of checkpoint kinase 1 (Chk1) [26]. The Rad17–replication factor C complex, the 9-1-1 complex, and the adaptor protein claspin are also required for Chk1 activation [25,27]. The Rad17–replication factor C complex acts as a clamp loader for the 9-1-1 complex [25] and claspin links ATR and Chk1, allowing for the phosphorylation of Chk1 on serine 317 and serine 345 [28]. Of these phosphorylation sites, serine 345 is essential for Chk1 activation, while serine 317 plays a contributory role [29].

Once active, Chk1 prevents the activation of Cdk1 by phosphorylating Cdc25A and Cdc25C, targeting them for cytoplasmic sequestration by the 14-3-3 proteins [30] or for ubiquitination and degradation by the proteasome [31]. This prevents the removal of inhibitory phosphates on threonine 14 and tyrosine 15 of Cdk1, preventing Cdk1 activity. Active Chk1 also stabilizes the Wee1 kinase, which is responsible for phosphorylating tyrosine 15 of Cdk1 [32].

It has been reported that Chk1, but not Chk2, is essential for the activation of the G2/M checkpoint. In 2000, Liu et al. generated an inducible Chk1 deficient line of murine embryonic stem cells [28]. They found that when this cell line was irradiated and Chk1 depleted, these cells abrogated the G2/M checkpoint [28]. It has also been demonstrated that H1299 human lung carcinoma cells treated with doxorubicin (a topoisomerase II inhibitor) and transfected with Chk1-silencing RNA (siRNA) abrogate the G2/M checkpoint [33]. Furthermore, when $p53^{-/-}$ HCT116 human colon carcinoma cells were treated with the genotoxic agent lidamycin, the cells transfected with Chk1 siRNA abrogated the G2/M checkpoint [34]. By contrast, the cells transfected with Chk2 siRNA remained arrested at the G2/M checkpoint [34]. Chk1 phosphorylation on serine 345 is a good measure of whether cells are arrested at the G2/M checkpoint or not. Loss of Chk1 serine 345 phosphorylation in mitotic cells collected by mechanical shake-off from interphase cells with Chk1 phosphorylated on serine 345 is a good indication that the cells have activated and abrogated the G2/M checkpoint, the second step of checkpoint adaptation (discussed in the following) [7].

4. GENOTOXIC AGENTS AS ANTICANCER DRUGS

Genotoxic agents are a mainstay of cancer therapy that cause cytotoxic levels of DNA damage. By inducing DNA damage, these agents cause cells to arrest at cell-cycle checkpoints leading to cell-cycle arrest and/or cell death. Genotoxic agents are widely used to treat cancer patients but are not always curative. Many of these agents are limited by the development of resistance to treatments, and genomic changes can be responsible for this acquisition of resistance to treatment [35]. For example, cisplatin is a widely used anticancer drug that has revolutionized the treatment of some types of cancers. Specifically, cisplatin and combination treatments with cisplatin have increased the cure rate for testicular cancer to 90%, if tumors are diagnosed early [36]. Cell lines and tumor samples that are resistant to cisplatin exhibit chromosomal abnormalities that distinguish them from cisplatin-sensitive cell lines and tumor samples. The genetic difference between cisplatin-sensitive and cisplatin-resistant cell lines and tumor samples has been studied using comparative genomic hybridization (CGH). This technique allows the detection of a change in chromosomal copy number [37]. In 1997, Wasenius et al. compared six ovarian carcinoma cell lines selected for resistance to cisplatin to two cisplatin-sensitive parental cell lines (2008 and A2780) using CGH [38]. They found that acquired resistance in the 2008 cells was associated with many chromosomal gains and

losses by comparison to the parental cell line, and the average number of chromosome aberrations per resistant cell was 15. By contrast, acquired resistance in the A2780 cell line was only associated with five chromosomal aberrations, and all of these were losses [38]. These data suggest that acquired resistance to cisplatin is not associated with specific genetic changes. This has also been demonstrated in testicular germ-cell tumor (TGCT) cell lines using CGH. Three cisplatin-resistant TGCT cell lines (resistant GCT27, 833K, and Susa) were found to contain more gains and losses of chromosomal regions by comparison to the parental cell lines [39]. However, these losses and gains were different in each of the three resistant cell lines.

5. CELL DEATH

Cell death or permanent growth arrest are the desired outcomes of treating patients with anticancer genotoxic agents. It is often assumed (incorrectly) that cancer cells die by apoptosis following treatment with anticancer drugs, and apoptosis has been extensively studied with regards to cancer development and treatment. However, other modes of cell death and growth arrest exist, and an understanding of these may be equally or more important to understand how cancer cells respond to current cancer treatments. Three types of cell death frequently discussed in the literature with regard to cancer therapies are apoptosis, necrosis, and mitotic catastrophe (Fig. 22.2). Senescence is also a desired outcome of cancer treatment because senescent cells undergo permanent growth arrest.

