Cell cycle control in cancer

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Abstract | Cancer is a group of diseases in which cells divide continuously and excessively. Cell division is tightly regulated by multiple evolutionarily conserved cell cycle control mechanisms, to ensure the production of two genetically identical cells. Cell cycle checkpoints operate as DNA surveillance mechanisms that prevent the accumulation and propagation of genetic errors during cell division. Checkpoints can delay cell cycle progression or, in response to irreparable DNA damage, induce cell cycle exit or cell death. Cancer-associated mutations that perturb cell cycle control allow continuous cell division chiefly by compromising the ability of cells to exit the cell cycle. Continuous rounds of division, however, create increased reliance on other cell cycle control mechanisms to prevent catastrophic levels of damage and maintain cell viability. New detailed insights into cell cycle control mechanisms and their role in cancer reveal how these dependencies can be best exploited in cancer treatment.

Cyclin-dependent kinases (CDKs). CDKs depend on cyclins for their kinase activity. The levels of cyclins increase during the cell cycle and cyclin–CDK complex activity drives cell cycle progression by phosphorylating protein targets.

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Cell cycle control is focused mainly on two events, the replication of genomic DNA and its subsequent segregation between daughter cells, which in eukaryotic cells occur during distinct cell cycle phases. It is often assumed that cancer cells undergo uncontrolled cell cycle progression and that most, if not all, cell cycle checkpoints need to be defective for a cell to become cancerous. However, a large body of recent work has provided strong evidence that only specific aspects of cell cycle control need to be disrupted for cancer cells to continue to divide. This work indicates that cancer cells are compromised mainly in their ability to exit the cell cycle, and consequently continue to divide, rather than undergoing uncontrolled cell division. Importantly, it also suggests that most cell cycle control functions are essential for cancer cell viability. These findings underscore the fundamental difference between the DNA damage checkpoint and DNA replication stress checkpoint responses: to prevent the accumulation and propagation of DNA damage and to prevent replication stress-induced DNA damage, respectively. Understanding these differences is essential in the context of cancer. The DNA damage checkpoint is often compromised in cancer cells, allowing continuous cell division despite the accumulation of genetic errors. In contrast, genes involved in the replication stress checkpoint are rarely mutated in cancer cells, as many cancers become increasingly dependent on checkpoint function to tolerate high levels of replication stress. Similarly, cancer cells also rely on a functional mitotic checkpoint to prevent catastrophic chromosome mis-segregation[1]. This shift in the paradigm of the role of checkpoints in cancer provides a better understanding of cell cycle control in cancer and reveals particular dependency on specific checkpoint functions, creating new therapeutic opportunities.

In this Review, we discuss our current understanding of cell cycle control mechanisms and outline the specific functions of the various cell cycle checkpoints that prevent or correct errors to avoid propagation of genetic alterations. We briefly describe cell cycle control pathways, such as cyclins and cyclin-dependent kinases (CDKs), G1–S transcriptional regulation, checkpoint signalling and the ubiquitin ligase regulatory pathways, but refer readers to more comprehensive reviews on these specific topics (see references for G1–S transcription[2,3], replication stress[4], DNA damage[5], CDKs[6] and mitotic checkpoint[7]). We point out which aspects of cell cycle control are frequently compromised in cancer cells to allow continued cell division, and on which cancer cells become increasingly dependent for their viability. Finally, we share our views on how these dependencies on cell cycle control and checkpoint mechanisms can be targeted therapeutically.

The cell division cycle

In unicellular and multicellular eukaryotes, cell division is controlled by a complex network of regulatory mechanisms, checks and balances to ensure that no mistakes are made before a cell is allowed to enter and progress through the cell cycle to divide.

Cell cycle phases. The mitotic cell cycle is divided into two distinct stages, interphase and M phase (FIG. 1). This allows the temporal separation of the duplication of cellular content during interphase and its separation into two genetically identical daughter cells in mitosis. The complex network of regulatory elements that form the cell cycle has one goal: the timely and accurate duplication and segregation of the genomic DNA.

Quiescence
A reversible cellular state, outside G1 phase, from which cells can re-enter the cell cycle.

DNA replication occurs in interphase during S phase (synthesis phase), defined as the time in interphase during which DNA replication is initiated but not completed. The periods of interphase that separate S phase from M phase have historically been named 'gap phases', or G1 before S phase and G2 after S phase, on the basis of the evident observation that these are gaps in between the two main events, duplication and segregation of the DNA. However, these phases are key periods for cell cycle regulation and include the crucial decision to enter the cell cycle during G1, and to initiate the process that leads to chromosome segregation during G2.

Cell cycle entry and progression. Before S phase, in the prereplicative G1 phase, there is a decision window during which cells can commit to initiate DNA replication and enter the cell cycle or stay in G1 phase (FIG. 1). During G1 cells can also exit the cell cycle into a non-proliferative state known as quiescence, or G0. The vast majority of cells in an adult body are in a non-proliferative state and would need to transit into G1 before being able to initiate DNA replication and enter the cell cycle. Upon completion of DNA replication, there is another decision window during the postreplicative G2 phase. During this window, cells can commit to enter M phase by initiating chromatin condensation and the central alignment of chromosomes. M phase serves the dual function of both accurately separating the duplicated DNA (mitosis) and dividing the entire cellular content into two new daughter cells (cytokinesis). During M phase, cells commit to the segregation of the genetic material and reset the cell cycle to return to interphase.

CDKs and cell cycle-regulated transcription drive cell cycle progression. The key regulator of cell cycle processes is CDK activity (BOX 1). Specific cyclins accumulate during different stages of the cell cycle, driven by cell cycle-regulated transcription and the inhibition of protein degradation. In turn, cell cycle-regulated transcription depends on CDK activity for activation.

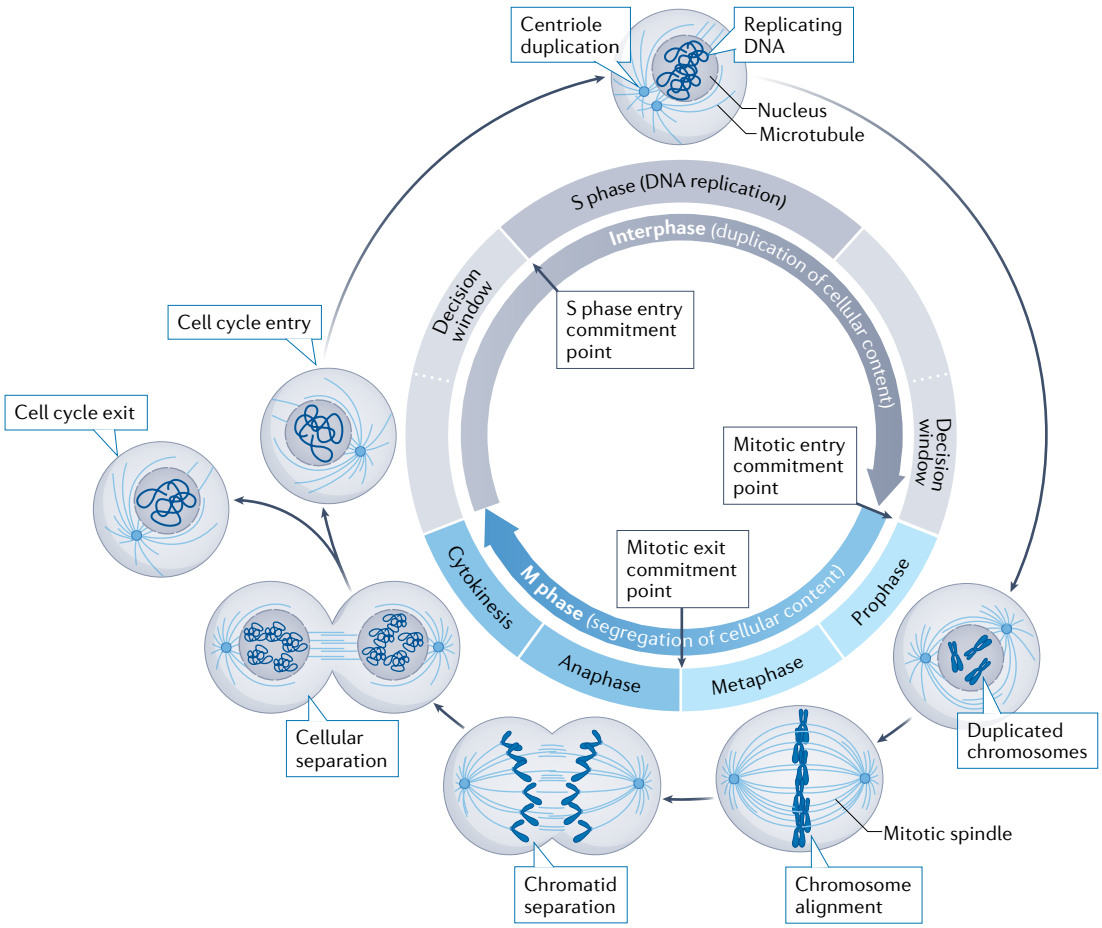
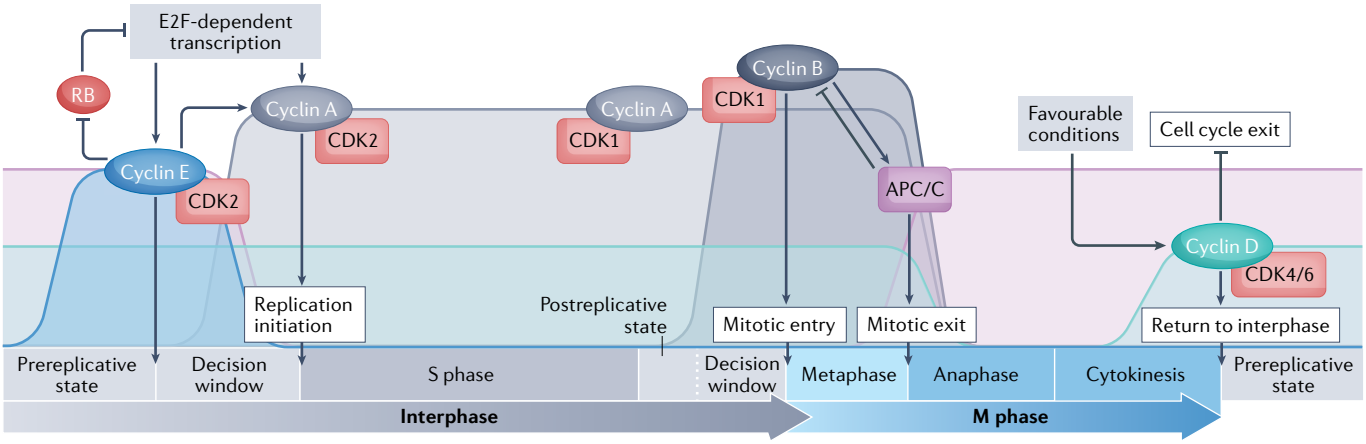


