Cell Cycle–Targeted Cancer Therapies

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Annu. Rev. Cancer Biol. 2017. 1:41–57

First published online as a Review in Advance on August 24, 2016

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

This article's doi: 10.1146/annurev-cancerbio-040716-075628

Keywords

cyclin-dependent kinases, CDKs, genotoxic damage, oncogene addiction, synthetic lethality, cell cycle checkpoints, cancer treatment

Abstract

A cardinal feature of cancer cells is the deregulation of cell cycle controls. Targeted drug therapy is designed to take advantage of specific genetic alterations that distinguish tumor cells from their normal counterparts. Mutated oncogenes and inactivated tumor suppressors can increase the dependency of cancer cells on G_1-phase cyclin-dependent kinases, augment replication stress and DNA damage during S phase, and dismantle checkpoints that monitor progression through $S/G_2/M$. These acquired defects generate cancer cell–specific vulnerabilities that provide a window of opportunity for targeted cancer treatments. We review the basic principles underlying the design of targeted therapies with emphasis on two main features: oncogene addiction and synthetic lethality. We discuss how traditional cytotoxic agents may depend, with relatively less specificity, on these same features and then point to examples of the successful application of newly developed, targeted therapeutic agents that offer reduced, dose-limiting toxicities to normal cells.

1. INTRODUCTION

S phase: the DNA synthesis phase of the cell cycle

The cell division cycle functions in an oscillatory manner to couple cellular DNA replication with chromosomal segregation, thereby ensuring that duplicated genetic material is distributed equally to two daughter cells (Norbury & Nurse 1992). In cycling somatic cells, the intervals between DNA synthesis (S phase) and mitosis (M phase) are separated by two gap phases (G_1 and G_2, respectively) (**Figure 1**). Cyclins expressed during different phases of the cycle allosterically regulate a family of cyclin-dependent kinases (CDKs), whose phosphorylation of key substrates enforces cell cycle progression (**Figure 1**) (Hunt 1991). Additional checkpoint controls act to guarantee that one process is completed before another begins (Hartwell & Weinert 1989). These

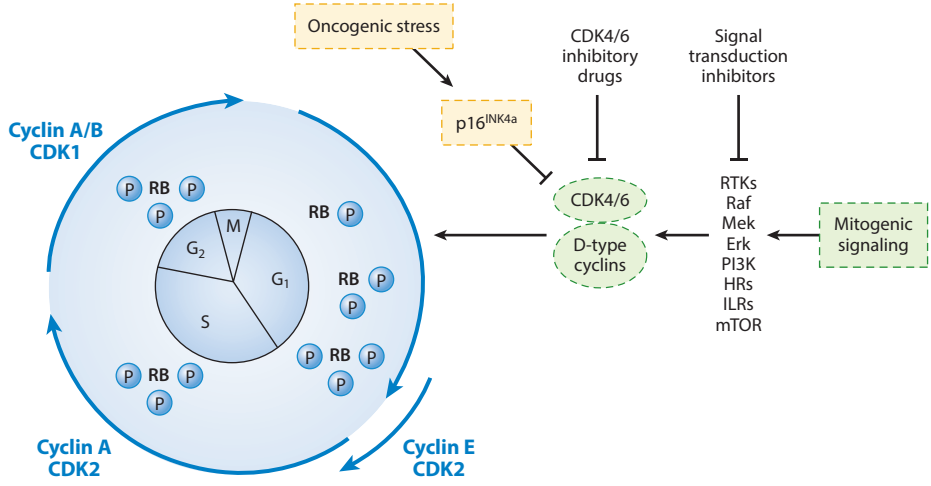

Figure 1

Cell cycle dynamics. The four phases of the mitotic cell division cycle are indicated at the left. Cells with unphosphorylated RB (retinoblastoma protein, a corepressor of E2F-responsive genes) enter into G_1 phase from a quiescent state (G_0) (not shown) and progress toward S phase in response to the mitogen-dependent, cyclin D–dependent kinases (CDKs) 4 and 6. RB phosphorylation by CDK4 and CDK6, and later by cyclin E– and A–dependent CDK2, inactivates RB, which is maintained in its hyperphosphorylated state by CDKs until cells exit mitosis and RB activity is restored by dephosphorylation. Total CDK activity increases throughout the cycle, but the degradation of cyclins A and B during M phase restores the G_1 state, in which low CDK activity is required for the licensing of the origins of replication that fire during S phase. Mitogenic signal transduction pathways that induce D-type cyclins and regulate their assembly with CDK4 and CDK6 (*right*) include receptor tyrosine kinases (RTKs), components of the RTK/Ras signaling pathways (Raf, Mek, Erk, PI3K), hormone receptors (HRs), and interleukin cytokine receptors (ILRs); nutrients promote increases in cellular mass and G_1 progression by stimulating the mechanistic target of rapamycin (mTOR). Cyclin D is degraded during S phase (Baldin et al. 1993, Diehl et al. 1998) but restored during G_2 in response to Ras signaling (Hitomi & Stacey 2001, Stacey 2003). In continuously cycling cells, the reaccumulation of cyclin D during G_2 results in the contraction of ensuing G_1 intervals and cell cycle generation times throughout later mitotic divisions. Mitogen withdrawal leads to cyclin D degradation, regardless of the position in the cycle, and cells exit the cycle from G_1. Depending on the biological context, cells can exit G_1 into G_0 or undergo definitive cell cycle withdrawal (senescence). INK4 proteins [including $p16^{INK4a}$ (shown) and $p15^{INK4b}$] are induced in response to hyperproliferative mitogenic stress, primarily resulting from oncogenic mutations affecting signaling pathway components. The INK4 proteins specifically inhibit CDK4 and CDK6 to promote cell cycle arrest; their effects are mimicked by chemical CDK4 and CDK6 inhibitory drugs. Signal transduction inhibitors, which block the accumulation of D-type cyclins, synergize with CDK4 and CDK6 inhibitors to arrest cell cycle progression. Adapted from figure provided by Ashley Broussard, St. Jude Children's Research Hospital Biomedical Communications Department, Memphis, TN.

mechanisms ensure, for example, that G_1 cells that acquire DNA damage do not enter S phase, that replicative DNA damage during S phase is repaired before cells enter mitosis, and that duplicated chromosomes are correctly aligned on the mitotic spindle before they segregate to daughter cells.