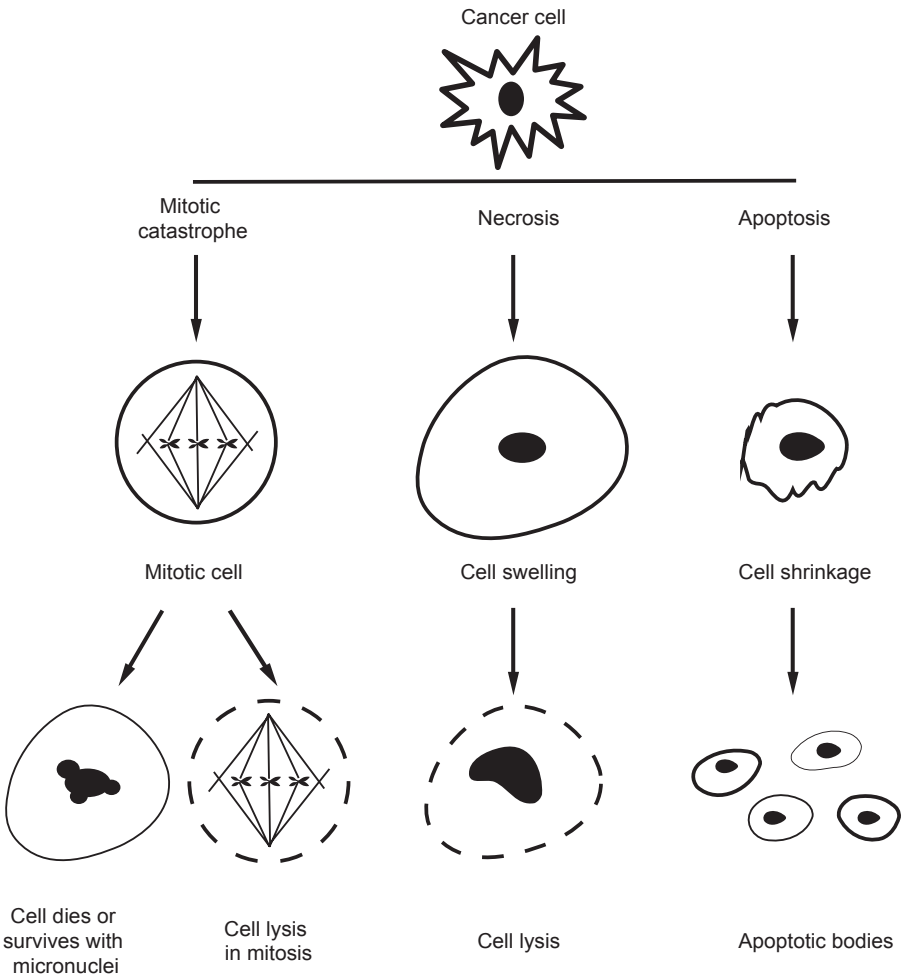

FIGURE 22.2 An overview of three different modes of cell death. Three of the major modes of cell death are apoptosis, necrosis, or mitotic catastrophe. Apoptosis is characterized by cell shrinkage and the formation of apoptotic bodies. Necrosis is characterized by cell swelling and lysis. Mitotic catastrophe is characterized by entry into mitosis prior to cell death, but cells may survive and contain micronuclei. Of these modes of cell death, mitotic catastrophe is not well understood, and it may be an outcome of different phenomena, including that of checkpoint adaptation. In this image, a *dashed line* indicates lysis and the mitotic cells are represented by a simple image of a mitotic spindle.

Apoptosis is considered to be the major mode of cell death in cancer cells treated with genotoxic agents. This is partly because the genes for many of the proteins that regulate apoptosis are mutated in human cancers [40]. However, there is increasing evidence that other cell death pathways have a major role in cancer cell death when solid tumors are treated with genotoxic agents. Inhibiting apoptosis is reported to have little or no effect on the clonogenic survival of cancer cells following treatment with anticancer drugs or ionizing radiation [41,42]. This has been demonstrated by several studies where the antiapoptotic protein Bcl-2 is overexpressed [43–48]. It was predicted that if Bcl-2 was overexpressed, then cells would be resistant to apoptosis and would therefore be less sensitive to treatment with genotoxic agents [42]. However, in these studies, although the overexpression of Bcl-2 prevented cells from undergoing apoptosis, it did not have a significant impact on clonogenic survival, indicating that the cells died by a mode of cell death other than apoptosis [43–48]. Wouters et al. also found that there was no difference in cell viability when apoptosis-proficient and apoptosis-deficient HCT116 cells were treated with either 5 μg/mL etoposide or 10 Gy ionizing radiation [49]. Ruth and Roninson made similar observations in cells engineered to express multidrug resistance protein 1 (MDR1), a P-glycoprotein that inhibits apoptosis [50]. HeLa-derived HtTA–MDR1 cervical adenocarcinoma cells and NIH 3T3 murine fibroblasts were treated with 9 Gy ionizing radiation and induced to express MDR1. Ruth and Roninson found that MDR1 expression prevented cells from undergoing apoptosis but did not change overall cell survival after treatment. Instead, the treated cells either died by mitotic catastrophe or initiated a senescence-like growth arrest [50].

6. MITOTIC CATASTROPHE

Mitotic catastrophe is a form of cell death related to mitosis; however, the exact definition of mitotic catastrophe is still debated [51]. It is debated whether mitotic catastrophe occurs as a direct result of a failed mitosis or if cells die by other cell death pathways following entry into mitosis. The induction of other cell death pathways due to a failed mitosis has three different consequences: (1) cell death during mitosis; (2) cell death once a cell has exited mitosis; and (3) senescence following exit from mitosis [51]. It is also debated whether mitotic catastrophe should be classified as a distinct form of cell death [41,52] or if cell death occurs by apoptosis or necrosis following aberrant mitosis [53,54].

It has long been known that cells treated with ionizing radiation enter mitosis. In 1961, Yamada and Puck found that the following irradiation with sub-cytotoxic concentrations of X-rays (either 0.3, 0.7, or 1.4 Gy), there was a decrease in the mitotic index of HeLa cells followed later by an increase in the mitotic index [55]. Because the cells delayed entry into mitosis, this suggests that they arrested at the G2/M checkpoint before entering mitosis. These cells could therefore have been undergoing checkpoint adaptation, however it is not known if they entered mitosis with damaged DNA or if they entered mitosis following the repair of damaged DNA.

By comparison to apoptosis, there are few distinguishing characteristics of mitotic catastrophe, other than mitosis itself. This means it is difficult to detect mitotic catastrophe as a form of cell death [52]. The main characteristic associated with mitotic catastrophe is the presence of micronuclei [51], but micronuclei only indicate that cells have undergone an aberrant mitosis. Some cells with micronuclei may not undergo mitotic catastrophe because they survive and do not die. Furthermore, cells may die directly in mitosis, and micronuclei cannot be used as a marker for this type of cell death. One of the best ways to study mitotic catastrophe is therefore time-lapse video microscopy, to observe cells in real time [56].

Mitotic catastrophe can be induced by DNA damage that directly affects the integrity of chromosomes by interference with the mitotic spindle [51] or by deficiencies in proteins and protein complexes involved in the process of mitosis itself [57]. Drugs such as the taxanes and vinca alkaloids induce mitotic catastrophe without damaged DNA by interfering with the mitotic spindle. This induces a mitotic arrest followed by cell death. The taxanes stabilize microtubules and induce a metaphase arrest, whereas the vinca alkaloids induce mitotic catastrophe by disrupting the dynamics of microtubule polymerization and depolymerization. Nocodazole is a compound that inhibits microtubule polymerization and is widely used as a positive control for mitotic cells in the laboratory because it arrests cells in mitosis.