Fig. 1 | **The cell division cycle.** The eukaryotic cell cycle is the process during which a cell duplicates its entire cellular content during interphase, and through division in M phase creates two genetically identical cells. The two main events, DNA replication and the segregation of the replicated DNA, are separated during the cell cycle. DNA replication happens during a distinct phase in interphase, called 'S phase', and DNA separation happens in mitosis (M phase). Segregation of the cellular content happens during cytokinesis at the end of M phase to complete a cell cycle, after which a cell can either exit the cell cycle or enter a new round of cell division. During interphase, cell cycle progression is controlled before and after S phase. Commitment to enter a new cell cycle (that is, S phase entry) is made during a decision window preceding S phase. Similarly, during a decision window following S phase, a cell can commit to mitotic entry. Commitment to mitotic exit happens during M phase at the metaphase–anaphase transition.

Box 1 | **CDK activity and APC/C activity are central to the control of cell cycle progression**

Cell cycle progression is driven by the accumulation of cyclin-dependent kinase (CDK) activity during interphase and M phase (see the figure). Loss of this activity, through APC/C in complex with the activator protein CDC20 (APC/C^{CDC20}) during mitosis, and then with CDH1 (APC/C^{CDH1}) during G1 leads to the degradation of cyclins, marking the return back to interphase. Cyclin D–CDK4/6 accumulation allows entry into the cell cycle, thereby preventing cell cycle exit. E2F-dependent transcription results in the accumulation of both cyclin E and cyclin A, which creates a decision window to enter S phase. Cyclin E–CDK2 activity further activates E2F-dependent transcription, creating a positive feedback loop that results in increased cyclin E–CDK2 activity and cyclin A–CDK2 complex. This process allows the accumulation of cyclin A–CDK2 activity, through the inactivation of APC/C^{CDH1} activity (not shown), and replication initiation and S phase entry. Subsequent accumulation of cyclin A/B–CDK1 complex creates a second decision window, following S phase completion, for mitotic entry. Accumulation of cyclin A/B–CDK1 activity drives mitotic entry and allows APC/C^{CDC20} activation, which is required for mitotic exit and targeted degradation of cyclins, to complete a cell cycle. Under favourable conditions, accumulation of cyclin D–CDK4/6 activity allows cells to re-enter the cell cycle.



RB, retinoblastoma protein.

This interdependence creates a regulatory network that ensures that cell cycle progression is sequential and unidirectional[8,9]. Cell cycle-dependent changes in CDK activity drive cell cycle entry, progression and completion. CDK activity in the prereplicative G1 phase is required for initiation of DNA replication and thereby commitment to cell cycle entry. Subsequently, during the postreplicative G2 phase, CDK activity plays a key role in preparing for chromosome segregation, via initiation of condensation, permeabilization of the nuclear envelope and central alignment of the replicated chromosomal DNA. Inactivation of CDK activity, via the anaphase-promoting complex/cyclosome (APC/C^{CDC20})-dependent targeted destruction of cyclins, coincides with chromosome segregation and return to interphase[10].

Choice to enter the cell cycle. There are several stages during the prereplicative G1 phase. In early G1 phase, the cell faces a 'choice' to either remain in a cell cycle state or exit the cell cycle into quiescence. A large body of research has shown that CDK activity plays a central role in this decision[2,3]. Growth-dependent CDK activity (D-type cyclin–CDK) creates a decision window during which the cell can commit to initiate replication and enter a new cell cycle (BOX 1). Inhibition or absence of this CDK activity can take the cell out of the G1 state and into quiescence.

Commitment to replication initiation and S phase entry is closely linked to activation of the E2F-dependent transcriptional network (reviewed in[11]). The transcriptional network is activated in G1 phase, drives S phase entry and is subsequently inactivated during S phase. The E2F-dependent transcriptional network includes many genes that encode key proteins in cell cycle and DNA replication control but also genome protection mechanisms and growth. E2F-dependent transcriptional regulation depends on a family of transcription factors (E2F1–E2F8) and their co-regulators the pocket proteins (retinoblastoma protein (RB), p107 and p130). Activation of E2F-dependent transcription is initiated by growth signals and mitogens that stimulate CDK activity through antagonizing CDK inhibitor activity and by the expression of D-type cyclins. The upstream signalling depends largely on the MAPK pathway (RAS–RAF–MEK–ERK) feeding into the transcriptional regulator MYC, which controls the expression of crucial positive cell cycle regulators.

Decision to enter the cell cycle. The E2F inhibitor RB plays a central role in the 'decision' to enter a new cell cycle[2,3]. During the prereplicative G1 phase, RB keeps E2F-dependent transcription inactive. However, RB is inactivated by CDK-dependent phosphorylation as these enzymes become more active, resulting in E2F-dependent transcription. This results in the expression of E-type cyclins, which further increases the overall activity of CDKs. The increased CDK activity induces further phosphorylation of RB, which fully inactivates this E2F inhibitor and allows the expression of E2F-dependent genes. This positive feedback loop creates a decision window to enter S phase by driving the accumulation of E-type cyclin and A-type cyclin–CDK activity required to initiate DNA replication and cell cycle entry[12].

Initially, RB inactivation was thought to be driven by an increase in CDK activity associated with D-type

Anaphase-promoting complex/cyclosome (APC/C). Ubiquitin ligase complex activity that is restricted to mitosis and G1 phase and is required to initiate exit from mitosis and indirectly for DNA replication.

Box 2 | **DNA replication control in S phase**

Initiation of DNA replication marks S phase entry and commitment to a new cell division cycle. DNA replication is initiated in a bidirectional manner from a large number of discrete sites spread around the genome, called 'replication origins'[210]. This is to ensure that the genome is replicated in a timely manner. It is essential for the preservation of the genome that DNA replication occurs 'once and only once' during typical cell cycles[211]. Preventing re-initiation from an origin where replication has already been accomplished is vital since it is thought that re-replication from even a single origin can cause DNA damage[212,213]. To prevent re-initiation of DNA replication, the process is temporally separated into two steps: origin licensing in G1 phase, followed by replication initiation, called 'origin firing', in S phase (reviewed in[214]).