Many oncogenic mutational processes exert their effects by targeting signaling pathway components, enforcing constitutive mitogen-independent progression in lieu of normally regulated controls. Other mutations disrupt the DNA damage response (DDR) and checkpoint mechanisms, allowing inappropriate consolidation of genomic damage and triggering aneuploidy or catastrophic mitotic failure, or both. Targeted therapy is designed to take advantage of specific vulnerabilities acquired by cancer cells as a result of the driver mutations in oncogenes and tumor suppressors that they sustain. By exploiting the acquired genetic dependencies of cancer cells, targeted therapies should reduce the dose-limiting toxicities affecting normal cells and act with greater precision than conventional genotoxic agents in killing tumor cells.

2. TARGETING G_1 PHASE

2.1. Regulators of G_1 Progression

Following cell cycle entry from quiescence (G_0), G_1 progression is initially controlled by one or more mitogen-regulated D-type cyclins (D1, D2, or D3) that assemble combinatorially with CDK4 or CDK6; cyclin E–CDK2 complexes act later, near the G_1/S transition (**Figure 1**). Because the expression of D-type cyclins, their assembly into functional holoenzyme complexes with CDKs 4 and 6, and their stability and nuclear import are all mitogen-dependent events, mitogen withdrawal results in rapid turnover of cyclin D, loss of CDK4 and CDK6 activity, and arrest during G_1/G_0 (Alt et al. 2000, Choi & Anders 2014, Musgrove et al. 2011, Sherr 1995, Vaites et al. 2011).

CDK4 and CDK6 are fastidious kinases that phosphorylate relatively few substrates (Anders et al. 2011), the most critical being members of the retinoblastoma (RB) family [RB, RBL1 (p107), and RBL2 (p130)] (Ewen et al. 1993, Kato et al. 1993, Matsushime et al. 1992, Meyerson & Harlow 1994). RB-family proteins act as transcriptional corepressors and adaptors of chromatin modifiers that coordinate the activities of many genes required for cell cycle progression, metabolism, differentiation, apoptosis, and permanent cell cycle withdrawal (senescence) (Burkhart & Sage 2008, Manning & Dyson 2012, Nicolay et al. 2015, Sadasivam & DeCaprio 2013). A key set of proteins negatively regulated by RB includes the E2F transcription factors that upregulate a suite of genes whose activities are required for cell cycle progression and chromosomal DNA synthesis (Chen et al. 2009, Dyson 1998, Nevins 1998, Trimarchi & Lees 2002). E2F targets include cyclins E and A; geminin, an inhibitor of DNA rereplication during S phase; and EMI1, which, together with CDK2, inhibits an E3 ubiquitin ligase (APC^{CDH1}) that degrades cyclins A and B throughout the G_1 interval (Diffley 2004, Hsu et al. 2002, McGarry & Kirschner 1998, Miller et al. 2006, Teixeira & Reed 2013). APC^{CDH1} maintains the low levels of net G_1 CDK activity required for loading prereplication complexes onto DNA and for licensing origin firing before DNA synthesis begins. The activation of CDK2 by the RB- and E2F-regulated cyclins E and A and the inhibition of APC^{CDH1} by EMI1 promote the irreversible G_1/S transition and are required for the firing of replication origins and for centrosome duplication as cells enter S phase (Diffley 2004, Yeeles et al. 2015). RB is then maintained in its hyperphosphorylated inactive state by CDK2 and CDK1 until it again becomes hypophosphorylated during mitosis (Ludlow et al. 1990). Another form of APC, which uses the substrate recognition factor CDC20 in lieu of CDH1, assembles during S phase, but is inhibited by EMI1 until cyclin B–CDK1 complexes are reactivated during mitotic entry (see Section 4.2).

M phase: mitosis, including stages of nuclear membrane breakdown, chromosome condensation, alignment of chromosomes on the mitotic spindle, chromosome segregation, and cytokinesis

G_1 and G_2: cell cycle gap phases separating M and S phase (G_1) and S and M phase (G_2)

Retinoblastoma (RB) family: corepressors of E2F-responsive genes required for S phase entry and progression

Anaphase-promoting complex (APC): also called the cyclosome; a multiprotein E3 ubiquitin ligase driven by the substrate selection factors CDC20 (G_2/M) and CDH1 (M/G_1)

INK4 proteins bind directly to CDK4 and CDK6, but not to other CDKs, to inhibit their kinase activities. In humans, $p16^{INK4a}$ and $p15^{INK4b}$, encoded by closely linked *CDKN2A* (*INK4a*) and *CDKN2B* (*INK4b*) genes on chromosome 9p21, are induced by hyperproliferative stress, whereas unlinked genes encoding $p18^{INK4c}$ and $p19^{INK4d}$ negatively regulate CDK4 and CDK6 during normal tissue development (Ortega et al. 2002, Roussel 1999). The $p16^{INK4a}$ and $p15^{INK4b}$ proteins are not expressed in tissues of young, healthy animals, but are induced in response to aberrant oncogenic signals conveyed by mutant and constitutively active receptor tyrosine kinases, Ras proteins, or their downstream effectors (Kim & Sharpless 2006). The $p16^{INK4a}$, cyclin D and CDK, and RB proteins constitute the RB pathway, in which the negative regulators ($p16^{INK4a}$ and RB) are potent tumor suppressors, and the positive regulators (D-type cyclins and CDK4 and CDK6) have proto-oncogenic activity (Bartek et al. 1996, Sherr 1996). Not surprisingly, inactivation of *INK4A* by mutation, deletion, or silencing; RB loss of function; and amplification of the genes encoding D-type cyclins, CDK4, or CDK6 are frequent events in human cancers (Hall & Peters 1996). Because the final target of the pathway is RB itself, $p16^{INK4a}$ arrests RB-proficient cells during G_1 but lacks activity in that subset of tumor cells that are RB deficient (Koh et al. 1995, Lukas et al. 1995, Medema et al. 1995).