Entry into mitosis with damaged DNA induces mitotic catastrophe through the SAC. HeLa cells treated with 1.5 μM aphidicolin entered mitosis with damaged DNA and arrested at metaphase [21]. Following this metaphase arrest, cells entered what the authors describe as a "precatastrophic phase" where chromosome segregation was attempted. This was followed by cell death. When either of the SAC proteins, Mad2 or BubR1, were depleted by siRNA in HeLa cells treated with aphidicolin, then the cells did not arrest at metaphase and continued with mitosis. This increased cell viability following treatment with aphidicolin by comparison to cells transfected with control siRNA [21].

Mitotic catastrophe has been observed in response to treatment with a variety of genotoxic agents that have different mechanisms of action (Table 22.1). These data demonstrate that mitotic catastrophe is an important and widely observed mode of death in response to treatment with genotoxic agents [58]. It is likely that these cells undergo checkpoint adaptation to enter mitosis with damaged DNA, but this was not addressed in the majority of these studies. Checkpoint adaptation

TABLE 22.1 A Table of Treatments That Induce Mitotic Catastrophe in Cell Lines

Treatment	Agent Type	Cell Type	Features of Mitotic Catastrophe	References
Aphidicolin	DNA replication inhibitor	HT0180 fibrosarcoma	Micronucleation	[103]
		$P53^{-/-}$ HCT116 colon carcinoma	Analysis of cell-cycle phase, increased mitotic index	[21]
Bleomycin	Radiomimetic, induces DSBs	DC-3F Chinese hamster lung fibroblast	Analysis of cell-cycle phase, micronucleation	[65]
Cisplatin	Cross-linking agent	CHO/UV41 Chinese hamster ovary	Rounded morphology, analysis of cell-cycle phase	[104]
		CHO	Micronucleation	[105]
		HCC metastatic hepatocellular carcinoma	Ser10 phospho-H3 positive, analysis of cell-cycle phase, micronucleation	[106]
		HT0180	Micronucleation	[103]
		SKOV-3 ovarian carcinoma	Lack of caspase activation, micronucleation	[63]
CPT	Topoisomerase I inhibitor	HT-29 human colon carcinoma	Checkpoint adaptation	[7]
		M059K	Checkpoint adaptation	
Cytarabine	Antimetabolite	HT0180	Micronucleation	[103]
Doxorubicin	Topoisomerase II inhibitor	HT0180	Micronucleation	[103]
		Huh-7 hepatocellular carcinoma (HCC)	Micronucleation, analysis of cell-cycle phase, lack of caspase activation	[64]
		SNU-354, -398, -449, -475 HCC	Micronucleation	
Etoposide	Topoisomerase II inhibitor	HT-29	Checkpoint adaptation	[7]
		HT0180	Micronucleation	[103]
5-FU	Antimetabolite	COLO320DM, HCT116, SW480	Analysis of cell-cycle phase, increased levels of cyclin B	[62]
		Colorectal adenocarcinoma		
Ionizing radiation	Physical agent that induces direct DSBs	U2OS osteosarcoma	Checkpoint adaptation	[99]
		MOLT4 leukemia	Checkpoint adaptation	[100]
		HeLa cervical adenocarcinoma	Analysis of cell-cycle phase, increased levels of cyclin B	[107]
		HT0180	Micronucleation	[103]
Oxaliplatin	Cross-linking agent	TE7 oesophageal adenocarcinoma	Analysis of cell-cycle phase, multinucleation	[108]
S23906	Atypical alkylating agent	HeLa	High levels of cyclin B, increased Cdk1 activity, Ser10 phospho-H3 positive	[58]
		HT-29		

may therefore be a common pathway that leads to cell death following treatment with genotoxic agents [59]. It is necessary to understand whether cells undergo checkpoint adaptation or not because checkpoint adaptation may contribute to cells surviving treatment with rearranged genomes. It may be possible to target the final step of checkpoint adaptation to prevent cells from entering mitosis with damaged DNA, preventing them from surviving treatment with rearranged genomes.

Cells that have undergone aberrant mitoses have also been observed in clinical samples. Micronuclei have been detected in clinical cervical [60] and oral carcinoma samples [61] after patients were treated with ionizing radiation. However, because mitotic catastrophe is difficult to detect, there is a lack of clinical data about mitotic catastrophe as a mode of cell death in vivo.

7. DUAL MODES OF CELL DEATH BY THE SAME GENOTOXIC AGENT

The mode of cell death may depend on different factors including the tissue of origin of a cell and the amount of DNA damage that a cell contains [62,63]. The effect of treatment concentration on the mode of cell death has been demonstrated in studies where the same cell lines treated with different concentrations of the same genotoxic agent died by different modes of cell death.

Three human colorectal adenocarcinoma cell lines, SW480, COLO320DM, and HCT116, were treated with relatively low and relatively high concentrations of the antimetabolite 5-fluorouracil (5-FU) [62]. SW480 and COLO320DM cells treated with 1000 ng/mL 5-FU and HCT116 cells treated with 100 ng/mL 5-FU died by apoptosis, whereas SW480 and COLO320DM cells treated with 100 ng/mL 5-FU and HCT116 cells treated with 10 ng/mL 5-FU died by mitotic catastrophe [62]. The same results were observed when hepatocellular carcinoma cells were treated with doxorubicin [64]. Low-dose doxorubicin treatment (15–60 ng/mL depending on the cell line) of five human hepatocellular carcinoma cell lines, Huh-7, SNU-354, -398, -449, and -475, induced entry into mitosis, followed by a senescence-like phenotype. By contrast, Huh-7 cells treated with a high dose of doxorubicin (10 μg/mL) died by apoptosis [64]. Similarly, DC-3F Chinese hamster lung fibroblast cells treated with bleomycin died by mitotic catastrophe when treated with a low concentration of bleomycin (10 nM), whereas DC-3F cells treated with a high concentration of bleomycin (10 μM) died by apoptosis [65]. High concentrations of genotoxic agents likely induce high levels of damaged DNA. These results therefore suggest that the amount of damaged DNA affects which cell death pathway is activated following treatment.