After completing a cell division cycle, cells return to interphase with low cyclin-dependent kinase (CDK) activity. The lack of CDK activity allows CDC6/CDT1-dependent loading of inactive MCM helicase complex onto replication origins, 'licensing' all the origins. DNA replication can now be initiated from these licensed origins by the formation of a transient pre-initiation complex, followed by the activation of the MCM replicative helicase, which is called 'origin firing'. This step requires CDK activity, which accumulates as detailed above. At the same time, the increase in CDK activity blocks the loading of inactive MCM helicase complex, preventing origin (re)licensing.

cyclin–CDK4/6, and subsequently with E-type cyclin–CDK2 during G1 phase[13]. However, recent data suggest that D-type cyclin–CDK might not be involved in the initial inactivation of RB, but instead 'primes' a cell for entry into the cell cycle by preventing its exit from, or promoting its entry into, G1 phase, potentially by controlling the metabolic state of the cell[14–16]. In this model, monophosphorylation of RB by D-type cyclin–CDK activity does not activate E2F-dependent transcription but creates a state between cell cycle exit (quiescence) and the cell cycle entry decision window. During this state, hyperphosphorylation of RB by E-type cyclin–CDK activity activates E2F-dependent transcription, initiating a positive feedback loop, which drives cells into a decision window to commit to a new cell cycle. D-type cyclin–CDK interaction with RB may be required for hyperphopshorylation[17]. Importantly, this recent insight supports a new model in which an increase in D-type cyclin–CDK activity, seen in many cancers, may not directly drive proliferation but instead 'primes' a cell for cell cycle entry, by preventing exit[18]. Most recently the sequential cooperation between D-type cyclin–CDK and E-type cyclin–CDK in the G1–S transition has been challenged, suggesting that E-type cyclin–CDK does not play a significant role[19,20]. Overall, the specific role of D-type cyclin–CDK activity and E-type cyclin–CDK activity in the timing of cell cycle events through the G1–S transition remains an active field of research, and for more details on the different models proposed, we refer the reader to more specific reviews[2,3].

Commitment to enter the cell cycle. Once the decision to enter a new cell cycle has been made, the initiation of DNA replication (that is, firing of replication origins) and thereby the 'commitment' to initiate S phase depends on A-type cyclin together with CDK2 activity. Accumulation of A-type cyclin–CDK activity depends not just on E2F-dependent induction but also on the inactivation of the G1 phase-specific APC/C (APC/C^{CDH1}), which targets A-type cyclins for destruction[21]. Inactivation of the APC/C^{CDH1} depends on both the E2F-dependent accumulation of E-type cyclin–CDK activity and the APC/C^{CDH1} inhibitor EMI1 (REFS[21,22]). Only when E2F-dependent transcription is active and APC/C^{CDH1} is inactive can a cell pass the commitment point. So activation of E2F-dependent transcription creates a decision window where cells can accumulate enough A-type cyclin–CDK activity to initiate replication and commit to a new cell cycle (BOX 1). CDK activity plays a key role in the separation of licensing and firing of replication origins to ensure that the genome is replicated in a timely manner and occurs 'once and only once' per cell cycle (BOX 2).

Decision to enter mitosis. Activation of CDK1 plays a central role in the decision to enter mitosis. The activity of CDK1 is subject to multiple levels of regulation to ensure rapid and timely mitotic entry (reviewed in[23]). First, CDK1 activation requires association with A-type or B-type cyclins, which gradually accumulate in the cell from S phase onwards owing to cell cycle-specific transcription. Second, CDK1 is maintained in an inhibited state via phosphorylation by the kinases WEE1 and MYT1. Activation is triggered by removal of these inhibitory phosphorylations by the phosphatase CDC25. The balance between WEE1/MYT1 and CDC25 activity levels ultimately determines when a cell progresses into mitosis and is itself regulated by multiple regulatory circuits[23]. In parallel, phosphatase inhibition by the Greatwall kinase pathway enables phosphorylation of CDK1 substrates[24]. These multiple layers of regulation mean that the steady increase of the levels of A-type and B-type cyclins throughout G2 phase is translated into a fast, bistable switch in CDK1 activity at mitotic entry[25] (BOX 1).

Commitment to mitotic entry. Once the threshold levels for CDK1 activity are reached, entry into mitosis is triggered by widespread phosphorylation of more than a thousand CDK1 substrates[26,27]. In parallel, CDK1 activates the mitotic kinases PLK1, Aurora A and Aurora B, which phosphorylate additional mitotic substrates[28,29]. The wave of mitotic phosphorylation triggers structural changes to every cell compartment and primes the cell for DNA separation and division. During prophase, rising CDK1 activity[30] in the cytoplasm triggers cell rounding[31] and centrosome separation[32]. At the same time, rapid nuclear import of activated CDK1–cyclin B induces condensation of chromosomes, activation of the APC/C, nucleolar disassembly and permeabilization of the nuclear envelope[33–35]. Loss of nuclear membrane integrity at the start of prometaphase allows free mixing between nuclear and cytoplasmic compartments and brings the condensed chromosomes into contact with microtubules to assemble the mitotic spindle. Microtubule filaments emanating from each centrosome form bilateral attachments to each sister chromatid at specialized central regions called 'kinetochores'[36,37], resulting in the eventual alignment of the chromosomes in the centre of the cell at metaphase.

Commitment to mitotic exit. During prometaphase, cyclin A is degraded by the 26S proteasome, following ubiquitylation by the APC/C^{CDC20} (REFS[38,39]) (BOX 1).

DNA end resection
Removal of nucleotides by exonucleases involved in repairing DNA, exposing tracks of single-stranded DNA at sites of double-strand break repair. It is required for homologous recombination.

Non-homologous end joining
DNA double-strand break repair mechanism based on the juxtaposition of two pieces of DNA.

Homologous recombination
Mechanism of DNA double-strand break repair requiring the presence of duplicated chromatids, occurring only in S and G2 phases. It requires the resection of DNA ends at the break site.

CDK1 activity remains high, owing to its continued interaction with B-type cyclins[33]. Following chromosome alignment at metaphase, mitotic exit is initiated by the APC/C^{CDC20} (reviewed in[40]), which at the same time promotes ubiquitylation of B-type cyclins and targets them for proteolytic destruction. Protein phosphatases reverse the action of CDK1 by widespread substrate dephosphorylation[41]. As with mitotic entry, exit from mitosis is rapid and irreversible. APC/C^{CDC20} activation triggers a chain of events including separation of sister chromatids[42], spindle elongation to pull them apart[43] and formation of an actomyosin contractile ring to divide the cell into two[44]. At the same time, destruction of the sole remaining cyclin type (B) reduces CDK activity to zero and effectively resets the cell cycle in the two daughter cells to the prereplicative G1 phase.

Cell cycle checkpoints

Cells rely on cell cycle checkpoints to prevent the accumulation and propagation of genetic errors during cell division. These cell cycle control checkpoints depend on evolutionarily conserved signalling pathways that monitor DNA damage during interphase, loss of DNA replication fork integrity during S phase and incomplete spindle assembly during M phase (BOX 3; FIG. 2).

DNA damage checkpoint. Throughout interphase, the occurrence of DNA double-strand breaks (DSBs) triggers a rapid signalling response that depends on the checkpoint protein kinase ataxia telangiectasia mutated (ATM). The response results in changes to ongoing transcription levels and patterns, mobilization of DNA repair machinery and interplay with the cell cycle regulators, resulting in slowing or arrest of movement through the cell cycle[5,45]. The primary role of this cellular response to DNA damage is to prevent the accumulation and propagation of genetic errors during cell division. ATM is activated by the DNA damage sensor complex MRN (MRE1, RAD50 and NBS1) and phosphorylates a great number of substrates[46,47], but key targets for cell cycle control include the protein kinase CHK2 and the transcription factor p53 (REFS[48–50]). p53 activates the CDK inhibitor p21, leading to the inhibition of cyclin–CDK complexes mainly in G1, to prevent S phase entry. In S and G2 phases, CHK2 degrades CDC25[51], thus reinforcing WEE1-dependent inhibitory phosphorylation of CDK1 to prevent mitotic entry. p53 and ATM are not as critical during S and G2 phases for slowing or halting cell cycle progression owing to a certain level of redundancy with the DNA replication checkpoint.