CDK2 activity is antagonized by another class of CDK inhibitors, the so-called CIP/KIP proteins (Sherr & Roberts 1999). The $p27^{Kip1}$ protein accumulates in quiescent cells (Polyak et al. 1994) but is phosphorylated, ubiquitinated, and degraded as cells that have entered the cycle approach S phase (Carrano et al. 1999, Nakayama & Nakayama 2006, Pagano et al. 1995). Notably, $p21^{CIP1}$ is a canonical target of p53 activation (El-Deiry et al. 1993). The p53 transcription factor acts as a potent tumor suppressor to arrest the cell cycle during G_1 in response to DNA damage or aberrant oncogene signaling, which is itself a source of replicative DNA damage during S phase (see Section 3.2). DNA damage during G_1 phase induces the ATM and CHK2 kinases, both of which phosphorylate and activate p53 (**Table 1**) (Ahn et al. 2004, Banin et al. 1998, Bartek & Lukas 2003, Canman et al. 1998, Kastan & Bartek 2004). In turn, p53-responsive genes, such as *CDKN1A*, encoding $p21^{CIP1}$, can arrest the cell cycle, whereas other p53 target genes can induce apoptosis or boost DNA repair (Kruiswijk et al. 2015, Levine & Oren 2009). Mutations targeting p53 are among the most frequent in cancer (Vogelstein et al. 2000), so that p53-deficient cancer cells defective in G_1 checkpoint control need to rely more strongly on intra-S-phase and G2/M controls for survival and may potentially be vulnerable to drugs targeting downstream checkpoints (Zhou & Bartek 2004).

INK4 proteins: specific polypeptide inhibitors of CDK4 and CDK6

CIP/KIP proteins: inhibitors of CDK2 and CDK1, including $p27^{KIP1}$ and $p21^{CIP1}$, the latter encoded by *CDKN1A*, a target of p53

p53: stress-induced transcription factor, frequently inactivated in cancer, which induces genes that trigger cell cycle arrest, apoptosis, and suppress metabolic reprogramming

Ataxia telangiectasia mutated (ATM) and ATM- and RAD3-related (ATR) kinases: kinases that sense DNA damage and recruit DNA damage response effectors

CHK1 and CHK2: kinases targeted by ATR and ATM that phosphorylate other DNA damage response substrates to trigger DNA repair and cell cycle arrest

2.2. Chemical Inhibitors of CDK4 and CDK6, and Oncogene Addiction

Although early-generation pan-CDK inhibitors failed to offer a strong therapeutic index for cancer cells versus normal cells, and were unacceptably toxic in the clinic (Asghar et al. 2015, Diaz-Moralli et al. 2013), potent and specific orally available chemical inhibitors of CDK4 and CDK6 have recently emerged as targeted therapeutics for cancer (Asghar et al. 2015, Sherr et al. 2016). These CDK4 and CDK6 inhibitors arrest tumor cells during G_1; however, tumors lacking functional RB are resistant, mimicking their refractoriness to $p16^{INK4A}$. One such drug, palbociclib (Pfizer) (Fry et al. 2004, Toogood et al. 2005), was approved by the US Food and Drug Administration in early 2015 for treating estrogen receptor (ER)-positive, HER2 (ERBB2)-negative breast cancer after it significantly extended progression-free survival of women with advanced disease without precipitating undue toxicity (Finn et al. 2015, VanArsdale et al. 2015). Notably, although palbociclib monotherapy exhibited limited activity, it acted synergistically in combination with the aromatase inhibitor letrozole (Novartis) to arrest breast cancer progression (Finn et al. 2015). This study underscores the concept that combinatorial therapies targeting both estrogen-dependent D-type

Table 1 Selected protein targets of drugs that impact cell cycle progression[a]