Different modes of cell death were also observed when two different ovarian carcinoma cell lines (Caov-4 and SKOV-3) were treated with 33 μM cisplatin [63]. Caov-4 cells died by apoptosis, whereas SKOV-3 cells died by entry into mitosis followed by necrosis-like lysis. However, the authors did not provide cytotoxicity data for Caov-4 and SKOV-3 cells treated with cisplatin, and so it may be that 33 μM cisplatin was a high dose of cisplatin for Caov-4 cells but not for SKOV-3 cells [63]. Overall, these studies have identified that the mode of cell death induced following treatment with genotoxic agents can be different and depends on either concentration of treatment or cell type. Furthermore, in all of the studies, cells underwent either cell death following entry into mitosis or cell death by apoptosis, highlighting the importance of these two cell death pathways in cells treated with genotoxic agents.

8. THE RELATIONSHIP BETWEEN ENTRY INTO MITOSIS WITH DAMAGED DNA AND GENOMIC INSTABILITY

Common types of genomic rearrangement are base substitutions, DNA insertions, DNA deletions, DNA translocations, and a change in copy number [66]. Genomic instability can occur following DNA damage, and two different events can induce this: (1) DNA-damage misrepair and (2) entry into mitosis with damaged DNA. DNA damage can be misrepaired during interphase inducing genomic rearrangements. For example, because non-homologous end joining does not require homologous DNA sequence to repair DNA double-strand breaks (DSBs), the ends of breaks from different chromosomes can be joined together, resulting in chromosomal translocations [67].

Entry into mitosis with damaged DNA can also be a source of genomic instability. In 2006, Nakada et al. found that when ATM-deficient primary fibroblast cells prematurely entered mitosis after treatment with etoposide, some cells survived with chromosomal translocations, including the 11q23 translocation associated with topoisomerase II inhibitor–induced secondary leukemia [68]. Entry into mitosis with damaged DNA induced by replication stress can also be a source of genomic instability. DNA damage induced by replication stress that occurred because of the overexpression of the oncogene E2F1 induced chromosome bridge formation and aneuploidy [69]. Replication stress can also occur in chromosomal instability positive (CIN^+) (aneuploid) human colorectal carcinoma cells lines and this was also found to induce structurally altered chromosomes that were subject to mis-segregation in mitosis, leading to genomic instability [70]. Cells treated with cisplatin can also enter mitosis with damaged DNA leading to the induction of chromosome aberrations [71].

Entry into mitosis with damaged DNA can lead to genomic instability by several different mechanisms. When DNA strand breaks occur, acentric fragments can be created. These fragments can be lost during cell division because they lack centromeres and are unable to attach to the mitotic spindle. These fragments can also be incorporated into micronuclei and either be lost or subjected to further genomic rearrangement [72], discussed later. The loss of genetic material following treatment with genotoxic agents has been detected experimentally. LA-9 murine cells containing a stable chromosome with integrated green fluorescent protein (GFP) were treated with either ionizing radiation or etoposide and assessed for loss of the GFP signal [73]. An increase in the percentage of non-fluorescent cells was observed when cells were either irradiated (3 or 5 Gy) or treated with etoposide (0.5 and 1 μM) by comparison to untreated cells [73].

DNA strand–break repair is inhibited in mitosis, once sites of damaged DNA induced by irradiation have been marked by the formation of histone γH2AX and mediator of DNA-damage checkpoint 1 (MDC1) foci [74]. Cdk1 activity is responsible for preventing DSB repair in mitosis by phosphorylating the key DSB-repair protein RNF8 (a ubiquitin ligase) at threonine 198, preventing it from interacting with MDC1 [74]. 53BP1 is also phosphorylated in mitosis, at threonine 1609 and serine 1618, preventing its recruitment to sites of damaged DNA. Because cells do not repair damaged DNA in mitosis, this means that when cells enter mitosis with damaged DNA, this damage can be transmitted to daughter cells. Instead of repairing damaged DNA in mitosis, mitotic cells progress to G1 where DNA-damage repair can occur. Although the repair of damaged DNA may occur in G1, it is possible that some genetic material could be lost or that micronuclei could form in cells in mitosis with damaged DNA, leading to genomic rearrangements. Furthermore, many cancer cells have a defective G1/S DNA-damage checkpoint. It is therefore plausible that a cancer cell can continue through a second cell cycle with damaged DNA following entry into mitosis with DNA damage (the final step of checkpoint adaptation), thus contributing to genomic instability.

Aberrant mitoses are also frequently associated with the formation of micronuclei and this can induce further genomic rearrangements in cells. In 2012, Crasta et al. demonstrated that micronuclei formed by errors in chromosome segregation during mitosis contribute to genomic instability [72]. Crasta et al. studied micronuclei in RPE-1-untransformed retinal pigment epithelial and U2OS osteosarcoma cells. They generated micronuclei in cells and then followed them through the cell cycle. The authors found that DNA contained in the micronuclei was damaged by DNA replication, and that 7.6% of chromosome spreads prepared from cells with micronuclei contained pulverized chromosomes. In addition, they reported that micronuclei persisted for several generations, and that the chromosomes contained in micronuclei could be reincorporated into the nuclei of daughter cells [72].

8.1 Chromothripsis

The chromosome shattering observed by Crasta et al. is called chromothripsis [75]. Chromothripsis describes a catastrophic event where tens to hundreds of genomic rearrangements are acquired in one or several regions of chromosomes [75]. A number of possibilities for how chromothripsis occurs have been suggested, including that chromothripsis occurs due to a high-energy ionizing radiation event during mitosis, that DNA fragmentation occurs as a result of aborted apoptosis, or that DSBs induced by genotoxic agents create dicentric fusions between sister chromatids that can be broken during mitosis [76]. However, the model for chromosome pulverization described by Crasta et al. is currently considered the most likely model for how chromothripsis arises [76]. This model of chromothripsis is supported by a 2015 study from the same group of researchers where live cell imaging and single-cell genome sequencing were used to characterize micronucleated cells [77]. Combining these techniques allowed the researchers to sequence cells where micronuclei were reincorporated into the main nucleus after one round of cell division. Zhang et al. (2015) used copy number analysis of the paired daughter cells present after the one round of cell division to determine which chromosomes were present in the micronuclei. They found that the mis-segregated chromosomes had a large number of genomic rearrangements in 8 of the 9 daughter cell pairs studied, by comparison to the normally segregated control chromosomes [77]. Till 2016, these are the only studies that provide experimental evidence for how chromothripsis can occur, although chromothripsis has been observed in a number of different cancer types including glioma [78], melanoma [75], multiple myeloma [79], medulloblastoma, acute myeloid leukemia [2], and breast cancer [80]. Additionally, chromothripsis has been detected in patients with congenital abnormalities [81]. Cells that undergo checkpoint adaptation enter mitosis with damaged DNA, and it is likely that this induces aberrant mitoses that may lead to genomic rearrangements and the induction of micronuclei, which can contribute to chromothripsis.