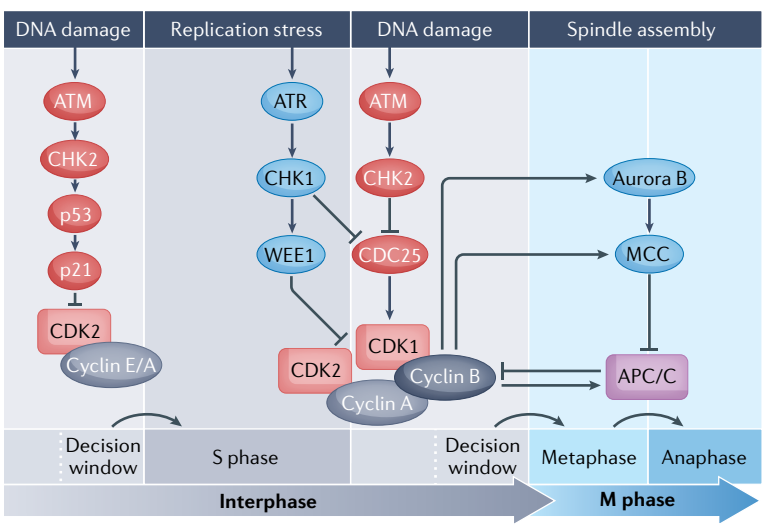
DNA end resection at DSBs is regulated by the cell cycle, and this influences the choice of both the repair pathway and the DNA damage signalling cascade[52]. During G1, DSB repair mechanisms are based largely on non-homologous end joining owing to lack of DNA end resection. In S and G2 phases, however, in response to DSBs the resection of DNA ends during DSBs allows repair via homologous recombination. DNA end resection generates single-stranded DNA at DSBs and this signals to ataxia telangiectasia and Rad3-related protein (ATR) and CHK1, the main kinases of the replication stress checkpoint response pathway, thus reinforcing the checkpoint response to DNA damage.

The severity of the damage dictates the choice of cell fate (FIG. 2). However, the threshold of what constitutes 'severe' varies depending on the environment and cell type, so cell fate decisions are not uniform or always easily predictable. Cells can re-enter the cell cycle, permanently exit the cell cycle and become senescent, or undergo apoptosis. In the prereplicative G1 phase, if the cell does not undergo apoptosis, the cell cycle arrest is either reversible (quiescence) or irreversible (senescence)[53]. In contrast, in S and G2 phases, long-term arrest results mainly in an irreversible exit from the cell cycle through senescence or apoptosis. The inability to re-enter the cell cycle is largely through mechanisms controlled by p53 (for reviews, see[53,54]).

DNA replication stress checkpoint. The DNA replication stress checkpoint functions only during S phase. DNA replication forks encounter many potential obstacles that obstruct their progression for successful and faithful genome duplication. Such impediments can cause DNA replication forks to progress slowly or stall, which exposes single-stranded DNA, and is defined as

Box 3 | **Cell cycle checkpoints and cell cycle control**

In response to DNA damage during interphase, replication stress during S phase or incomplete spindle assembly during M phase, specific cell cycle checkpoints arrest or slow down the cell cycle by inhibiting the activity of cyclin-dependent kinases (CDK) and the anaphase-promoting complex/cyclosome (APC/C) (see the figure). The DNA damage checkpoint can be activated by double-strand DNA breaks (DSBs) throughout interphase and depends on the checkpoint protein kinases ataxia telangiectasia mutated (ATM) and CHK2 to block cell cycle progression. Depending on the phase of the cell cycle, it can either prevent the accumulation of cyclin E/A–CDK2 activity to block replication initiation in pre-S phase or block mitotic entry during S phase and G2 phase. The replication stress checkpoint is activated in response to the presence of single-stranded DNA and depends on the checkpoint protein kinases ataxia telangiectasia and Rad3-related protein (ATR) and CHK1 to prevent the accumulation of cyclin A/B–CDK1/2 activity to block mitotic entry. Downstream regulation depends on activation of the kinase WEE1, for inhibitory phosphorylation of CDK1/2, and inactivation of the phosphatase CDC25, which removes the inhibitory phosphorylation of CDK1/2. The spindle assembly checkpoint is activated by incomplete attachment of chromosomes to the mitotic spindle and depends on the mitotic checkpoint complex (MCC; BUB3 together with MAD2 and MAD3 bound to CDC20) to prevent APC/C activation and thus to block mitotic exit.



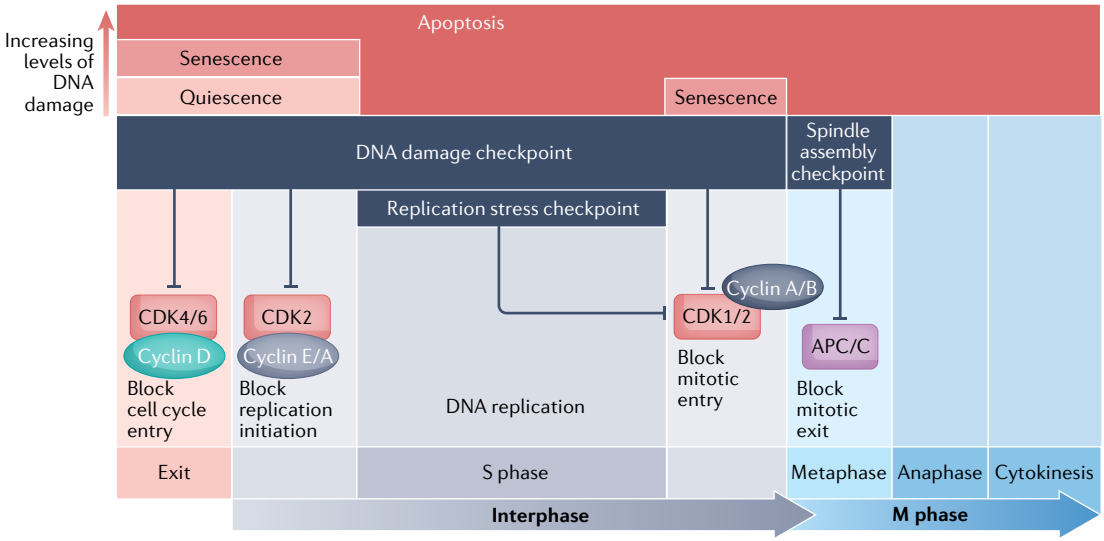


Fig. 2 | **Checkpoint-dependent cell cycle arrest and exit.** The replication stress checkpoint can block mitotic entry during S phase by preventing the accumulation of cyclin-dependent kinase 1/2 (CDK1/2)–cyclin A/B activity, and the spindle assembly checkpoint can block mitotic exit during M phase by preventing the activation of the anaphase-promoting complex/cyclosome (APC/C). By comparison, the DNA damage checkpoint operates throughout interphase. Depending on the phase of the cell cycle, the DNA damage checkpoint can either block mitotic entry during and following S phase, much like the replication stress checkpoint, or block replication initiation in pre-S phase by preventing the accumulation of cyclin E/A–CDK2 activity. It can also block cell cycle entry following mitotic exit, or during a prolonged pre-S phase, by preventing or inhibiting cyclin D–CDK4/6 activity, thereby inducing a reversible cell cycle exit known as quiescence. In response to high levels of DNA damage, the DNA damage checkpoint can induce an irreversible exit from the cell cycle, through senescence, outside S and M phases, or even cell death through apoptosis throughout the cell cycle.

replication stress[55]. DNA replication stress can be experienced by cells under normal physiological conditions or due to exogenous factors[56]. It can be induced by many factors, including deregulation of components required for DNA synthesis, a decrease or increase in the frequency of replication initiation and factors that block replication forks, such as unusual DNA structures, DNA damage forming bulky lesions or collision with transcription machinery[57].