Drug target	Cell cycle phase[b,c]	Major functions during the cell cycle
ATM (ataxia telangiectasia mutated)	G_1, S, G_2	A kinase sensor of DNA double-strand breaks and oxidative stress; phosphorylates hundreds of proteins required for cell cycle arrest (e.g., CHK2, p53), DNA repair (e.g., NBS1, BRCA1), chromatin structure (e.g., cohesin components), and other diverse cellular functions
ATR (ATM- and RAD3-related)	(G1), S, G_2	A kinase essential for both unperturbed cell cycle progression and as a checkpoint sensor of single-stranded DNA and stalled replication forks; phosphorylates CHK1 and other proteins required for diverse cellular functions, including homologous recombination
Aurora A and B kinases	G_2/M	A family of mitotic kinases that associate with centrosomes and mitotic spindle components; Aurora A functions during centrosome separation and chromosome alignment at kinetochores and recruits Aurora B to centromeres; Aurora B binds to spindle microtubules during anaphase; both enzymes function during cytokinesis
BRCA1 (breast cancer susceptibility type-1)	(G_1), S, G_2	Associates with many proteins on chromatin to modulate homologous recombination and repair of DNA double-strand breaks, transcription, and cell cycle checkpoint control
BRCA2 (breast cancer susceptibility type-2, equivalent to FANC-D1)	S, G_2	Binds single-stranded DNA and RAD51 to facilitate repair of strand breaks by homologous recombination; functions as a component of the Fanconi anemia complex (FANC) to repair DNA cross-links
CDK4 and CDK6 (cyclin-dependent kinases 4 and 6)	G_1, [G_2]	Phosphorylate and inactivate RB-family proteins to reduce cellular dependence on extracellular mitogens and to propel S-phase entry
CHK1 (checkpoint kinase-1)	S, G_2	A serine or threonine kinase phosphorylated and activated by ATR; induces cell cycle arrest by activating p53 and by inhibitory phosphorylation of CDC25 phosphatases; phosphorylates tousled-like kinases (TLKs) to regulate chromatin remodeling
CHK2 (checkpoint kinase-2)	G_1, S, G_2	A serine or threonine kinase phosphorylated and activated by ATM; facilitates DNA repair via phosphorylation of BRCA1 and facilitates cell cycle arrest by targeting p53, CDC25, and E2F1
Kinesin-5	G_2/M	A motor protein required for microtubule assembly, and mitotic spindle formation and function
MPS1 (monopolar spindle-1 kinase)	G_2/M	Recruits mitotic checkpoint BUB and MAD proteins to unattached kinetochores to inhibit the APC^{CDC20} ubiquitin ligase, thus halting mitotic progression and preventing premature mitotic exit
PARP1 [poly(ADP-ribose) polymerase-1]	G_1, S, G_2	Detects DNA breaks; binds to DNA; polymerizes poly(ADP-ribose) chains on many targets; recruits repair proteins
PLK1 (polo-like kinase-1)	S, G_2/M	Facilitates centrosome maturation during S/G_2; activated by Aurora kinases; regulates WEE1; facilitates CDC25 activation to dephosphorylate and activate CDK1 during mitotic entry and recovery from the G_2 checkpoint
WEE1 checkpoint kinase	S, G_2/M	Phosphorylates CDK1 and CDK2 to inhibit kinase activity; prevents S-phase progression and mitotic entry; opposed by CDC25 phosphatases

[a]Individual drug targets are referenced via in-text citations. Most of the available targeted agents either are in preclinical development or have been advanced to various stages of clinical trials. Notable exceptions include the CDK4 and CDK6 inhibitor palbociclib (Pfizer) and the PARP inhibitor olaparib (AstraZeneca), both of which have been approved by the US Food and Drug Administration.

[b]Parentheses indicate that the designated target has a lesser role during the G_1 phase.

[c]Square brackets indicate uncertainty about activity during G_2. Continuously cycling cells synthesize D-type cyclins during the G_2 phase, raising the possibility that cyclin D–dependent kinases phosphorylate substrates other than RB-family proteins during this interval (Stacey 2003). A potential G_2 substrate is FOXM1 (Anders et al. 2011).

cyclin synthesis and CDK4 and CDK6 activity are particularly efficacious. Because many cancers are driven by oncogenic mutations in signaling pathways that constitutively activate expression of D-type cyclins and for which targeted and specific inhibitory drugs have already been developed, opportunities abound in various tumor types for analogous combinatorial therapies with CDK4 and CDK6 and D-cyclin inhibitors (**Figure 1**). In addition to palbociclib, two similar drugs, abemaciclib (Eli Lilly) and ribociclib (Novartis), are in clinical trials for cancer treatment (Asghar et al. 2015, Sherr et al. 2016).

CDK7: component of the TFIIH transcriptional complex that phosphorylates RNA polymerase II and, separately, phosphorylates and activates other cyclin-dependent kinases

Why is inhibition of CDK4 and CDK6 well tolerated? Neither of these CDKs is essential for viability, and their combined absence in mouse gene knockout models is compensated for by CDK2 and CDK1 throughout much of fetal development (Malumbres et al. 2004). However, tumor cells driven by deregulated mutant oncoproteins may become addicted to the resulting high signaling thresholds and, thus, exhibit a heightened dependency on recruited downstream signal transducers (Luo et al. 2009, Pagliarini et al. 2015, Weinstein 2008). For example, cycling breast cancers expressing abnormally high levels of ERs become especially reliant on the pro-proliferative effects of cyclin D1–driven CDK4 activity (Musgrove et al. 2011). Moreover, ER-positive tumors that had sustained deletion of *CDKN2A* or amplification of *CCND1*, including those with inactivated p53, also responded well to combined palbociclib and letrozole treatment (Finn et al. 2015). In models of HER2-positive breast cancers, resistance to targeted receptor inhibition can be mediated by elevated cyclin D1 and CDK4 signaling, whereas CDK4 inhibition resensitizes these cancers to anti-HER2 therapy and delays tumor recurrence (Goel et al. 2016).

2.3. Cyclin-Dependent Kinases Governing RNA Transcription

Certain CDKs, including CDK7, CDK8, and CDK9, that are regulated by distinct cyclins have major roles as cofactors during polymerase II transcription and messenger RNA (mRNA) chain elongation. CDK7, CDK8, and CDK9 are components of, respectively, TFIIH, mediator, and pTEFb transcription complexes, and inhibitors of these CDKs are under development (Malumbres 2014). Unlike other CDK inhibitors that are adenosine triphosphate antagonists, the CDK7 inhibitor THZ1 binds covalently to a unique cysteine residue located outside of the canonical kinase domain (Kwiatkowski et al. 2014). Overexpressed *MYC* oncogenes, which are frequently amplified and targeted by activating translocations in many tumor types, maintain aberrant pro-proliferative activities by sustaining transcription in cancer cells. Chemical inhibitors of CDK7, CDK8, and CDK9 that regulate superenhancer-mediated transcription appear to exert more significant antiproliferative effects on cells that overexpress MYC family oncogenes than on their normal counterparts (Christensen et al. 2014, Huang et al. 2014, Lu et al. 2015, Pelish et al. 2015, Wang et al. 2015). Recent preclinical studies have pointed to their efficacy in myelogenous and T lymphoid leukemias, neuroblastoma, triple-negative breast cancer, hepatocellular carcinomas, and RB-inactivated small cell lung cancers driven by superenhancer-associated transcription factors, including MYC itself. Using these agents for indirect MYC targeting seems promising.