9. A HISTORY OF CHECKPOINT ADAPTATION

It is likely that the cells dying by mitotic catastrophe in the studies listed in Table 22.1 were undergoing checkpoint adaptation. However, this was not tested in the majority of the studies because checkpoint adaptation had not been identified in

human cell lines at the time these studies were undertaken. Checkpoint adaptation is the process of entering mitosis with damaged DNA and is defined by three sequential steps: (1) a cell-cycle arrest induced by DNA damage; (2) overcoming this arrest; and (3) resuming the cell cycle with damaged DNA (Fig. 22.1) [82]. Checkpoint adaptation was first observed in 1993 by Sandell and Zakian [83]. DNA-damage repair-deficient *Saccharomyces cerevisiae* cells initiated and then overcame a G2 arrest following the loss of telomeric DNA from an extra dispensable chromosome [83].

Since the discovery that *S. cerevisiae* cells undergo checkpoint adaptation, several different research groups have explored this process in yeast cells. Because the DNA-damage response is highly conserved in eukaryotes, some of this research may provide an insight into how checkpoint adaptation is induced in other eukaryotic organisms such as humans. However, there are differences between G2/M checkpoint control in humans by comparison to those in *S. cerevisiae*. As described earlier, inhibitory phosphorylation of Cdk1 is maintained by the activation of Chk1 in human cells arrested at the G2/M checkpoint. This prevents cells from entering mitosis with damaged DNA. In *S. cerevisiae*, an arrest at the DNA-damage checkpoint does not require inhibitory phosphorylation of Cdk1, and two distinct pathways are involved in the activation of the DNA-damage checkpoint [84]. One pathway involves Chk1 and the other one involves Rad53, a second checkpoint kinase that is homologous to human Chk2. These pathways have different roles in the checkpoint following DNA damage; the Chk1 pathway acts pre-anaphase to prevent chromosome segregation, whereas the Rad53 pathway prevents mitotic exit [84]. Both of these kinases are activated by Mec1, the yeast homolog of ATR. Sanchez et al. (1999) found that Chk1 can prevent entry into anaphase by controlling phosphorylation and levels of Pds1 by preventing cohesion cleavage. Furthermore, they suggest that Rad53 induces cell-cycle arrest through inhibitory phosphorylation of its substrate Cdc5 and show that the overexpression of Cdc5 overrides checkpoint arrest [84]. Cdc5 is a polo-like kinase that induces mitotic exit by phosphorylating and inactivating proteins such as the Bfa1–Bub2 complex. Bfa1–Bub2 are part of the mitotic exit network (MEN), and they prevent mitotic exit until mitosis is complete [85].

That Cdc5 has a role in checkpoint adaptation in *S. cerevisiae* was first observed in 1997. Toczyski et al. (1997) identified two *S. cerevisiae* mutants that were checkpoint adaptation deficient in response to a single double-stranded DNA (dsDNA) break induced using the same *S. cerevisiae* model and method as Sandell and Zakian [82]. One of the mutants identified contained mutated CDC5 and the other mutated CKB2. Cdc5 is a member of the polo-like kinase (Plk) family of proteins. CKB2 encodes a nonessential subunit of casein kinase II (CKII), a serine–threonine kinase that is implicated in a number of pathways including the phosphorylation of the PP2-like phosphatase Ptc2 [86].

In 2001, Galgoczy and Toczyski used the Cdc5 mutant checkpoint adaptation-deficient *S. cerevisiae* strain to investigate the effect of checkpoint adaptation on cell viability and genomic instability [87]. They found that checkpoint adaptation increased cell viability when DNA damage was induced in a nonessential chromosome. Furthermore, they demonstrated that checkpoint adaptation proficient cells irradiated with 30 Gy of X-rays contained more chromosomal losses and translocations, by comparison to checkpoint adaptation-deficient cells. This indicates that checkpoint adaptation has a role in the induction of genomic instability in yeast. Pellicioli et al. also used *S. cerevisiae* checkpoint adaptation-proficient and -deficient cells to investigate checkpoint adaptation and found that the kinase activity of Rad53 was elevated for over 24 h in Cdc5 checkpoint adaptation deficient mutants, whereas Rad53 kinase phosphorylation and activity was lost in cells that underwent checkpoint adaptation [88]. Overexpression of Rad53 also prevented cells from undergoing checkpoint adaptation in response to the induction of a DSB. These data support the results from Sanchez et al., which demonstrated that Rad53 inhibited Cdc5, preventing mitotic entry [84].

Further studies have since confirmed that both Rad53 and Cdc5 have important roles in checkpoint adaptation in *S. cerevisiae*. Rad53 is dephosphorylated and inactivated by the PP2C-like phosphatase Ptc2, promoting checkpoint adaptation [86]. When the PTC2 gene was deleted, checkpoint adaptation proficient cells were unable to undergo checkpoint adaptation and when Ptc2 was overexpressed in checkpoint adaptation-deficient cells, the ability to undergo checkpoint adaptation was restored [86]. To dephosphorylate Rad53, Ptc2 must be phosphorylated on threonine 376 by CKII kinase [89]. This might explain the discovery of mutated CKII as a checkpoint mutant by Toczyski et al. [82]. In addition to Rad53 phosphorylation, Rad53 deacetylation also has a role in checkpoint adaptation. The deletion of histone deacetylase Rpd3 prevents checkpoint adaptation and leads to an increased level of acetylation on Rad53 [90]. Checkpoint adaptation is therefore promoted by deacetylation and inhibition of Rad53.

Till 2016, the precise biochemical pathway that induces checkpoint adaptation in *S. cerevisiae* has not been identified. However, it has been demonstrated that the checkpoint kinase Rad53 and the polo-like kinase Cdc5 have central roles in this process. This reflects the important roles of Rad53 and Cdc5 in the control of the cell cycle in budding yeast. Recently, proteins involved in several cellular responses that are not directly involved in checkpoint control have been identified as having a role in the biochemical pathway(s) that induce(s) checkpoint adaptation. Many of these studies have used checkpoint adaptation-deficient mutants to identify proteins that might be involved in checkpoint adaptation. However, these studies are not usually capable of elucidating the precise role of these proteins in checkpoint adaptation.

