Targeting CDK2 in cancer: challenges and opportunities for therapy

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Cyclin-dependent kinase 2 (CDK2) plays a pivotal part in cell cycle regulation and is involved in a range of biological processes. CDK2 interacts with and phosphorylates proteins in pathways such as DNA damage, intracellular transport, protein degradation, signal transduction, DNA and RNA metabolism and translation. CDK2 and its regulatory subunits are deregulated in many human cancers and there is emerging evidence suggesting CDK2 inhibition elicits antitumor activity in a subset of tumors with defined genetic features. Previous CDK2 inhibitors were nonspecific and limited by off-target effects. The development of new-generation CDK2 inhibitors represents a therapeutic opportunity for CDK2-dependent cancers.

Targeting cell cycle CDKs in cancer and the case for inhibiting CDK2

Over the past decade, the clinical development of CDK4/6 inhibitors has led to practice-changing outcomes in breast cancer treatment [1]. This has energized the field and increased interest in therapeutically targeting other members of the CDK family. CDK2 was an early focus for anticancer drug discovery in the 1990s but the initial excitement was dampened owing to off-target effects of the early drugs. Interest in CDK2 inhibition has now reignited with the identification of more functions for CDK2 that impact cancer biology and the possibility of developing inhibitors with greater specificity to CDK2. Accumulating evidence also suggests that CDK2 is fundamentally linked to the proliferation of particular cancer types, such as ovarian cancer [2,3]. Here, we shall discuss advances in our understanding of CDK2 biology and the role it takes across the proliferative and pro-survival mechanisms exploited by cancer cells. We will review how this new level of understanding is being leveraged to propose novel therapeutic strategies across a range of cancer types, exploiting the vulnerabilities of CDK2 function in different cancer contexts.

Understanding CDK2 function

Cell cycle regulation and DNA replication

In dividing cells, CDK2 is a core cell-cycle regulator that is active from the late G_1-phase and throughout the S-phase. CDK2 is activated by the binding of cyclin E1 or E2, and cyclin A2, and phosphorylation by the CAK complex (CDK7, MAT1, cyclin H) and removal of inhibitory phosphorylations by Cell division cycle 25 A (CDC25A). During late G_1, activated CDK2–cyclin-E together with CDK4/6–cyclin-D phosphorylate Rb to release E2F from Rb, and this initiates the transcription of genes required for cell cycle progression [1]. Beyond Rb, CDK2 governs the phosphorylation of other regulatory proteins, thereby linking other processes to cell cycle progression. For instance, the phosphorylation of SMAD3 by CDK2–cyclin-E limits its transcriptional activity and eventually slows cell-cycle progression [4]. CDK2 also phosphorylates several components of the pre-replication complex, which is required to initiate DNA synthesis. This includes CDC6, which is required for loading minichromosome maintenance (MCM) proteins onto the DNA and MCM helicase proteins to control

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initiation of DNA replication [5]. Finally, CDK2 has an important role in coordinating the cell cycle to centrosome duplication [6], because it is necessary for the phosphorylation of target centrosome proteins such as nucleophosmin (NPM) [7] and Centriolar coiled-coil protein of 110 kDa, CP110 [8] to initially release centrioles and then sustain centriole duplication.

The earliest studies on CDK2 identified it as being required for normal cell cycles, and this was supported by results showing that expression of a dominant-negative CDK2 attenuates growth of cells in culture and that inhibition of CDK2 blocks S-phase initiation [9]. The supposed essential nature of CDK2, however, was challenged by the observation that CDK2 knockout mice are viable with normal mitotic cell-cycle progression, although meiosis is disrupted [10]. It was then found that CDK2 is dispensable for proliferation in some human cancer cell lines [11], which dampened the early enthusiasm for CDK2 as a therapeutic target in cancer.

Major difficulties in interpreting these results, and other studies, involve the different methods used to investigate CDK2 function, including dominant-negative CDK2 mutant protein, CDK2 knockout, CDK2 siRNA transfection and CDK2 inhibition by small-molecule inhibitors. CDK1 binds and is activated by cyclin E and cyclin A when CDK2 is low or absent, which makes it difficult to define CDK2-specific functions in $CDK2^{-/-}$ models, or with siRNA or shRNA approaches. The currently available CDK2 inhibitors also have limitations owing to their promiscuity toward other kinases, including CDK1, CDK4/6, CDK7 and CDK9 (Table S1, see supplementary material online). To address these deficiencies, Merrick et al. used an elegant chemical genetic strategy where CDK2 was replaced with a version that enlarged the ATP-binding pocket, allowing normal CDK2 activity but also specific inhibition by bulky adenine analogs [12]. This showed that CDK2 is required to enter S-phase, especially in conditions of growth factor deprivation, and that it is required for oncogene-mediated anchorage-independent proliferation [13]. Consequently, CDK2 targeting has reemerged as a therapeutic avenue to restrict cancer cell proliferation, especially because there is the potential to co-target the functions of CDK2 in DNA damage and apoptosis, as described below.