3. TARGETING S PHASE

3.1. The DNA Damage Response

DNA damage occurs more frequently throughout S phase than during other cell cycle phases in normal cells, setting dependencies on the complex protein machinery called upon to recognize and repair the thousands of different lesions that arise during each division cycle, including DNA strand breaks, abnormally modified bases or abasic sites, and mismatched bases (Bauer et al. 2015, Ciccia & Elledge 2010, Jackson & Bartek 2009, Kunkel 2015). Although the p53-dependent G_1/S

checkpoint arrests the cell cycle to allow DNA repair prior to S-phase entry (see Section 2.1), other mechanisms can delay DNA replication and prevent late-origin firing during the S-phase interval (the intra-S-phase checkpoint) or can recognize unrepaired DNA damage during G_2/M to prevent aneuploidy or mitotic catastrophe (Bartek & Lukas 2007, Kastan & Bartek 2004). Each of these checkpoint pathways involves the recruitment of protein complexes that sense and signal the presence of damaged DNA and engage effectors to repair different lesions (Goldstein & Kastan 2015, Hoeijmakers 2001, Jackson & Bartek 2009, O'Connor 2015). Mechanisms—such as base and nucleotide excision repair, mismatch repair, homologous recombination (HR), nonhomologous end joining (NHEJ), and translesion synthesis—have evolved to protect the genome (Bauer et al. 2015, Jasin & Rothstein 2013, Kunkel 2015, Malkova & Haber 2012, Reardon & Sancar 2005, Reyes et al. 2015, Sale 2013).

***BRCA1* and *BRCA2*:** breast cancer susceptibility genes that have key functions in homologous recombination and DNA repair

Persistent double-strand (ds)DNA breaks are the most deleterious forms of damage because if they are carried into mitosis, improper chromosome segregation may occur and essential genetic material may be lost. During G_1 phase, before the duplication of chromosomal DNA occurs, cells must resort to NHEJ, a relatively inaccurate mechanism that predisposes the genome to mutations and chromosomal rearrangements. However, during S phase, when one sister chromatid can be used as a repair template for another, cells can utilize accurate HR in addition to NHEJ in an attempt to correct dsDNA breaks (Jackson & Bartek 2009, Jasin & Rothstein 2013, Moynahan & Jasin 2010). In general, deleterious mutations impinging on one DDR pathway can be compensated for by another, albeit with reduced fidelity and efficiency.

3.2. Replication Stress and the DNA Damage Response in Tumor Cells

A hallmark of cancer cells is their acquisition of defects in one or more DNA damage checkpoint or repair pathways, forcing them to rely on secondary, suboptimal mechanisms for maintaining genome integrity. Taking advantage of this feature affords a rationale for targeted cancer therapy. Although germ-line mutations of DDR components, such as *BRCA1* and *BRCA2* tumor suppressor genes, predispose to cancer (**Table 1**), most cancer-associated DDR defects are acquired during tumor progression. A major force that selects for abnormalities in the DDR machinery in nascent tumor cells is replication stress (RS), which is driven by the activation of oncogenes or the loss of certain tumor suppressors (Bartkova et al. 2005, Di Micco et al. 2006, Gorgoulis et al. 2005, Halazonetis et al. 2008, Hills & Diffley 2014). Indeed, the most frequently encountered examples of tumor-promoting aberrations that enhance RS include mutations affecting *Ras*-driven signaling pathways, disruption of the RB pathway, amplification and overexpression of c-*MYC* or cyclins D and E, and haploinsufficiency of $p27^{KIP1}$, all of which promote S-phase entry. The increased threshold of RS is accompanied by the aberrant accumulation of diverse replication intermediates, stalled or collapsed replication forks, or even unscheduled genome rereplication (Bartek et al. 2012, Branzei & Foiani 2010, Zeman & Cimprich 2014). RS may reflect collisions of replication forks with oncogene-driven transcription complexes, and it occurs when the progress of DNA polymerase is uncoupled from the helicase that unwinds DNA. Extended regions of single-stranded (ss)DNA near the replication fork and the binding of replication protein A to the single strands to prevent DNA cleavage induce the DDR mediated by the ATR and CHK1 kinases (**Table 1**). ATR–CHK1 signaling recruits the repair machinery to suppress replication fork collapse, and it inhibits new-origin firing to slow DNA replication and allow more time for repair. Failure to exert these functions can instead generate more severe dsDNA breaks that prevent replication and allow cells to enter mitosis with fragmented or misarranged chromosomes.

RS alarms both the ATR–CHK1 and ATM–CHK2 kinase cascades that initially provide an intrinsic anticancer barrier, commonly leading to elimination of incipient tumor cells from the

proliferative pool. However, RS in nascent cancer cells also provides a strong pressure that selects for the inactivation of DDR components, among them the ATM and CHK2 checkpoint kinases and their prime target, p53 (Ahn et al. 2004, Bartek et al. 2007, Halazonetis et al. 2008). The loss of p53, for example, compromises tumor suppression by allowing cells with DNA damage to endure, insulating them from undergoing apoptosis or from permanently exiting the cell cycle into a senescent state (Halazonetis et al. 2008, Vogelstein et al. 2000, Vousden & Prives 2005). Notably, although ATM and CHK2 are dispensable for cell proliferation, and their inactivating mutations are tolerated in cancer, ATR and CHK1 perform vital cell cycle–regulatory tasks, including supporting replication fork stability and the timing of mitotic entry. These functions are often essential for cancer cell viability and can be exploited by ATR and CHK1 inhibitors in the treatment of advanced tumors (Bartek et al. 2012, Murga et al. 2011, Velic et al. 2015). Because *ATR* is an essential gene, there had been concerns that its pharmacological inhibition might not be tolerated in vivo. However, cancer cells are considerably more sensitive than normal cells to partial ATR inhibition, and drugs that inhibit ATR or CHK1 are now being clinically tested, particularly as radiosensitizers, for treating several types of malignancies, including breast, pancreatic, and non-small-cell lung cancers (Velic et al. 2015).