The accumulation of single-stranded DNA, arising from the decoupling of the polymerase and the helicase, is the signal for activation of the checkpoint protein kinase ATR and its downstream effector CHK1[58–60] (BOX 2). This triggers an elaborate local and global cellular response. Replication stress is not DNA damage, and the main function of the DNA replication checkpoint response is actually to prevent replication stress-induced DNA damage. An important part of the response is preventing mitotic entry to allow more time for replication to be completed. The checkpoint controls cell cycle progression through restricting CDK activity, primarily through CHK1-dependent phosphorylation of CDC25, which results in its proteasomal degradation[61], and WEE1, by promoting 14-3-3 binding[62,63]. This results in WEE1-dependent inhibitory phosphorylation of CDK1. To prevent replication stress-induced DNA damage and ensure complete duplication of genomic material before entry into mitosis, the checkpoint response regulates at least three aspects of DNA replication: first, by blocking late-origin firing; second, by stalling ongoing replication forks; and thirdly, by stabilizing stalled replication forks[64–71]. This prevents the initiation of replication at new sites, limits the overall rate of replication and allows replication forks to recover and resume once the impediments are dealt with, ensuring all areas of the genome are replicated[65,72–76]. This is a temporary solution, as a more prolonged stalling of a replication fork can cause fork collapse, whereby replisome components dissociate from the DNA, leading to DSBs. Once this happens, the DNA damage checkpoint response will be activated to repair the damage.

Spindle assembly checkpoint. The spindle assembly checkpoint (SAC) functions in M phase to ensure that replicated DNA is partitioned equally between the two daughter cells. Errors in mitotic spindle formation (for example, uncaptured chromosomes or two sister chromatids attached to the same spindle pole) result in incorrect chromosome segregation and chromosomal gains or losses in daughter cells. The SAC prevents this by acting as a surveillance mechanism for unattached or incorrectly attached kinetochores. The SAC machinery is a multiprotein complex that is recruited to any kinetochores not bound to microtubules following phosphorylation by Aurora B and CDK1 (REFS[7,77,78]). The SAC catalyses the formation of the diffusible mitotic checkpoint complex (consisting of MAD2, BUBR1 and CDC20), which acts as a potent inhibitor of APC/C^{CDC20} and anaphase initiation[79–82]. Once all kinetochores are attached and bioriented, lack of SAC activity leads to disassembly of the mitotic checkpoint complex, freeing up CDC20 to act as a co-activator of APC/C[83,84]. A single unattached kinetochore gives sufficient signal to maintain APC/C inhibition and M phase arrest[85]. In this way,

Senescence
A non-reversible cellular state, outside G1 phase, from which cells cannot re-enter the cell cycle.

the SAC acts as a 'delay' signal, prolonging mitosis until bipolar spindle attachment is achieved by all chromosomes. Since mitotic entry is irreversible, cells cannot exit mitosis until the SAC is satisfied. This means that prolonged SAC activation (for example, in cells treated with microtubule disrupting agents) becomes arrested in mitosis, for as long as 24 h (REF.[86]). In cases where chromosome biorientation is not resolved following prolonged mitotic arrest, cells follow two paths: either apoptosis via caspase activation[87] or 'slippage', whereby cells exit M phase without chromosome segregation and enter the next cell cycle as a single tetraploid cell[88]. Mitotic slippage occurs because basal levels of B-type cyclin degradation during prometaphase eventually lower CDK1 activity to below the threshold for M phase exit[89]. Slippage frequently results in cell death or cell cycle exit through the activation of p53 (REFS[90,91]).

Cell cycle control in cancer

Sustained proliferative signalling, which drives continuing and excessive rounds of cell division, is the hallmark of cancer. Recent insight has revealed that this continuous cell division is driven by mutations that both prevent apoptosis and compromise cell cycle exit, rather than driving uncontrolled cell cycle progression. These include mutations in the signalling pathways that initiate exit from the cell cycle or promote S phase entry[92], but much less frequently in those that prevent mitotic entry[93–96] and exit[97–101] (FIG. 3). This suggests that, as the cell cycle is an exquisitely finely regulated process, endless division cycles present fundamental challenges to cancer cells that require some checkpoints (for example, replication stress or mitotic checkpoint) to remain functional[102]. Understanding how cancer cells overcome these challenges reveals vulnerabilities that can be targeted therapeutically.

Cell cycle checkpoints in cancer. Cells can exit the cell cycle either reversibly, through initiating quiescence, or non-reversibly, by senescence or apoptosis. The decision to exit the cell cycle depends on just one of the cell cycle checkpoints — the DNA damage checkpoint (BOX 2). Throughout interphase, in response to irreparable DNA damage, the DNA damage checkpoint can initiate quiescence, senescence or programmed cell death largely through p53-dependent pathways[103,104]. Unsurprisingly, p53 mutations are the most common mutations found in cancer[54]. However, even if cancer-associated mutations prevent cell cycle exit, continuous proliferation can still be prevented by blocking cell cycle entry in the prereplicative G1 phase, which depends on activation of E2F-dependent transcription. In line with this, cancer-associated mutations in this pathway have been found in all types of cancer[105], and include mutation in

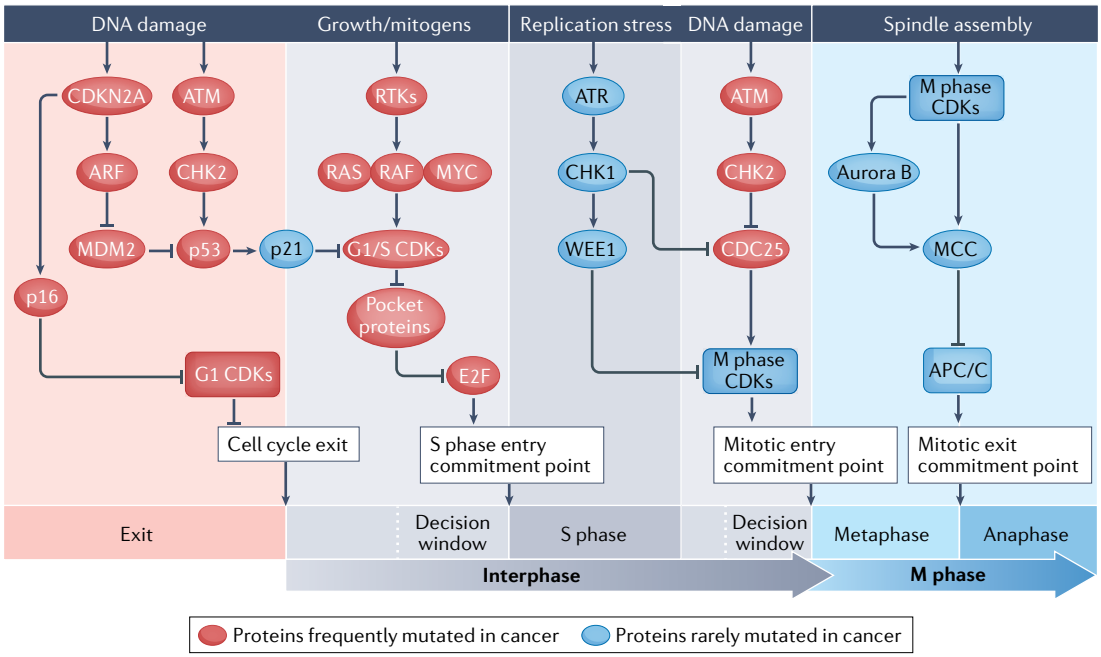


Fig. 3 | **Key signalling pathways involved in cell cycle control and cancer.** Continued cell cycle progression in cancer cells is driven mainly by mutations, or deregulation, of proteins involved in cell cycle-control signalling pathways. However, these mutations are associated with specific cell cycle control pathways more so than others. Mutations commonly found in cancer cells are shown in red: they affect mainly cell cycle control in response to DNA damage and growth signals in pre-S phase. These mutations drive S phase entry and prevent cell cycle exit. Very few cancer-associated mutations are found in proteins involved in the response to replication stress or incomplete spindle assembly; proteins that are rarely mutated in cancer are shown in blue. In the context of cancer treatment, these pathways represent therapeutic opportunities. Pocket proteins include retinoblastoma protein (RB), p107 and p130. E2F includes the activating E2Fs E2F1–E2F3 and indicates E2F-dependent transcription. G1/S cyclin-dependent kinases (CDKs) include cyclin D–CDK4/6 and cyclin E–CDK2. M phase CDKs include cyclin A/B–CDK1/2. APC/C, anaphase-promoting complex/cyclosome; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; MCC, mitotic checkpoint complex (BUB3 together with MAD2 and MAD3 bound to CDC20); RTKs, receptor tyrosine kinases.

Genome instability
Stochastic acquisition of genetic change over many cell divisions that can result in mutations and chromosomal rearrangements and aneuploidy.

Chromosomal instability
(CIN). Type of genome instability involving structural and/or numerical chromosome aberrations.