CDK2 in the DNA damage response (DDR)

Following DNA damage, the DDR arrests cells at the G_1/S boundary to allow repair to the damaged DNA and maintain genomic fidelity in daughter cells. Two mechanisms at the G_1/S DNA damage checkpoint converge to inhibit proliferation via CDK2. Accumulation of p53 leads to upregulated transcription of $p21^{Cip1/Waf1}$ and subsequently cell-cycle arrest via cyclin-D1–CDK4/6 and cyclin-E–CDK2 inhibition [14]. The second pathway targets CDC25A for degradation, leading to a persistent inhibitory phosphorylation of CDK2 at Thr14 and Tyr15 by WEE1, thereby blocking entry into S-phase [15].

Although CDK2 is inactivated by the G_1/S DDR checkpoint, $CDK2^{-/-}$ cells still undergo G_1/S arrest in response to DNA damage [16]. This is caused by the inhibition of CDK1 by $p21^{Cip1/Waf1}$, because CDK1 can substitute for CDK2 to drive G_1/S transition in its absence [16]. However, $CDK2^{-/-}$ cells show an additional DDR defect as initiation of DNA repair and resumption of proliferation is delayed, leading to more-extensive DNA damage following irradiation and reduced survival of irradiated $CDK2^{-/-}$ mice [16].

The additional role for CDK2 in the DDR occurs at least partially through the homologous recombination (HR) pathway. HR is defective in $CDK2^{-/-}$ mouse embryonic fibroblasts – a finding reinforced by

similar data from CDK2 siRNA or CDK2 small-molecule inhibition [17]. In these systems, the interruption of CDK2 function abrogated recruitment of Eukaryotic homologue of Escherichia coli RecA (RAD51) to the DNA repair foci [17]. This is probably because CDK2 is crucial for activation of the MRN-CtIP-BRCA1 (MRN: Mre11-Rad50-NBS1; CtIP: C-terminal binding protein 1 interacting protein; BRCA1: Breast cancer 1) complex which is required for normal DNA damage repair and checkpoint signaling following DNA damage. As shown by yeast two-hybrid assays, CDK2–cyclin-A complex directly interacts with the C terminus of Mre11 of the MRN complex (Mre11-Rad50-NBS1) to phosphorylate CtIP of the MRN-CtIP-BRCA1 complex [18,19], so that complex can be recruited to Double strand break (DSBs). The nibrin (NBS1) component of MRN is also phosphorylated by CDK2 [20], and this assists in converting DSBs into structures that are suitable for repair by HR [21]. Possible roles for CDK2 exist in other DDR pathways, including non-homologous end-joining repair through phosphorylation of its central protein, Ku [22], and p53 response through timely p53 and checkpoint kinase 1 (CHK) phosphorylation following DNA damage [17]. Altogether, these observations indicate a direct role of CDK2 in DDR that functions alongside its cell-cycle role, and has the potential for clinical targeting.

Roles for CDK2 in apoptosis

CDK2 regulates core regulatory and functional components of the apoptotic pathways. The CDK2 target protein, Forkhead box protein O1 (FOXO1), plays a vital part in triggering DNA-damage-induced apoptosis following dsDNA breaks [23]. Upon DNA-damage-induced G_1/S-phase arrest, CDK2 no longer phosphorylates FOXO1 at inhibitory sites, and this allows FOXO1 to become transcriptionally active [23] and trigger apoptosis via upregulation of numerous proapoptotic proteins such as Fas ligand (FasL), Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) and B-cell lymphoma 2 (BCL-2) interacting mediator of cell death (Bim) [24].

CDK2 also protects against apoptosis through phosphorylation of the pro-survival factor myeloid leukemia cell differentiation protein (MCL-1). CDK2–cyclin-E binds MCL-1 to phosphorylate it on Thr92 and Thr163 to increase its stability, and CDK2 also facilitates the phosphorylation of MCL-1 on Ser64 to sequester the proapoptotic factor Bim [25,26]. CDK2 expression is elevated in human diffuse large B cell lymphoma, a large proportion of which is MCL-1-dependent; and inhibition of CDK2 by CDK2 siRNA or CVT-313 (CDK2 inhibitor with some efficacy to CDK1/CDK5) in diffuse large B cell lymphoma cell lines induces apoptosis in association with a decrease in MCL-1 levels [27].

Biomarkers of CDK2 activity

With a few exceptions, CDK2 is generally not upregulated or amplified in cancers (Table 1). Rather, CDK2 activity is altered through its binding partners or by alterations to post-translational modifications.