Synthetic lethality: cell death resulting from disruption of two genes functioning in compensatory pathways in which disruption of either alone is tolerated

The emergence of clones with DDR checkpoint defects allows for tumor progression at the expense of decreased genomic stability owing to the persistent, unresolved consequences of ongoing oncogene-fueled RS. Such volatile, genomically unstable states favor tumor heterogeneity and progression, limiting the repertoire of DDR mechanisms for halting the cell cycle and allowing sufficient time for repair (Burrell et al. 2013a,b; Halazonetis et al. 2008; Lord & Ashworth 2016). In turn, it is these specific vulnerabilities that selectively sensitize cancer cells to traditionally used irradiation and S-phase poisons (Goldstein & Kastan 2015, Moding et al. 2013), providing a therapeutic window that distinguishes tumor cells from actively self-renewing populations of normal cells—which include hematopoietic progenitors in the bone marrow, the epithelial cells of the gut, or hair follicle stem cells—and that confers dose-limiting toxicities to these agents. One negative consequence of chemo- and radiotherapy is that cytotoxic treatment regimens can further mutagenize subpopulations of cells that have already lost such checkpoint controls, leading to the emergence of drug-resistant and even more genetically unstable clones. This is a major reason why multiple cytotoxic agents are frequently combined to kill cancer cells by simultaneously attacking different DDR pathways and thereby avoiding the re-emergence of resistant populations (Al-Lazikani et al. 2012, Burrell et al. 2013b, Velic et al. 2015).

3.3. Synthetic Lethality

The fact that tumor cells originate from cells with inherited DNA repair defects, or acquire somatic defects in particular DDR pathways that force reliance on compensatory mechanisms, provides an opportunity to induce cell death by drug-induced inactivation of backup pathways. The underlying principle of such synthetic lethality is that although inactivation of one of two compensatory pathways alone does not lead to a loss of cell viability, the disruption of both promotes cell death. Hence, cancer cells mutant in one DDR pathway should be more susceptible than their normal counterparts to the disruption of a second (Kaelin 2005, Lord & Ashworth 2012). The success of conventional cytotoxic therapies largely depends on the targeting of S- and M-phase cell cycle checkpoints that survey the fidelity of DNA replication and mitotic progression and that induce cell death when repair mechanisms fail (Goldstein & Kastan 2015). For example, accelerated entry into S phase may occur before the accrual of sufficient metabolic building blocks for DNA replication (Beck et al. 2012). In this case, the clinical efficacy of chemotherapeutic nucleoside analogs that further reduce nucleoside triphosphate pools and amplify replication stress represents an example of drug-induced synthetic lethality.

A parallel example of more precisely targeted therapy involves tumor cells with SETD2 mutations that result in deficiency of histone H3 lysine 36 trimethylation ($H3K36^{me3}$) (Pfister et al. 2015). This histone mark clusters within the bodies of actively transcribed genes, allowing them to recruit specific chromatin readers that regulate proper precursor mRNA processing (Guo et al. 2014). Cells with SETD2 mutations that are defective in $H3K36^{me3}$ exhibit significantly reduced synthesis of the M2 subunit of ribonucleotide reductase, causing a shortage of nucleotide triphosphates. Because the phosphorylation of CDK2 (and CDK1; see Section 4.1) by the WEE1 kinase (**Table 1**) negatively regulates its activity and prevents the firing of replication origins, drug-induced WEE1 inhibition in SETD2-deficient cells further increases RS and kills $H3K36^{me3}$-deficient tumor cells (Pfister et al. 2015, Shoaib & Sorensen 2015).

WEE1: a kinase that phosphorylates S-phase and M-phase cyclin-dependent kinases to inhibit their activities

Synthetic viability: a consequence of mutations in a target gene that restore its fitness and bypass synthetic lethality

Synthetic lethality has been exploited in the treatment of familial breast and ovarian tumors that lack BRCA1 and BRCA2 tumor suppressor function and are defective in HR, which is used during S phase for double-strand break (DSB) repair (Bryant et al. 2005, Farmer et al. 2005). The poly(ADP-ribose) polymerase (PARP) is required for ssDNA repair (**Table 1**). The formation of ADP-ribose chains and the subsequent dissociation of PARP from DNA facilitate the repair process, whereas drugs such as olaparib (AstraZeneca) that inhibit PARP dissociation cause replication fork stalling and collapse, thereby initiating DSBs. Because HR requires functional BRCA proteins, tumors lacking these are highly sensitive to PARP inhibition, whereas normal cells are not (Lord et al. 2015). *BRCA1* and *BRCA2* are canonical tumor suppressors requiring the loss of both alleles to manifest phenotypic deficiency. Hence, cells from patients who inherit one normal and one mutated *BRCA* gene are relatively resistant to PARP treatment, whereas tumor cells that lose the wild-type allele are much more sensitive.