Aneuploidy
Abnormal number of chromosomes in a cell.

many oncogenes and tumour suppressors. These mutations induce E2F-dependent transcription, promoting S phase entry and compromising the ability of a cell to exit the cell cycle in the prereplicative phase.

Importantly, the main functions of other cell cycle checkpoints (the DNA replication stress checkpoint and the SAC) remain vital in cancer cells and thus these are not frequently mutated (FIG. 3). This includes the temporal checkpoint-dependent cell cycle arrest before and during mitosis, which is essential to prevent catastrophic levels of DNA damage resulting from replication stress or incomplete spindle assembly.

Oncogene-induced replication stress. While deregulated E2F-dependent transcription promotes cell cycle entry and compromises the ability of a cell to exit the cell cycle, this has a flip side. By accelerating S phase entry, it is thought to generate replication stress, known as oncogene-induced replication stress. For example, prolonged activation of MYC and cyclin E–CDK, expression of the human papillomavirus E7 oncoprotein, or loss of RB all induce E2F-dependent transcription and have been linked to deregulated replication initiation[106–110]. Oncogene-induced replication stress is thought to be an important source of genome instability and might therefore be the basis of intratumour heterogeneity. This is one of the main barriers to improving cancer outcome, in terms of both reliable biomarker identification and cancer multidrug resistance[111–113]. In some cases chromosomal instability (CIN) can be attributed to replication stress[114]. Owing to the presence of persistent replication stress, cancer cells have an increased dependence on the replication stress checkpoint response to prevent catastrophic levels of replication stress-induced DNA damage[115]. In addition, oncogene-induced replication stress also creates a particular dependency of cancer cells on the SAC[116].

Maintaining the key functions of the replication stress checkpoint response ensures the completion of DNA replication, a condition that is crucial for viability. Indeed, the kinases ATR and CHK1 are essential in all cell types, including cancer cells[115]. This represents a fundamental difference from the DNA damage checkpoint, in which key components are often mutated in cancer. DNA replication stress and DNA damage response are intertwined. It has been shown that replication stress-induced DNA damage ultimately triggers activation of the DNA damage checkpoint, which inhibits tumour development in its early stages by preventing proliferation[117–119].

Mitotic checkpoint in cancer. Aneuploidy is common in cancer[120], and it has long been known that abnormal chromosome configurations can arise through mitotic errors[121,122]. An obvious source of chromosome segregation errors would be a defective SAC. However, while mutations in SAC genes have been observed in cancer[123], they are rare[97]. More common are changes in gene expression, with overexpression of SAC components (including MPS1, MAD1, MAD2, BUBR1 and CDC20) being reported in several cancer types[98,124–126]. The effect of gene overexpression on SAC function or mitotic progression is not clear and may depend on the protein overexpressed[127]. For example, MAD1 overexpression has been shown to weaken the checkpoint by mislocalizing the SAC complex[124], while MAD2 overexpression can result in checkpoint hyperactivation[125]. The mixed evidence for SAC dysfunction in cancer indicates that SAC loss is not a major driver of tumorigenesis.

Cancer cells may be more dependent on the SAC than normal cells. Cancer cell lines typically spend three to five times longer in metaphase than normal cells[128,129], likely owing to sustained checkpoint activation delaying mitotic exit. Removing the SAC entirely in cancer cells results in catastrophic chromosome loss that is invariably lethal[1]. On the other hand, non-transformed cells are less sensitive to death induced by SAC inhibition[130,131]. This is largely because of the presence of aneuploidy as well as ongoing karyotypic changes, known as CIN, in cancer cells. CIN can involve either loss or gain of whole chromosomes (numerical CIN) or rearrangements of portions of chromosomes (structural CIN)[132]. Both types can be generated by segregation errors in mitosis[133]. In addition, replication stress in S phase[114] and DNA damage response activation in mitosis[134] have been shown to be a source of CIN.

Recent evidence suggests that low levels of CIN are beneficial to cancer cells, driving cancer evolution by generating a wider range of possible karyotypic combinations[135,136]. On the other hand, too much CIN is harmful, leading to cell death and growth suppression owing to the loss of essential genes[137,138]. High CIN can be associated with improved prognosis of patients[139,140]. A functioning SAC is crucial in cells with low CIN to extend mitosis for long enough to allow spindle capture of abnormal chromosomes. In line with this, experimental induction of CIN in diploid cells leads to a decrease in APC/C^{CDC20} function, which prolongs mitosis and guards against the development of further excessive CIN[141]. Thus, the compromised cell cycle exit checkpoints in cancer that fuel continuous cell division may lead to greater reliance on the mitotic checkpoint to allow cells to tolerate aneuploidy without developing excessive CIN.

Cell cycle control in cancer: therapeutic opportunities

Cancer cells continue to divide as a result of mutations that allow cell cycle progression and prevent exit. An important consequence is that all cancers depend on continuous cell division and many become increasingly dependent on remaining cell cycle control mechanisms to prevent the excessive accumulation and propagation of genome instability.

Exploiting cancer reliance on cell cycle control pathways. Generating excessive levels of DNA damage, through either radiotherapy or chemotherapy, is more likely to result in catastrophic levels of genome instability and therefore cell death in cancer cells than in healthy cells[142]. This approach remains the most effective way to treat cancer[143], but also harms healthy cells by inducing DNA damage.

The increased reliance of cancer cells on cell cycle control regulatory pathways provides opportunities to

Senolytics
Compounds that selectively kill cells that are in a state of senescence.

target pathways or processes that are essential in cancer cells but dispensable in healthy cells. However, the complexity of the cell cycle control network suggests that different pathways or processes should be targeted in different cancers. Therefore, to increase the effectiveness of targeting cell cycle control mechanisms by anticancer agents, we need to understand the network dynamics in normal cells compared with cancerous cells in the context of specific cancer-associated mutations. This could be used to guide better drug design, therapeutic combinations and patient selection. Here, we focus on the control of cell cycle progression, the response to replication stress and the control of M phase entry and progression, and refer to other reviews that highlight the potential targeting of specific aspects of DNA repair mechanisms (FIG. 4; TABLE 1).

Forcing cancer cells to permanently exit the cell cycle. Tumour growth depends on continued cell proliferation. The main driver of cell cycle progression is CDK activity, and increased CDK activity has been widely reported in various cancers, making CDKs attractive targets for new treatments[144]. CDK inhibitors could potentially prevent continued cell cycle progression by forcing cancer cells to permanently exit the cell cycle into a senescent state. Such cytostatic drugs would prevent tumour growth, but also offer potential opportunities to eradicate senescent cancer cells using senolytics[145]. There is much interest in such a 'one–two punch' approach where cytostatic drugs induce senescence in cancer cells and sequential treatment with senolytics kills senescent cancer cells[146].

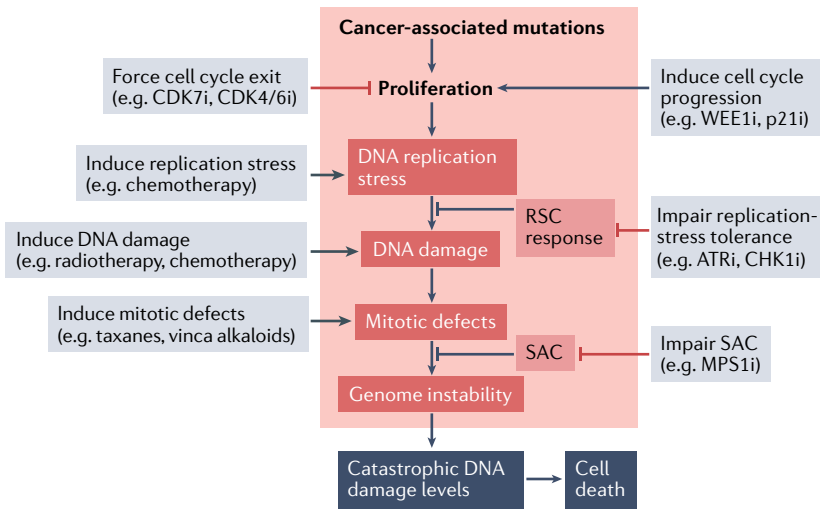


Fig. 4 | **Cancer's continued proliferation presents many therapeutic opportunities.** Cancer is initiated by mutations in the DNA that allow cells to continuously divide. An important outcome is that all cancers depend on continuous cell division and specific aspects of the cell cycle checkpoint responses to prevent catastrophic genome instability and cell death, which can be exploited in cancer treatment. The stepwise consequences of cancer-associated mutations that allow continued cell proliferation and the cellular responses that prevent or minimize (blocking arrows) processes that can lead to catastrophic genome instability and cell death are depicted in red boxes. Continued proliferation itself, its consequences and the cellular responses to these represent therapeutic opportunities (indicated in grey boxes) that can be exploited through the use and development of anticancer drugs and/or therapies (indicated in parentheses). ATRi, ataxia telangiectasia and Rad3-related protein inhibitor; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; CDK7i, cyclin-dependent kinase 7 inhibitor; CHK1i, CHK1 inhibitor; MPS1i, MPS1 inhibitor; p21i, p21 inhibitor; RSC, replication stress checkpoint; SAC, spindle assembly checkpoint; WEE1i, WEE1 inhibitor.