In 2015, Ghospurkar et al. identified that phosphorylation of replication factor A2 (Rfa2), the yeast homolog of RPA, induced *S. cerevisiae* cells to undergo checkpoint adaptation [91]. Cells with a phosphomimetic form of Rfa2 where all serine/threonines in the N-terminal domain (9 amino acids) were mutated to aspartic acid underwent checkpoint adaptation. Furthermore, checkpoint adaptation-deficient cells expressing these phosphomimetic proteins also underwent checkpoint adaptation. The authors therefore propose that the induction of checkpoint adaptation occurs when the Rfa proteins (Rfa1 and Rfa2) are modified following a prolonged arrest at the DNA-damage checkpoint [91].

Chromatin remodeling proteins are also involved in checkpoint adaptation. This was first demonstrated by Lee et al., who found that checkpoint adaptation-deficient *S. cerevisiae* cells contained mutated Rdh54/Tid1 [92]. In 2006, Papamichos-Chronakis et al. found that the chromatin remodeling protein Ino80 was required for checkpoint adaptation in *S. cerevisiae* following the induction of a DSB [93]. In 2012, Eapen et al. found that checkpoint-deficient *S. cerevisiae* cells contained mutant Fun30, a chromatin remodeling protein involved in homologous recombination (HR) [94]. The role of these chromatin remodeling proteins in checkpoint adaptation remains to be elucidated.

These *S. cerevisiae* studies indicate that checkpoint adaptation is an important area of research of interest to researchers worldwide. Because checkpoint adaptation increases the number of *S. cerevisiae* cells that survive a DNA-damaging event and also increases genomic instability [87], it has been suggested that checkpoint adaptation may be important in the development of tumorigenesis [95]. Additionally, these studies indicate that a polo-like kinase and checkpoint kinases are central to checkpoint adaptation in *S. cerevisiae*. These data provide starting points for elucidating the biochemical pathway(s) that induce(s) checkpoint adaptation in higher eukaryotes. However, these studies also highlight the complexity of checkpoint adaptation and the need for more research in this area. Furthermore, although proteins involved in the DNA-damage response and cell-cycle regulation are largely evolutionarily conserved across eukaryotes, the regulation of cell-cycle checkpoints in *S. cerevisiae* is not identical to the regulation of cell-cycle checkpoints in humans. To understand better the process of checkpoint adaptation in humans, it is therefore necessary to use human model systems such as human cancer cell lines.

Initially, it was proposed that checkpoint adaptation would only occur in unicellular organisms, as a last attempt to survive if DNA-damage repair was not successful [96]. This is because entry into mitosis with damaged DNA in multicellular organisms may have a detrimental effect on the survival of an organism as a whole, by increasing the risk of genomic instability. By contrast, one could rationalize that unicellular organisms have nothing to lose by attempting cell division when DNA damage is irreparable [96]. However, in 2004, Yoo et al. described checkpoint adaptation in *Xenopus* oocyte extracts [97]. Yoo et al. reported that when they blocked DNA replication with aphidicolin, cell-free extracts were arrested in interphase and then entered mitosis with only partially replicated chromosomes. Because *Xenopus* are multicellular organisms, this, for the first time, suggested that checkpoint adaptation may also occur in other metazoans such as humans [96,97]. However, *Xenopus* oocyte extracts are different from somatic cells because they are a cell-free system without intact cell membranes. They also rapidly alternate between S phase and mitosis without G1 or G2 phases of the cell cycle.

Checkpoint adaptation was next identified in plant cells in early 2006. The pathways involved in DNA-damage repair and cell-cycle checkpoints are largely conserved in all eukaryotes, including plants [98]. Root cells from *Allium cepa* were irradiated with 2.5–40 Gy X-rays and analyzed for entry into mitosis with damaged DNA. X-ray irradiation with either 5, 10, 20, or 40 Gy produced G2/M arrest in the cells. An increase of apoptotic cells was also observed when cells were treated with 20 and 40 Gy X-rays. The number of apoptotic cells relative to the not treated control cells increased at 16 h following treatment with 20 Gy and at 4 h following treatment with 40 Gy. However, some cells treated with both of these doses of X-rays still underwent checkpoint adaptation following treatment, albeit at later times by comparison to cells treated with 5 and 10 Gy X-rays. The authors also scored mitotic cells for aberrant mitoses. Broken chromatids and acentric chromosomal fragments (lacking a centromere) were observed in some cells that were in either metaphase, anaphase, or telophase. When the percentages of aberrant mitoses were quantified following treatment with either 5, 10, 20, or 40 Gy X-rays, between 20% and 90% of mitoses were aberrant. Furthermore, the percentage of aberrant mitoses depended on the dose of X-ray irradiation and time after treatment. Cells were fixed at 0, 2, and 4 h and then at 4 h intervals to 24 h. A consistent number of mitoses were aberrant when cells were treated with 5 Gy X-rays between 2 and 24 h following treatment (between 50% and 70%). By contrast, 90% of mitoses were aberrant 24 h after treatment with either 20 or 40 Gy. Cells treated with 20 and 40 Gy X-rays did not enter mitosis between 4 and 20 h following treatment, suggesting that they were arrested at the G2/M checkpoint for a long time before entering mitosis by comparison to cells treated with 10 Gy, which entered mitosis at 16 h, and cells treated with 5 Gy which entered mitosis at all times tested between 2 and 24 h [98]. This study demonstrates that chromosome aberrations are present when cells undergo checkpoint adaptation which may lead to genomic instability in cells that survive this process. Additionally, these results suggest that cells enter mitosis at different times when they have different levels of damaged DNA; cells treated with higher doses of X-rays required more time to enter mitosis with damaged DNA (the final step of checkpoint adaptation) by comparison to cells treated with lower doses of X-rays.