These examples underscore the need to determine the underlying mutations in a patient's tumor so as to pinpoint specific oncogenic drivers and pre-existing defective pathways, thus allowing targeted agents to be employed that precisely exploit the vulnerabilities of cancer cells. Combining targeted DDR inhibitors, with each other or with chemotherapy or irradiation, may prove effective in attacking specific repair mechanisms to induce synthetic lethality. Precision therapy of this type depends upon determining the mutational spectrum encountered in particular tumors, information that may be difficult to obtain, given random biopsies and clonal evolution that leads to variegated gene expression and to significant differences among primary and metastatic lesions. Still, drugs in clinical trials that specifically target S-phase regulators—such as PARP and the CDK2, ATM and ATR, CHK1 and CHK2, and WEE1 kinases (**Table 1**)—may emerge as new targeted therapeutics (O'Connor 2015, Velic et al. 2015).

3.4. Synthetic Viability and Treatment Resistance

Despite the growing list of promising drugs that target cancer cell vulnerabilities, acquired drug resistance remains a problem. The successful application of PARP inhibitors, approved in the United States and Europe for clinical use for ovarian cancer patients whose tumors harbor *BRCA1* or *BRCA2* mutations, illustrates the particular complexities of acquired drug resistance. Some cases of PARP inhibitor resistance are attributable to secondary reverse mutations in *BRCA1* or *BRCA2* that undermine synthetic lethality by restoring partially or fully functional *BRCA* genes (Lord & Ashworth 2013). In addition, there is a growing range of additional cancer abnormalities that occur in *BRCA1*-defective cancers and lead to adaptation of the DDR machinery in a way that can be described as synthetic viability. These candidate resistance mechanisms reflect the loss of DDR factors—such as 53BP1, RIF1, JMJD1C, or MAD2L2 (Rev7)—that normally limit the extent of DSB end resection and hence HR repair, and whose loss results in at least partially restored HR capacity in cancer cells lacking *BRCA1* (Bouwman et al. 2010, Bunting et al. 2010, Watanabe et al. 2013, Xu et al. 2015, Zimmermann et al. 2013).

Another example of drug resistance involves the presence of two parallel and redundant stress-support checkpoint pathways that target an overlapping spectrum of substrates. Specifically, *KRAS* oncogene-driven cancers that feature enhanced endogenous RS depend on CHK1 signaling; however, in subsets of such tumors, the MK2 checkpoint kinase is aberrantly activated and substitutes for CHK1, thereby causing resistance to CHK1 inhibitors. Consistent with the redundant stress-support roles of CHK1 and MK2 (Reinhardt & Yaffe 2013), concomitant pharmacological inhibition of both kinases efficiently kills such mutant *KRAS* tumors, providing a rationale for overcoming this mode of resistance (Dietlein et al. 2015).

CDC25: a family of phosphatases that reverses WEE1-induced inhibitory phosphorylation of cyclin-dependent kinases

4. TARGETING G_2 PHASE AND MITOSIS

4.1. The G_2 Checkpoint

Cyclin A, induced at the G_1/S transition, is degraded at prometaphase, whereas cyclin B accumulates in S/G_2 after cyclin A and is degraded later during anaphase. Transcription factors that induce *CCNB1* are activated by CDK activity, placing a dependency of cyclin B accumulation during G_2 on cyclin A (Fung et al. 2007, Fung & Poon 2005). Mitotic entry is largely controlled through the activation of cyclin B–driven CDK1, but G_2 cells that express cyclin B do not yet express active CDK1, which is restrained through inhibitory phosphorylation by the WEE1 and MYT1 kinases and is activated by the removal of inhibitory phosphates mediated by the CDC25C phosphatase (Domingo-Sananes et al. 2011, Lindqvist et al. 2009, Nigg 2001). Rising levels of cyclin B and CDK1 complexes during the G_2 phase eventually overcome the WEE1 inhibitory threshold, after which WEE1 itself is inactivated by CDK1-mediated phosphorylation and CDC25C is activated (Perry & Kornbluth 2007). This process drives the sudden burst of CDK1 activity as cells enter mitosis.

DNA damage during G_2 activates the ATR/CHK1 signaling pathway, leading to phosphorylation and inactivation of CDC25 isoforms, CDK1 inhibition, and G_2 cell cycle arrest. If DNA damage is subsequently repaired, arrest can be reversed. Other kinases, including polo-like kinase-1 (PLK1) and Aurora A, contribute to checkpoint reversal and mitotic entry (**Table 1**) (Aarts et al. 2013, Dominguez-Brauer et al. 2015, Keen & Taylor 2004, Nigg 2001, Strebhardt & Ullrich 2006). For example, PLK1 is activated by Aurora A–mediated phosphorylation. In turn, CDK1 phosphorylation mediates PLK1 recruitment to, and phosphorylation and degradation of, WEE1 and the parallel reactivation of CDC25. A prediction is that chemical WEE1, PLK1, or Aurora kinase inhibition would prematurely drive cancer cells with pre-existing DNA damage into mitosis, ultimately triggering mitotic catastrophe and cell death by synthetic lethality (Jackson et al. 2007). However, despite intensive efforts, the developed inhibitors of Aurora and PLKs have caused severe side effects owing to an inability to distinguish cancer cells from normal cells, and they have not so far demonstrated therapeutic clinical activity in solid tumors (Dominguez-Brauer et al. 2015, Huang et al. 2009).

A promising anticancer strategy is to ablate the G_2 DNA damage checkpoint by targeting the ATR/CHK1/WEE1 pathway. A number of small-molecule inhibitors of ATR, CHK1, and WEE1 are being evaluated in clinical trials, either in combination with DNA-damaging agents or with one another (Sorensen & Syljuasen 2012). Despite the fact that p53 mutations allow cells to tolerate aneuploidy, these drugs are showing particularly promising results in p53-mutant tumors (Hirai et al. 2009, Van Linden et al. 2013). Even without concomitant genotoxic treatments, a drug combination targeting CHK1 and WEE1 kinases not only deregulates CDK activity to generate DNA lesions during S phase (see Section 3.1) but also triggers escape from the G_2 checkpoint and produces mitotic catastrophe in cancer cells that have sustained enhanced RS and DNA damage (Aarts et al. 2012).