Changes to the cyclin–CDK complex

The major regulators of CDK2 activity are its binding cyclins: cyclin E1, cyclin E2, cyclin A1 and cyclin A2, and the cyclin-dependent kinase inhibitors of the cyclin–CDK2 complex: $p21^{Cip1/Waf1}$, $p27^{Kip1}$ and $p57^{Kip2}$. Cyclins E1/E2 and A1/A2 sequentially bind CDK2 through G_1- to late S-phase, and each can be dysregulated in cancer leading to changes to cell-cycle

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TABLE 1

Potential biomarkers for predicting high CDK2 activity

Potential biomarkers	Representative tumor types	Method used	Refs
CCNE1 amplification	High-grade serous ovarian cancer Endometrial cancer Hepatocellular carcinoma	• siRNA • shRNA • Chemical inhibition • Genomic data • Xenografts • ISH analysis of patient samples	[2,3] [77,78]
Cyclin E1/2/LMW-E overexpression	Inflammatory breast cancer High-grade serous ovarian cancer	Immunohistochemical analysis of patient samples	[79]
CDK2 overexpression	Glioblastoma	• shRNA • Xenografts	[63]
	Prostate cancer	• Multiple bioinformatics methods • siRNA • cas9-induced CDK2 knockout	[51]
	B cell lymphoma	• siRNA • Chemical inhibition	[27]
FBXW7 loss	Colon and breast cancer	• siRNA • CRISPR/Cas9 • Mouse models	[37]
PP2A-B56 amplification	Cervical cancer	• siRNA	[80]
USP28	High-grade serous ovarian cancer	• Immunohistochemistry of patient samples	[78]

progression, reviewed elsewhere [28,29], with the exception of cyclin A1, which is predominantly expressed in the male germline. Each of the cyclins and the cyclin-dependent kinase inhibitor proteins are themselves regulated by the cell cycle and the complex regulatory networks that control the expression of these proteins is also dysregulated in cancer. Notable important examples are: F-box and WD repeat domain containing 7 (FBXW7), part of the ubiquitin ligase that promotes degradation of cyclin E1; Ubiquitin-specific protease 28 (USP28), which deubiquitinates cyclin E1; and Skp2, which controls the ubiquitin-dependent degradation of p21$^{Cip1/Waf1}$ and p27^{Kip1} (Table 1).

Modifications to the cyclin–CDK complex

During cell-cycle progression, CDK2 is a conduit for increased growth factor signaling as a target of the Phosphatidylinositol 3-kinase/Protein kinase B (PI3K/AKT) pathway; AKT phosphorylates CDK2 at Thr39 during late S- and G_2-phases causing its cytoplasmic translocation, which is necessary for entry into G_2/M-phase [30]. CDK2–cyclin-A2 then phosphorylates AKT at Ser477–Ser479 to create a feed-forward loop for further CDK2 inactivation by priming full activation of AKT [31]. CDK2 is also activated by cyclin-activating kinase (CAK): CDK7, cyclin H, MAT1, through the addition of Thr160 phosphorylation and by CDC25A phosphatase through the removal of inhibitory Tyr15 phosphorylation. CDC25A [32] and CAK [33] are upregulated in a range of cancers and, notably, the CDK7 component of CAK is particularly deregulated in breast cancer [33].

Identifying cancers with CDK2 dependency

CDK2 inhibition in CCNE1 amplified and cyclin E1 high cancers

The presence of CCNE1 amplification in early lesions indicates that CCNE1 amplification is a driver event in high-grade serous ovarian cancer [34]. In this and other cancers with very high cyclin E1 expression (Table 2), CDK2–cyclin-E1 has roles in chromosomal instability as well as increased proliferation. Excess CDK2–cyclin-E1 increases Chromosomal instability (CIN) through multiple mechanisms. First, CDK2–cyclin-E1 mediates genomic instability via replication stress that leads to the under-replication of DNA in late S-phase and genomic deletions [35]. Excess CDK2–cyclin-E1 also inhibits the Anaphase promoting complex-C^{dh}1 complex during mitosis, causing the misalignment of chromosomes at the metaphase plate, resulting in chromosome mis-segregation and polyploidy [36]. Finally, using a combination of in vitro (CRISPR/Cas9, siRNA, shRNA) and in vivo assays it was shown that CDK2–cyclin-E1 phosphorylates Ser18 of centromere protein A (CENP-A) – a key protein in kinetochore assembly – and persistent CENP-A Ser18 phosphorylation results in mitotic defects and CIN [37]. Loss of FBXW7, which recognizes and primes cyclin E1 for destruction by the proteasome, also leads to increased cyclin E1 and, consequently, centromere dysfunction [37]. Therefore, CDK2 inhibitors, by halting CIN, could inhibit cancer cell growth and impede the development of drug resistance. One consideration in targeting cyclin-E1-high cancers is whether cyclin E1 exerts its oncogenic effect independently of CDK2. Studies on hepatocarcinogenesis indicate that cyclin E1 is required for advanced liver cancer progression but this activity is CDK2 independent [38]. Thus, caution must be exercised in using cyclin E1 as a biomarker for CDK2 because, in some cases, direct targeting of cyclin E1 could be more appropriate [39].