Various CDK-targeting drugs have entered clinical development, but until recently few have achieved their expected efficacy in clinical trials[147]. However, more detailed insight into the role of the various CDKs in cell cycle control is now being used to direct therapeutic strategies and increase the effectiveness of cell cycle kinase inhibitors. The most prominent examples of these are the CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib[148]. These drugs target the most commonly deregulated CDK activity in cancer, D-type cyclin–CDK[149], and show significant clinical benefit for the treatment of hormone receptor-positive metastatic breast cancer, leading to FDA approval for treatment of this disease. As discussed earlier, D-type cyclin–CDK activity is now thought to play a more direct role in preventing cell cycle exit than in driving cell cycle entry. This new fundamental understanding suggests that CDK4/6 inhibitors can force cancer cells to exit the cell cycle. In line with this, published data show that CDK4/6 inhibition can trigger senescence, apoptosis or quiescence in specific cancer types (for more detailed reviews, see[145,150,151]). This new understanding predicts that patients with cancer-associated mutations in proteins involved in cell cycle exit pathways, such as p16 (also known as CDKN2) are more likely to benefit from CDK4/6 inhibitors. Conversely, patients with mutations that drive cell cycle entry independently of CDK4/6 activity, such as oncogenic E-type cyclin–CDK or E2F1 or loss of RB, are less likely to benefit from CDK4/6 inhibition. However, there is still much to learn about the role of CDK4 and CDK6 in cell cycle control and how inhibitors such as palbociclib affect their function. Most recently, studies have indicated that palbociclib may also prevent cell cycle entry by indirectly inhibiting CDK2, via increasing p21 abundance, and as such arrests cell cycle entry even when CDK4's kinase activity is already impaired[152–154]. In addition, current studies show promising results by combining CDK4/6 inhibitors with MAPK pathway inhibitors in KRAS-driven lung and pancreatic cancer mouse models[155,156]. Senescence induced by the combination of these cytostatic drugs is much more pronounced than that induced by single drug treatment alone. In both cases, the senescence-associated secretory phenotype, which leads to a local inflammatory response, was a crucial driver of these responses. This opens new perspectives on the combinatorial use of cytostatic drugs.

In addition to CDK4/6 inhibitors, several studies have shown a marked sensitivity of many cancer types to selective CDK7 inhibition[157–165] CDK7 is the main kinase activity of CDK-activating kinase (CAK), which phosphorylates CDK1, CDK2, CDK4 and CDK6, the main cell cycle-dependent CDKs[166]. Importantly, cancer cells are sensitive to CDK7 inhibitors at doses at which normal cells are insensitive, suggesting that treatments might be well tolerated in a clinical setting. In comparison with CDK4/6 inhibitors, which are rendered ineffective in patients with mutations that drive cell cycle entry, CDK7 inhibitors inhibit CDK4/6 and CDK2 activity, both preventing cell cycle entry and promoting

Table 1 | **Drugs that target cell cycle regulatory factors as possible therapeutics for cancer**

Molecular target	Drugs	Clinical use	Refs
Forcing cell cycle exit			
CDK4/6	Palbociclib	Approved for ER^+ and $HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumours	147,201,202
	Ribociclib	Approved for ER^+ and $HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumours	147,203–205
	Abemaciclib	Approved for ER^+ and $HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumours	147,206–208
CDK7	ICEC0942 (CT7001)	Phase I/II, ER^+ breast cancer, AML	147
Forcing cell cycle progression			
WEE1	Adavosertib (AZD1775)	Phase II, relapsed SCLC, ovarian cancer, NSCLC, AML, gastric adenocarcinoma and various advanced solid tumours	94,181
Impairing replication stress tolerance			
ATR	VX-970	Phase II, recurrent ovarian, primary peritoneal or fallopian tube cancer and metastatic urothelial carcinoma	181
CHK1	LY2606368	Phase II, SCLC, *BRCA1/BRCA2*-mutated breast or ovarian cancer, TNBC, HGSOC, metastatic CRPC and advanced solid tumours with HRR defects or genetic alterations indicative of replication stress.	181
		Phase I, acute leukaemia, solid tumours or lymphoma	
	MK-8776	Phase II, relapsed AML (with or without cytarabine)	181
		Phase I, advanced solid tumours	
Inducing catastrophic genome instability			
Mitotic spindle	Taxanes (paclitaxel, docetaxel and nanoparticle albumin-bound paclitaxel)	Approved for use in a wide range of cancers, including ovarian cancer, breast cancer, lung cancer, bladder cancer, prostate cancer, melanoma and oesophageal cancer.	184–186
	Vinca alkaloids (vinblastine and vincristine)	Approved for use in a range of cancers, including ALL, AML, HL, neuroblastoma and NSCLC	184,187
SAC	MPS inhibitors (BAY 1161909 and BAY 1217389)	Preclinical studies in neuroblastoma, medulloblastoma and breast cancer (in combination with taxanes). Recently entered phase I clinical trials	194–198,209
	Aurora B inhibitors (various, including AZD1152 and AT9283)	Phase II in AML, multiple myeloma, SCLC and prostate cancer. Phase I in various solid tumours	101

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ATR, ataxia telangiectasia and Rad3-related protein; CDK, cyclin-dependent kinase; CRPC, castration-resistant prostate cancer; ER, oestrogen receptor; HGSOC, high-grade serous ovarian cancer; HL, Hodgkin lymphoma; HRR, homologous recombination repair; NSCLC, non-small-cell lung cancer; SAC, spindle assembly checkpoint; SCLC, small-cell lung cancer; TNBC, triple-negative breast cancer.

cell cycle exit. This suggests that CDK7 inhibitors could trigger cell cycle exit and apoptosis in cancers with mutations in either cell cycle exit or cell cycle entry pathways, potentially benefiting a wider range of patients. In addition to its role in CDK activation, CDK7 is also required for RNA polymerase II (Pol II)-dependent transcription, through Pol II and CDK9 phosphorylation[8]. With many cancers depending on transcriptional drivers, CDK7 inhibitors therefore kill two birds with one stone: the continued cell cycle progression via CDK inhibition and the addiction to transcriptional drivers via Pol II inhibition. However, it is not yet known whether the effectiveness of CDK7 inhibition in driving a cell cycle exit depends on the inhibition of CDK, Pol II or both. In addition, it is unclear why some cancer cells are more sensitive to CDK7 inhibition, and of those why some undergo arrest and others undergo apoptosis[167]. With CDK7 inhibitors moving ever closer to the clinic, it will be vital to gain a more fundamental understanding of how cancer-associated mutations modulate CDK7 inhibitor-induced cell cycle exit and apoptosis both for the effectiveness of CDK7 inhibitor treatment and for patient stratification.

Overall, it will be crucial to gain a detailed fundamental understanding of the role of the various CDKs and what makes specific cancer cells sensitive to CDK inhibition. This will guide identification of biomarkers that can be used for patient stratification for the clinical use and development of CDK inhibitors, identify how drugs previously shelved during drug development might be effective when combined in the right way and identify targetable vulnerabilities of senescent cancer cells.