4.2. Mitotic Progression and the Spindle Assembly Checkpoint

The activation of CDK1 by mitotic cyclins drives the mitotic state until the metaphase–anaphase transition. By this time, the nuclear membrane has broken down, centrosomes duplicated at G_1/S have matured and migrated to the spindle poles, and chromosomes with duplicated sister chromatids have condensed and aligned on the mitotic spindle (Nigg 2001). Although cyclin B–CDK1 phosphorylates numerous proteins, a critical substrate is the APC^{CDC20} E3 ubiquitin ligase, or cyclosome (Teixeira & Reed 2013). APC^{CDC20} degrades securin, a negative regulator of separase, the protease that cleaves cohesin components to allow sister chromatid separation during anaphase. APC^{CDC20} also degrades cyclin B, resulting in the loss of CDK1 activity, thus reversing the mitotic state as chromosomes segregate and cells prepare for cytokinesis. Later in mitosis, APC^{CDH1} is assembled and remains active through G_1, targeting cyclins A and B for degradation, until cycling cells re-enter S phase and APC^{CDC20} again begins to accumulate (see Section 2.1).

The APC^{CDC20}-dependent spindle assembly checkpoint (SAC) operates to ensure that chromosomes are properly aligned on the mitotic spindle before anaphase ensues. Although cyclin B–CDK1 activates APC^{CDC20}, its activity is suppressed by a mitotic checkpoint signaling complex (MPS1, BUB1, BUBR1) until spindles properly attach to kinetochores, thus preventing precocious chromosome segregation and aneuploidy (Kops et al. 2005, Sudakin et al. 2001). Microtubule inhibitors, such as taxanes and vinca alkaloids, long used for cancer treatment, affect spindle assembly and target the SAC to induce mitotic arrest and independently trigger mitochondrial outer membrane permeabilization and apoptosis. The recruitment of the mitotic checkpoint complex to unattached kinetochores depends on the SAC kinase MPS1, which is required for APC^{CDC20} inhibition and correct chromosome alignment (**Table 1**) (Bayliss et al. 2012, Dominguez-Brauer et al. 2015). Although inhibition of MPS1 is highly lethal, it may prove that more modest reductions in its level of activity can augment the effects of paclitaxel to selectively kill cancer cells (Janssen et al. 2009). Notably, although failure to satisfy the SAC retards cyclin B degradation and sustains mitotic arrest, only some cells die during mitosis, whereas others bypass cytokinesis and escape into a G_1 state, from which they either undergo p53-dependent arrest as viable tetraploid cells or, in the absence of p53, continue to divide as aneuploid cells (Lanni & Jacks 1998). The sensitivity of normal cells, and particularly neurons, to microtubule inhibitors, as well as the failure of tumor cells to initiate apoptosis during or after mitotic arrest, limits the efficacy of these agents (Huang et al. 2009). Unfortunately, none of the drugs that specifically target mitosis—such as inhibitors of kinesin-5, polo, or Aurora kinases (Keen & Taylor 2004, Marcus et al. 2005, Orth et al. 2008, Strebhardt & Ullrich 2006, Wood et al. 2001)—prevents mitotic slippage and specifically induces mitotic death in tumor cells. Potentially, drugs that inhibit CDC20 or cyclin B destruction, or both, would prolong mitosis and prevent G_1 escape (Rieder & Medema 2009), but such agents have not been developed.

Securin: an inhibitor of separase that is targeted for degradation during mitosis to allow chromosome separation during the metaphase–anaphase transition

Separase: a protease that cleaves cohesin to allow chromosome segregation

Spindle assembly checkpoint (SAC): mitotic protein complex (BUB1, BUBR1, MAD1, MAD2, MPS1) that inhibits APC^{CDC20} until kinetochores are properly aligned on the mitotic spindle

SUMMARY POINTS

1. Normal cell cycle progression depends on checkpoint controls that monitor S-phase entry, ensure faithful DNA replication, and govern entry into, and progression through, mitosis. Compromising these mechanisms generates cancer cell–specific vulnerabilities that can be pharmacologically exploited.
2. Inhibitors of mitogenic signaling pathways, which may be compensated for and tolerated in normal cells, may more forcefully block the proliferation of cancer cells that become addicted to aberrantly elevated oncogenic signaling thresholds.

3. Cancer cells experience increased replicative stress due to oncogene activation and other mutations affecting the DNA damage response, thus providing opportunities to kill them by disabling residual compensatory mechanisms.
4. Many inhibitory drugs now in clinical use or under development target G_1 CDKs, other cell cycle kinases (ATM and ATR, CHK1 and CHK2, WEE1, Auroras, polos, MPS1), DNA damage sensors (PARP), kinesins, and epigenetic regulators (SETD2 methylase). These agents are being widely exploited in combination with one another or with conventional cytotoxic agents to advance cancer therapy.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We sincerely thank Norman Sharpless and Michael Kastan for comments. Due to length restrictions and because of the considerable complexities of cell cycle dynamics, we have cited many previously written reviews that we hope will provide a comprehensive conceptual background for this article. We ask our colleagues to forgive us for not directly highlighting some of their individual contributions. C.J.S. is an investigator of the Howard Hughes Medical Institute and is also supported by a National Cancer Institute Comprehensive Center Core Grant (CA-21765) and ALSAC at St. Jude Children's Research Hospital. J.B. is supported by grants from the Danish Cancer Society, the Swedish Research Council, the Danish Council for Independent Research, the Novo Nordisk Foundation, Cancerfonden, and Lundbeckfonden.

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