CDK2 inhibition in C-MYC-overexpressing cancers

The MYC gene drives uncontrolled cell division via activation of the cell cycle including CDK2 activity [40]. In tumors driven by MYC overexpression, CDK2 activity appears to be vital for preventing

TABLE 2

Cancer subtypes with particular susceptibility to CDK2 inhibition and their biomarkers.

Biomarkers	Tumor types	Methods	References
C-MYC overexpression	Colon cancer	CDK2 knockout mouse embryo fibroblasts	[41,80]
Microphthalmia-associated transcription factor (MITF) expression	Melanoma	• Adenoviruses expressing wild-type MITF • Dominant-negative MITF • siRNA	[81]
pThr416-Enhancer of zeste homolog 2 level	Triple negative breast cancer	• shRNA • Orthotropic breast cancer mouse model • Human TNBC tissue specimens	[51]
KRAS mutation	Lung cancer	• siRNA • Chemical inhibition	[8]
BRCA1- and ATM-deficiency	Breast cancer	• siRNA • Chemical inhibition • Immunohistochemistry of patient samples	[17]

senescence and permitting immortalization of cancer cells [41]. In MYCN-amplified neuroblastoma cell lines, selective silencing of CDK2 using RNAi led to apoptosis associated with the upregulation of p53 target genes, but not in non-MYCN-amplified cells [42].

CDK2 also engages a positive feedback loop with MYC, where CDK2 stabilizes the MYC protein. CDK2 phosphorylates MYC on Ser62 to block recognition and ubiquitination by the S-phase kinase associated protein 1 (SKP1), Cullins, and F box proteins (SCF) ubiquitin–protein-ligase complex, and this prevents the subsequent degradation of MYC [43]. Consequently, the targeting of MYC expression via inhibition of bromodomain-containing protein (BRD)4 and MYC stabilization via CDK2 inhibition is currently being evaluated in MYC-amplified cancers (Fig. 1a). Murine-derived MYCN-amplified medulloblastoma cell lines co-treated with JQ1 (a BRD4 inhibitor) and miliciclib (a CDK2 inhibitor specific at low dose range) have demonstrated drastic downregulation of N-MYC and significant apoptosis compared with control or JQ1 treatment alone [44].

CDK2 inhibition for KRAS-mutant lung cancer

CDK2 inhibition is also being considered in the context of highly aneuploid cancers such as KRAS-mutant lung cancer [45]. In models of KRAS-mutant lung cancer, inhibition of CDK2 kinase activity either by using siRNA or seliciclib (a CDK2/7/9 inhibitor) or CCT68127 (a CDK2/9 inhibitor) results in anaphase catastrophe and apoptosis [8], and reduced growth of lung cancer xenografts [46]. Anaphase catastrophe occurs because a key centrosome regulator, CP110, is no longer able to maintain unstable aneuploidy cancer cells with supernumerary centrosomes. The phosphorylation of CP110 by CDK2 allows CP110 to induce centrosome clustering, but in the presence of CDK2 inhibitors centrosome clusters no longer form, and anaphase catastrophe ensues. KRAS-mutant lung cancer is particularly sensitive to CDK2 inhibition because these cancers have reduced CP110 [47]. Because many cancers have aneuploid cells with supernumerary centrosomes, there might be potential to extend CDK2 inhibition to other settings beyond KRAS-mutant lung cancer [45].

CDK2 inhibition in hormone-dependent cancers

An important role for CDK2 in hormone-dependent cancers is supported by the finding that CDK2 mediates the phosphorylation of the androgen [48], estrogen [49] and progesterone [50] receptors increasing their transcriptional activation. Given that estrogen and progesterone regulate breast development and contribute to breast cancer progression [50], and androgen receptor phosphorylation is strongly implicated in prostate cancer [48], CDK2 therefore represents a clinically relevant molecular target in hormone-dependent breast and prostate cancers. For example, in prostate cancer, the overexpression of CDK2 is associated with recurrence risk, and its expression was found to be more than double in metastases when compared with levels in the primary tumor [51].