10. CHECKPOINT ADAPTATION IN HUMAN CELLS

Checkpoint adaptation was first observed in human cancer cells in 2006 [99]. Syljuåsen et al. treated U2OS cells with 6 Gy of ionizing radiation and cells accumulated in G2 phase of the cell cycle. Several of the key cellular features of checkpoint adaptation are described in Fig. 22.3. In the original report, cells were arrested at the G2/M checkpoint, as shown by flow cytometry and by Chk1 phosphorylation on serine 345 [99]. The arrested cells then began to enter mitosis with damaged DNA, observed by the detection of histone γH2AX and phosphorylated serine-10 histone H3 staining using immunofluorescence microscopy. The authors found that when Chk1 was inhibited in the irradiated cells by the Chk1 inhibitor UCN-01, more cells were in mitosis 18 h following treatment by comparison to cells treated with 6 Gy ionizing radiation alone [99]. Furthermore, cells that over-expressed Chk1 arrested in G2 for longer than cells with wild-type levels of Chk1 following treatment with 6 Gy ionizing radiation. Since the polo-like kinase Cdc5 has a role in checkpoint adaptation in *S. cerevisiae* [82] and polo-like kinase 1 (Plk1) was implicated in checkpoint adaptation in *Xenopus* egg extracts [97], Syljuåsen et al. tested if Plk1 has a role in checkpoint adaptation in human cancer cells. Plk1 was depleted by siRNA in U2OS cells which were then treated with ionizing radiation. These cells accumulated in G2/M 19 h after treatment, but did not enter mitosis, suggesting that Plk1 has a role in checkpoint adaptation in human cancer cells [99]. However, the authors did not determine whether checkpoint adaptation was prevented or just delayed as a result of inhibiting Plk1. Similar to the studies in *S. cerevisiae*, the results of Syljuåsen et al. implicated Chk1 and Plk1 in the pathway of checkpoint adaptation in human cells. However, it was unclear if this process was clinically relevant because humans are unable to tolerate a single dose of 6 Gy of ionizing radiation and are treated with fractions of ionizing radiation in the clinic.

The question of whether or not checkpoint adaptation occurs in cancer cells treated with fractionated doses of ionizing radiation was addressed in 2011. Rezacova et al. reported that 26% of MOLT4 leukemia cells treated with fractionated radiation initiated a G2/M arrest 48 h after treatment before entering mitosis with damaged DNA [100]. However, it was still unknown whether checkpoint adaptation occurred in response to treatments other than ionizing radiation.

In 2012, checkpoint adaptation was observed in HT-29 human colorectal adenocarcinoma cells treated with either camptothecin (CPT) or etoposide and in M059K human glioma cells treated with CPT [7]. Kubara et al. demonstrated that HT-29 cells treated with 25 nM CPT for 48 h were arrested in the G2/M phase by analyzing DNA content using flow cytometry and by the detection of serine 345–phosphorylated Chk1. These CPT-treated cells then entered mitosis with

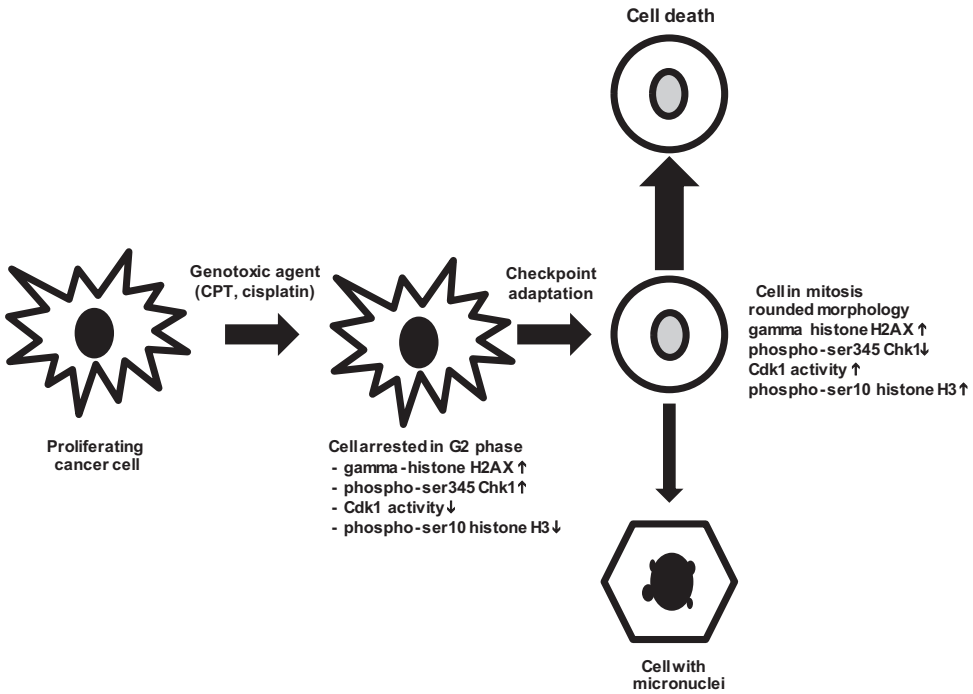

FIGURE 22.3 **A model of checkpoint adaptation.** In this figure we highlight several of the key cellular events that are characteristic of checkpoint adaptation. Proliferating cancer cells treated with genotoxic cancer drugs, such as camptothecin (CPT) or cisplatin, will arrest at the G2/M cell-cycle checkpoint with damaged DNA. The cells then enter mitosis with damaged DNA by undergoing checkpoint adaptation. The majority of cells die but some survive, likely with changes to their genome as demonstrated by micronuclei. The *arrows* list either increases or decreases in the biochemical and cellular events at each step in the process.

damaged DNA; they contained high levels of cyclin B1, had decreased levels of Cdk1 phosphorylated on tyrosine 15, and were positive for histone γH2AX and phosphorylated serine-10 histone H3 staining. Furthermore, Kubara et al. also demonstrated that entry into mitosis with damaged DNA could be prevented by co-treatment with the Cdk1 inhibitor CR8 [7]. Since HT-29 and M059K cells treated with CPT undergo checkpoint adaptation, CPT can be used as a positive control for checkpoint adaptation in these cell lines.

Kubara et al. (2012) also investigated the roles of Chk1 and Plk1 in checkpoint adaptation. HT-29 cells treated with CPT for 24 h and then with CPT and a Plk1 inhibitor for a further 24 h were partially prevented from entering mitosis with damaged DNA. However, there was only a slight decrease in the percentage of mitotic cells present in cells cotreated with CPT and a Plk1 inhibitor by comparison to cells treated with CPT alone [7]. This suggests that proteins other than Plk1 are involved in checkpoint adaptation in human cancer cells.