Forcing uncontrolled cell cycle progression in cancer cells. An alternative to restricting cancer cell cycle progression by targeting CDK activity would be to put cell cycle progression into overdrive by increasing CDK activity. CDK inhibitors such as WEE1 and p21 (REF.[104])

delay or arrest the cell cycle until certain criteria are met. Whereas WEE1 delays mitotic entry, p21 mainly restricts S phase entry. With many cancer-associated mutations promoting S phase entry via deregulation of E2F-dependent transcription, cancer cells are thought to become increasingly reliant on these CDK inhibitors to prevent propagation of DNA damage[168]. In line with this, p21 is rarely mutated in human cancer[169], while WEE1 is expressed at higher levels in various cancer types[94]]. Therefore, targeting p21 or WEE1 could potentially compromise a cancer cell's ability to prevent propagation of DNA damage and thereby generate excessive levels of DNA damage to tip the balance towards cancer cell death. Inhibition of WEE1 forces cells with damaged DNA to prematurely enter mitosis, ultimately resulting in mitotic catastrophe and apoptosis[170]. Targeting WEE1 for inhibition is therefore currently being explored as an opportunity to further potentiate DNA damage-inducing therapies[171]. For example, a recent study found that carboplatin-resistant breast cancer cells are uniquely vulnerable to WEE1 depletion[172]. p21 is only now being considered as a potential intervention target for cancer treatment. This is based on a better fundamental understanding of p21's central role in preventing cell cycle entry and more translational studies, but much more work is needed to explore potential therapeutic approaches in the cancer field[173]. Future research will reveal whether increasing CDK activity in cancer cells, by targeting CDK inhibitory proteins, can selectively drive catastrophic genome instability without inducing genome instability in healthy cells.

Exploiting oncogene-induced replication stress. The replication stress response is often discussed as part of the DNA damage response, but, as pointed out already herein, they are distinctly different. The replication stress response prevents replication stress-induced DNA damage, while the DNA damage response prevents the propagation of DNA damage. This distinction is crucial in the context of cancer biology and therapeutics. Therapies targeting the replication stress response will cause an increase in replication stress-induced DNA damage in cancer cells already experiencing oncogene-induced replication stress. This alone can lead to catastrophic levels of genome instability in cancer cells, while leaving healthy cells unharmed. Targeting the replication stress response can also increase the efficacy of cancer therapies that induce DNA damage or target the DNA damage response.

Cancer cells commonly experience oncogene-induced replication stress[174]. Therefore, targeting the DNA replication stress checkpoint protein kinases or proteins involved in replication stress tolerance should be specifically toxic to cancer cells[115]. In line with this, ATR and CHK1 inhibitors are particularly toxic to cells experiencing oncogene-induced replication stress caused by deregulated S phase entry[175–178]; however, initial clinical trials suggest limited potential for their use as anticancer drugs[115]. It is now becoming clear that focusing these treatments on cancers with high levels of replication stress, due to a combination of genetic deficiencies, does have the potential to translate into clinical efficacy[178,179]. In particular, patients with mutations that drive cell cycle entry are likely to benefit[180]. Adjustment of treatments on the basis of additional genotypes is currently being tested, and phase II trials have started for triple-negative breast cancer, small-cell lung cancer, platinum-resistant ovarian cancer and haematological cancers[181]. This approach requires reliable biomarkers to determine replication stress levels in patient tissue, which at the moment are missing.

In many cells *ATR* and *CHK1* (also known as *CHEK1*) are essential genes[182], which limits the therapeutic window. An alternative approach, currently being explored, is to direct treatment to normally dispensable processes that acquire essential functions only during the replication stress response. One such process is replication stress tolerance, which involves many factors necessary to cope with increased replication rates and prevent catastrophic genome instability[115]. For example, increased deoxyribonucleoside triphosphate levels have been shown to reduce oncogene-induced replication stress[109]. This suggests that cancer cells experiencing high levels of replication stress might become reliant on increased deoxyribonucleoside triphosphate levels. Additionally, it was recently shown that the tolerance to replication stress depends on increased E2F-dependent transcription, which increases the expression of genes encoding many of the proteins involved in DNA replication and repair[183]. These findings suggest that cancer cells might rely on specific processes to tolerate high levels of oncogene-induced replication stress, which can be targeted to generate novel therapeutic strategies. To exploit replication stress tolerance in cancer, more work is needed to establish specific targetable aspects of replication stress tolerance. These include the proteins and processes involved in preventing oncogene-induced replication stress, those involved in preventing replication stress-induced DNA damage and those required for replication stress-induced DNA damage repair.

Overall, replication stress tolerance is emerging as a promising area of investigation for the discovery of novel targeted therapies. However, two major factors restrain our ability to exploit this successfully in cancer treatment: a limited understanding of the proteins and processes involved in the specific aspects of tolerance, and a lack of clear and robust biomarkers to guide the clinical use of existing and novel drugs that target the replication stress response. Without advances in these areas the promise of successfully targeting replication stress tolerance in cancer, alone or in combination with existing therapies, in the clinic will not be met.

Driving catastrophic CIN. Cancer cells rely on a functional SAC more than do normal cells to prevent catastrophic errors in chromosome segregation during M phase[1,130,131]. This makes the mitotic checkpoint an attractive target for cancer therapy. Two of the most successful classes of chemotherapy drugs work by disrupting formation of the mitotic spindle: vinca alkaloids, which destabilize microtubules, and taxanes, which stabilize them (reviewed in[184]). In both cases, the inability of cells to form a bipolar spindle means that the SAC remains active and cells stop in mitosis. In cultured cells,

prolonged mitotic arrest leads directly to apoptosis[185]. However, at clinically relevant taxane doses, spindle errors instead result in catastrophic chromosomal mis-segregation, leading to excessive CIN and ultimately the creation of non-viable daughter cells[186].

The success of microtubule-targeting drugs has led to the search for more specific agents to target mitosis therapeutically in cancer. However, these attempts have been so far mostly unsuccessful[187,188]. Targeting EG5, a kinesin required for bipolar spindle formation, recapitulates the effect of taxane treatment in cell culture but this has not translated to the clinic[189]. Similarly, inhibitors of CENPE, which mediates microtubule–kinetochore attachment, or the mitotic kinases Aurora A, Aurora B, CDK1 and PLK1 have shown promise in preclinical studies but remain stalled in late-stage clinical trials, mainly owing to adverse effects on normal proliferative tissues[101,184,187–192]. However, 'smarter' strategies such as combination with other therapies or the manufacture of prodrugs that become preferentially activated in cancer cells may provide greater success for these compounds in the future[188].

An alternative approach to targeting mitosis in cancer is to inhibit the SAC directly, inducing premature mitotic exit and excessive CIN. This strategy would likely hit cancer cells harder than normal cells owing to the pre-existing CIN in many tumours. Turning low CIN into high CIN has been shown to be an effective method of inducing cell death in many experimental systems[137,138,193]. For example, inhibition of the SAC component MPS1 (also known as TTK) or low-dose taxane treatment both induce low levels of CIN when used separately, but in combination drive high CIN, severe chromosome segregation errors and the resulting death of unviable progeny in a mouse breast cancer model[194]. Recent work has revealed how aneuploid cells can be uniquely vulnerable to SAC inhibition: although aneuploid cells were initially less sensitive than diploid cells to MPS1 inhibition, after 10 days of treatment the cumulative effect of multiple chromosomal aberrations resulted in increased death and decreased proliferation specifically in aneuploid cells[195]. Several MPS1 inhibitors have been developed and show promise particularly in treating paediatric neurological malignancies[100,196–198]. Inhibitors of MPS1 or other SAC components (such as CDC20 (REF.[99])) are good candidates for a new generation of antimitotic targeted therapies. However, preclinical work makes it clear that these drugs are most effective at killing cancer cells when used to exploit pre-existing vulnerabilities (such as aneuploidy) or in combination with other treatments. The effect of taxanes, in particular, can be enhanced by treatments that alter SAC activity[197,199,200]. Exploiting this in the clinic will require both identification of biomarkers for aneuploidy or CIN in patients and using our understanding of SAC function to design smart combinations of therapies to target cancer cell dependency on the mitotic checkpoint.

Conclusions and perspectives

We have discussed recent work uncovering the detailed roles of cell cycle checkpoints in cell cycle control and how only specific aspects of these checkpoints are compromised in cancer cells to allow continuous cell division. This work is guiding and improving existing therapeutics and highlights opportunities to develop novel and combinatorial treatments (TABLE 1). These specifically include targeting replication stress tolerance mechanisms, the mitotic checkpoint, and proteins and processes involved in delaying or arresting cell cycle progression. These new strategies could be used alone or in combination with existing drugs that induce DNA damage and replication stress. In addition, it opens up the prospect that cancer could be managed by drugs forcing cancer cells to permanently exit the cell cycle. Overall, our new understanding of cell cycle control and cancer is guiding current treatments and creating therapeutic opportunities to improving initial treatment with curative intent, either through greater precision or by extending treatment modalities and better-informed treatment decisions that will result in better outcomes for patients.

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