CDK2 inhibition can be particularly useful for endocrine-therapy-resistant breast cancer. Hormone-dependent breast cancer is normally treated with endocrine therapy but ~30% of patients will develop endocrine resistance leading to disease progression [52]. Given the cardinal role of estrogen in cell-cycle control, resistance mechanisms to endocrine therapies involve the modulation of cell-cycle-related genes and proteins such as: overexpression of C-MYC, cyclin E and D1; inactivation of Rb; and decreased expression of p21 or p27 [53–55]. Most of these resistance pathways culminate with activation of CDK2. For example, the overexpression of C-MYC drives endocrine therapy resistance through the repression of CDKN1A (gene encoding p21), which relieves CDK2–cyclin-E complexes to drive cell-cycle progression [53]. Another common event in endocrine resistance is cyclin D1 amplification or upregulation, which activates CDK2 either directly by upregulating the expression of cyclin E2 or indirectly by increasing the formation of CDK4–cyclin-D1 complexes, which sequesters p21$^{Cip1/Waf1}$ and p27^{Kip1} [56,57]. The possibility of targeting CDK2 to counteract these resistance mechanisms has been established by experiments showing that CDK2 inhibition prevents the proliferation of tamoxifen-resistant cells and restores their sensitivity to antiestrogen therapy [56,58].

CDK4/6 inhibitors are FDA approved for the treatment of advanced hormone-receptor-positive breast cancer in combination with endocrine therapy, and represent the current standard of care in this setting [1]. Although these inhibitors have demonstrated efficacy in the clinical setting in combination with endocrine therapy, resistance is emerging as a major challenge [59,60]. One of the mechanisms of resistance to CDK4/6 inhibitors in hormone-receptor-positive breast cancer occurs through
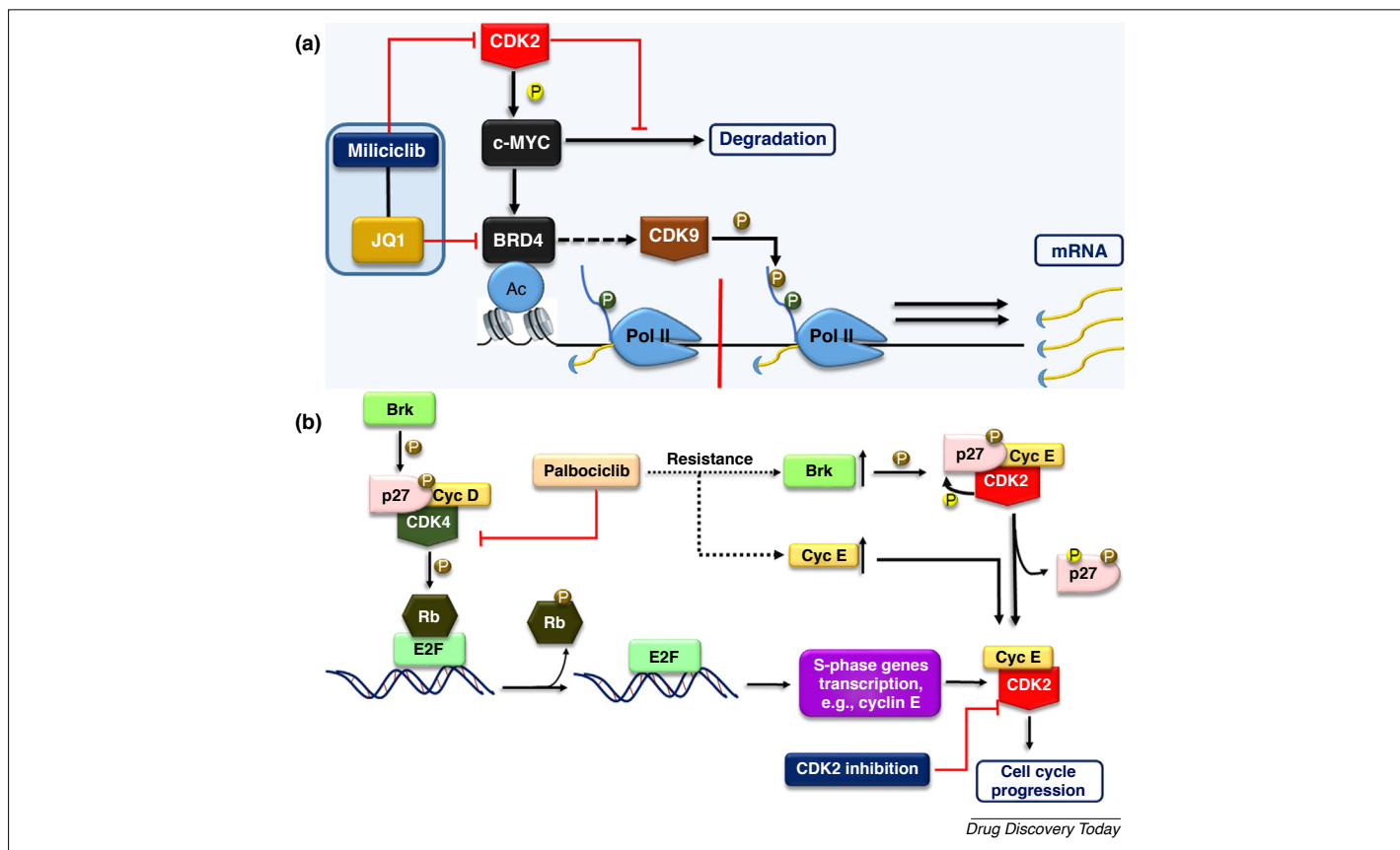
FIGURE 1