The role of Plk1 in human checkpoint adaptation remains unclear. In 2014, Liang et al. studied how single U2OS cells from a cell population treated with 1.5 Gy ionizing radiation responded to the activation of the G2/M checkpoint [101]. They found that different cells from the same population entered mitosis after a G2/M arrest at different times and with different levels of damaged DNA [101]. This entry into mitosis was reported to be dependent on levels of Plk1 and cyclin B. However, unpublished results from the laboratory of R.H. Medema indicate that inducing the expression of a constitutively active Plk1 mutant was not capable of overriding an established DNA damage–induced checkpoint [102]. The full molecular pathway(s) that are involved in the cellular induction of checkpoint adaptation remain to be elucidated.

11. THE CONSEQUENCES OF CHECKPOINT ADAPTATION

Checkpoint adaptation has several possible outcomes: (1) cells may die in mitosis; (2) cells may survive mitosis but die in subsequent phases of the cell cycle; or (3) cells may survive mitosis and divide with damaged DNA (Fig. 22.1) [7,99]. It has been demonstrated that the majority of human cancer cells that undergo checkpoint adaptation will die [7,99]. This is the desired outcome of treating cancer cells with a genotoxic agent. However, checkpoint adaptation is also a mechanism by which cells can transmit damaged DNA to daughter cells. It is therefore likely that checkpoint adaptation is a source of genomic instability in human cells that undergo this process (Fig. 22.3).

12. THE RELATIONSHIP BETWEEN CHECKPOINT ADAPTATION AND GENOMIC INSTABILITY

As described in Section 9, Galgoczy and Toczyski found that checkpoint adaptation in *S. cerevisiae* generated genomic rearrangements such as chromosome loss or chromosome rearrangement [87]. Additionally, these authors demonstrated that checkpoint adaptation increased yeast survival following DNA damage and that adaptation-deficient cells were less likely to survive a DNA-damaging event. These data suggest that, in yeast, checkpoint adaptation may contribute to cell survival following a genotoxic event and that cells surviving checkpoint adaptation are more likely to contain rearranged genomes. Some plant cells that enter mitosis following treatment with 5, 10, 20, and 40 Gy X-rays also contained broken chromatids, acentric chromosomal fragments, and chromosome bridges [98]. These data support the suggestion that checkpoint adaptation may induce genomic rearrangements in human cancer cells. However, these plant cells were fixed within 24 h of treatment, so it is unknown whether cells survived checkpoint adaptation with rearranged genomes or whether these chromosomal aberrations induced cell death.

To determine whether human cancer cells can survive checkpoint adaptation with rearranged genomes, it is first necessary to collect cells undergoing checkpoint adaptation and then culture these cells to assess cell viability. HT-29 cells can be used for this type of analysis because they display a pronounced rounded morphology in mitosis and spend a long time in mitosis following treatment. This allows cells that have undergone checkpoint adaptation and are in mitosis to be collected by mechanical shake-off (Fig. 22.1). These cells can then be assessed for cell survival by the clonogenic assay. Furthermore, once survival cells have been identified, these cells can be investigated to determine whether they contain rearranged genomes. Genomic rearrangements in these survival cells could be detected using cytogenetic techniques such as fluorescence in situ hybridization (FISH) to observe centromeres, telomeres, and chromosomal regions using fluorescent probes specific for these regions and by spectral karyotyping (SKY), which uses fluorescent probes of different colors to detect specific chromosomal regions. In addition to these techniques it will also be possible to sequence the genomes of individual cells surviving checkpoint adaptation when advances are made in single-cell DNA sequencing.

It has been reported that small numbers of HT-29 cells treated with CPT can survive entry into mitosis with damaged DNA [7]. Furthermore, preliminary data from our laboratory have suggested that HT-29 cells treated with CPT contain

mitotic chromosomes that are shattered into different pieces (Rahman, Kernéis, and Golsteyn, unpublished data). CPT-treated HT-29 cells that survived checkpoint adaptation contained fewer chromosomes by comparison to untreated cells. Our laboratory has identified that entry into mitosis increases the number of micronuclei present in M059K cells treated with cisplatin. Checkpoint adaptation may thus facilitate the formation of survival cells with rearranged genomes, as a consequence of these cells surviving entry into mitosis with damaged DNA.

The investigation of checkpoint adaptation can provide an insight into the specific role of each step upon cell survival and genomic changes. For example, cancer cells appear to need to enter mitosis following treatment with a genotoxic agent to facilitate cell death in other phases of the cell cycle [96]. It is currently unknown whether cells that undergo checkpoint adaptation will still die if they are prevented from entering mitosis. If treated cancer cells can die when entry into mitosis with damaged DNA (the final step of checkpoint adaptation) is inhibited, then this could prevent cells from surviving treatment with rearranged genomes.

Current genotoxic anticancer treatments such as cisplatin are often limited by acquired resistance to treatment, which is induced by genomic changes [38,39]. By preventing treatment-induced genomic changes, it should be possible to prevent cells from acquiring resistance to treatment. These cancer cells would then be susceptible to cell killing induced by further rounds of treatment, thus improving the efficacy of genotoxic anticancer agents.

GLOSSARY

Mitotic cell rounding When cells enter mitosis they undergo a morphology change and display a rounded morphology instead of the flattened morphology of cells in interphase.

Chromothripsis Tens to hundreds of clustered genomic rearrangements are acquired in an event.

LIST OF ABBREVIATIONS

ATM Ataxia telangiectasia mutated
ATP Adenosine triphosphate
ATR ATM and Rad3 related
ATRIP ATR-interacting protein
CAK Cyclin-dependent kinase-activating kinase
Cdk Cyclin-dependent kinase
CGH Comparative genomic hybridization
Chk Checkpoint kinase
CIN Chromosomal instability
CKII Casein kinase II
CPT Camptothecin
DSB Double-strand breaks
dsDNA Double-stranded DNA
FISH Fluorescence in situ hybridization
GFP Green fluorescent protein
GST Glutathione *S*-transferase
HR Homologous recombination
MDC1 Mediator of DNA damage–checkpoint protein 1
MDR1 Multidrug-resistance protein 1
MEN Mitotic exit network
Plk1 Polo-like kinase 1
Rb Retinoblastoma
Rfa2 Replication factor A2
RPA Replication protein A
SAC Spindle assembly checkpoint
siRNA Silencing RNA
SKY Spectral karyotyping
ssDNA Single-stranded DNA
TCGA The Cancer Genome Atlas
TGCT Testicular germ cell tumor
TOPBP1 DNA topoisomerase binding protein 1
5-FU 5-Fluorouracil
9-1-1 Rad9–Rad1–Hus1 complex

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