(a) Concurrent inhibition of CDK2 and BRD4 synergistically targets MYC oncogenic signaling. CDK2 maintains the stability of C-MYC by phosphorylating its Ser62 residue which blocks recognition by polyubiquitination complex and subsequent degradation. BRD4, through its interaction with acetylated histones (Ac, hallmark of active transcription), recruits CDK9 to promote the transcription of MYC and its target genes. Dual inhibition of CDK2 (miliciclib) and BRD4 (JQ1) provides an efficient way of silencing oncogenic signaling in MYC-amplified cancers. **(b)** CDK2 plays a part in resistance mechanisms to CDK4/6 inhibitors. Inhibition of Rb phosphorylation and arrest of cancer cells in the G_1-phase of the cell cycle as a result of CDK4/6 inhibition is not durable. Resistance mechanisms mainly involve overcoming the G_1 arrest through upregulation of CDK2 activity by amplification of cyclin E or downregulation of p27^{Kip1}. Phosphorylation of p27^{Kip1} at Tyr88 by breast-tumor-related kinase (Brk) is a necessary first step for relieving the inhibitory effect of p27^{Kip1} on CDK2 and as such upregulation of Brk is one of the mechanisms employed to drive resistance to CDK4/6 inhibitors. In this setting, given its 'druggability', targeting CDK2 provides a mechanism to restore the sensitivity of cancer cells resistant to CDK4/6 inhibitors (e.g., palbociclib). Abbreviations: Rb, retinoblastoma protein; E2F, E2 transcription factor; BRD4, bromodomain containing protein 4; Pol II, RNA polymerase II; mRNA, messenger RNA.

upregulated activity of CDK2, either through amplification of cyclin E [61] or downregulation of p27^{Kip1} [62] (Fig. 1b). Overexpression of cyclin E also occurs subsequent to upregulation of signaling pathways (e.g., PI3K/AKT) [61]. Another compensatory pathway involving CDK2 occurs through enhanced degradation of p27^{Kip1} and subsequent increased phosphorylation of Rb by CDK2, allowing G_1/S-phase transition [62]. Thus, triple inhibition of CDK2 and CDK4/6 could provide an opportunity to revert acquired therapeutic resistance to the dual inhibition of CDK4/6.

Developing combination therapy approaches with CDK2 inhibition

The involvement of CDK2 across numerous oncogenic pathways means that CDK2 inhibition has the potential to accentuate other therapies while also decreasing proliferation. Overall, the literature indicates that CDK2 inhibition can combine with different modalities of treatment, including radiotherapy [63], chemotherapy and targeted inhibitors. CDK2 inhibition appears to be effective in combination with a range of antimitotic chemotherapies. In triple-negative breast cancer (TNBC) cell lines, CDK2 inhibition

(using CYC065) was combined with eribulin – a non-taxane microtubule inhibitor approved for metastatic breast cancer. In this setting, downregulation of CDK2 activity was associated with inhibition of the transforming growth factor (TGF)-β pathway, a major driver of proliferation in TNBC [64], leading to restored chemosensitivity in resistant models. It has also been shown in inflammatory breast cancer (IBC) that overactivation of CDK2–cyclin-E activity within a stem-like subpopulation rendered them chemotherapy-resistant. The chemosensitivity of these cells was restored when paclitaxel was combined with CDK2 inhibitor SU9516, resulting in significant apoptosis compared with individual treatments [65]. The interpretation of the effect of CDK2 in these studies must be considered in the context that CYC065 is a pan CDK2/5/9 inhibitor, and SU9516 inhibits CDK2/1/4. Extra corroboration of targeting CDK2 in the TNBC model was shown by direct changes to phosphorylation of CDK2 target protein SMAD [64].

CDK2 inhibition has also been used in combination with targeted therapies, such as phosphoinositide 3 kinase (PI3K) inhibitors. Compared with single-agent treatment, the combination of
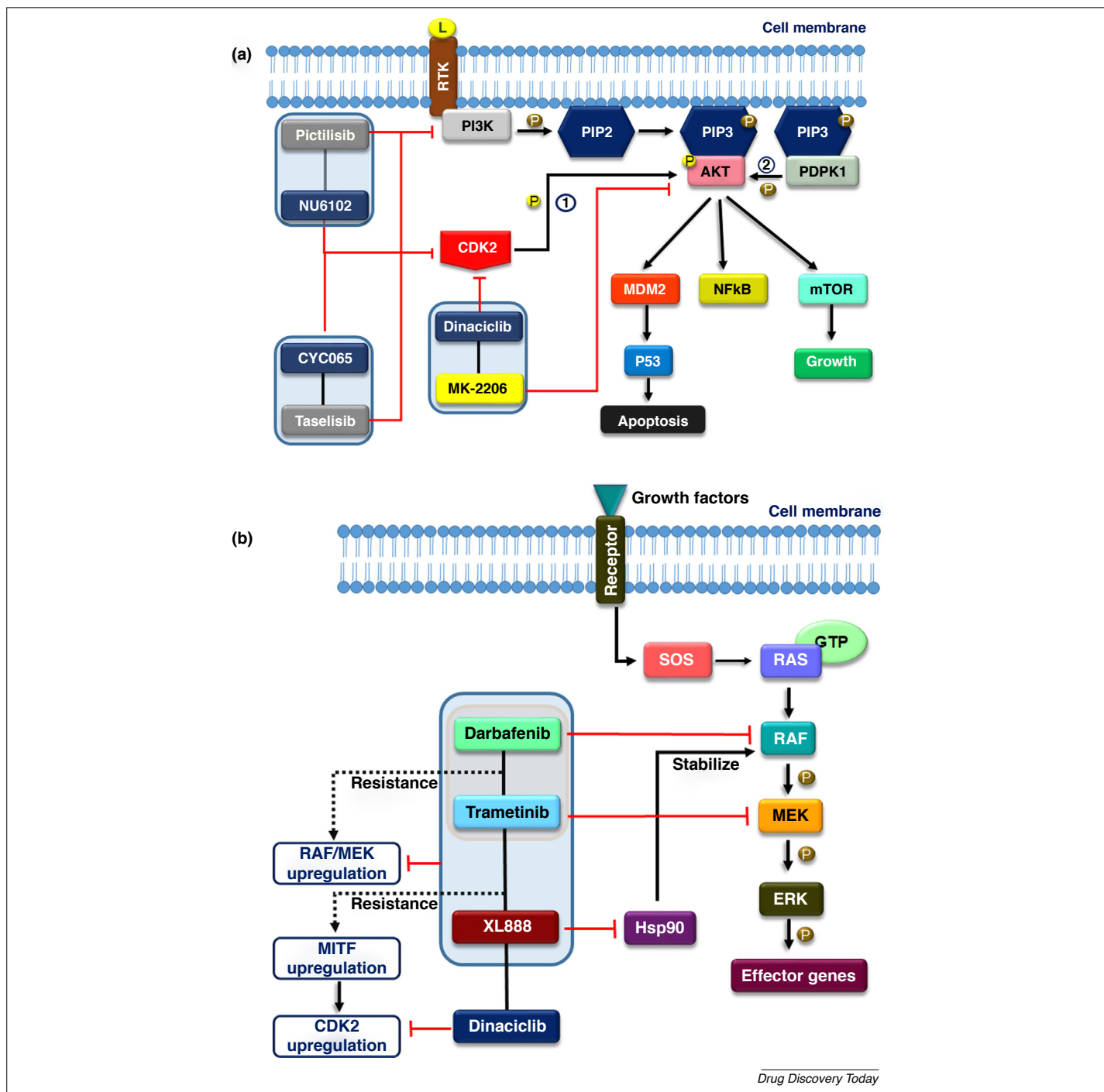
FIGURE 2

(a) CDK2 inhibitors enhance the antiproliferative effects of PI3K/AKT/mTOR inhibitors. The PI3K/AKT/mTOR pathway has various cellular functions and is highly deregulated in most cancers. This signaling pathway transmits a signal from receptor tyrosine kinase (RTK) to downstream effectors (e.g., mTOR, p53, NF-κB) via lipids (PIP2, PIP3) and kinases (PI3K, AKT, PDPK1). CDK2 phosphorylates AKT at Ser477 and Ser479, giving a stage for full activation of AKT through Ser473 phosphorylation. Thus, inhibition of CDK2 (NU6102) blocks complete activation of the PI3K/AKT pathway and gives enhanced antiproliferative activity when combined with inhibitors of the PI3K/AKT pathway (pictilisib). **(b)** CDK2 inhibition overcomes resistance to BRAF-MEK-hsp90 inhibitors. The RAS/RAF/MEK/ERK pathway is activated in multiple human cancers owing to its role in proliferation, survival and senescence. This complex pathway involves signal relay from a cell surface receptor to multiple effector genes through activation and phosphorylation of membrane-bound and cytoplasmic proteins (RAS, RAF, MEK, ERK). Resistance to inhibitors targeting individual components (e.g., darbafenib, RAF; trametinib, MEK) of the pathway is highly common making combination therapies a favored approach. Unfortunately, resistance persists in combination therapy settings [e.g., darbafenib + trametinib + XL888 (hsp90 inhibitor)]. In this setting, upregulation of CDK2 (subsequent to upregulation of MITF) is responsible for the acquired resistance and its inhibition (using dinaciclib) sensitizes resistant cells to BRAF-MEK-hsp90 combined inhibition. Abbreviations: PI3K, phosphoinositide 3 kinase; PIP_2, phosphoinositide-3,4-biphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; AKT, protein kinase B; *PDPK1*, 3-phosphoinositide-dependent kinase 1; NF-κB, nuclear factor κB; MDM2, mouse double minute 2 homolog; mTOR, mammalian target of rapamycin; p53, tumor protein 53; GTP, guanine triphosphate; RAF, rapidly accelerated fibrosarcoma; MEK, MAPK/ERK kinase; ERK, extracellular signal-regulated kinase; hsp90, heat shock protein 90; MITF, microphthalmia-associated transcription factor.

CDK2 (CYC065: CDK2/5/9 inhibitor) with PI3K inhibition demonstrated synergistic cytotoxicity in serous uterine carcinoma with *CCNE1* amplification [66] (Fig. 2a). The combination of AKT inhibitors with dinaciclib (CDK1/2/5/9/12) has also shown promise in preclinical models of *CCNE1*-amplified ovarian cancer [3]. Finally, B-Raf inhibitors and heat shock protein (Hsp)90 inhibitors in combination with dinaciclib were shown to be highly effective in melanoma cell lines that had acquired or intrinsic resistance to BRAF and Hsp90 inhibition [67]. In this study, an important piece of corroborating evidence that targeting was via CDK2 was provided by a proteome screen and a series of CDK siRNA studies that identified CDK2 as the responsible kinase for resistance [67] (Fig. 2b).

Concluding remarks: developing effective inhibitors of CDK2 for clinical use

The broad functionality of CDK2 in proliferative and pro-survival pathways highlights it as an ideal target for mechanism-based and low-toxicity therapeutic strategies in cancer treatment. In addition to the roles and potential avenues for intervention discussed above, there is developing evidence that CDK2 activity also impacts cell differentiation [68–71] and the adaptive immune response [72,73]. Importantly, CDK2 inhibition appears to have most potential in particular molecular landscapes or cancer subtypes.

Identification of small-molecule inhibitors of CDK2 is needed, but attaining the selectivity required remains one of the major hurdles [74]. Many early CDK2 inhibitors are classed as type I inhibitors that target the conserved ATP-binding site of the kinase in its active conformation. This pocket is similar between the CDK proteins, meaning that it is difficult to attain specificity, and most inhibitors that have progressed to clinical trials are from this category. The type II inhibitors are compounds that preferentially bind to the inactive CDK2 conformation to compete with binding of the activating cyclins. This group has greater potential for developing CDK2-specific inhibitors, but development of this class

of inhibitors is less progressed. Likewise, the type III allosteric inhibitors that target other binding regions of CDK2 are very early in development. The type II and type III inhibitors that have progressed have not been particularly potent; but these initial compounds highlight the potential for highly specific CDK2 inhibitors. Although developing an inhibitor with specificity to its target is a highly challenging task, medicinal chemistry strategies that exploit differences in molecular shape, electrostatics, conformation and flexibility, in conjunction with approaches including covalent, noncompetitive and allosteric binding, which can rationally control the undesirable off-targets effects, can be used.

All of the CDK2 inhibitors identified to date are nonselective leading to a promiscuous mode of action. In this context, it is important to note that specific cancers can benefit from the selective inhibition of CDK2 in combination with other CDKs, which broadens the spectrum for drug development. For example, endocrine-resistant cancers could benefit from a double blockade of CDK2/CDK4 action because these cancers are so highly dependent on the G_1/S axis. Cancers that show cyclin E1 amplification and CDK2 dependency could benefit from CDK2/CDK7 blockade, to directly inhibit CDK2 and then prevent the activation of residual CDK2 via CDK7 inhibition. There is already evidence that this approach will have some success because combination of CDK2 and PI3K inhibitors have demonstrated high *in vitro* efficacy in colorectal cancer cell lines [75], and the combination of CDK1/CDK2 inhibitors (e.g., roscovitine) with PI3K was synthetically lethal in human glioma xenograft models [76]. Consequently, the availability of a broad spectrum of inhibitors of either high specificity or selective targeting would be a major step forward in the search for new, effective, targeted cancer therapies, especially in those subtypes with CDK2 dependency, such as MYC-amplified cancers. Highly specific CDK2 inhibitors will bring additional benefit in defining further unique roles of CDK2 in cancer development, which will aid the ongoing identification of cancer subtypes that are susceptible to CDK2 inhibition.

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