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REVIEW ARTICLE

Intrinsic and acquired resistance to CDK4/6 inhibitors and potential overcoming strategies

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Abnormal activation of the cyclin-dependent kinases (CDKs), which result in aberrant cell proliferation, is one of the inherent characteristics of tumor. Thus targeting the activity of CDKs represents a promising tumor therapeutic strategy. Currently, the specific inhibitors that target CDK4 and CDK6 have been approved for the treatment of estrogen receptor positive, human epidermal growth factor receptor 2 negative (ER^+ $HER2^-$) breast cancer in combination with endocrine therapy; other combination strategies are being tested in a number of clinical trials. However, the acquired resistance to CDK4/6 inhibitors has emerged. As the cell cycle is orchestrated by a series of biological events, the alterations of other molecular events that regulate the cell cycle progression may be involved in intrinsic resistance to CDK4/6 inhibitors. In this review we mainly discuss the mechanisms underlying intrinsic resistance and acquired resistance to CDK4/6 inhibitors as well as combination strategies with other signal pathway inhibitors being tested in clinical and pre-clinical studies, to extend the use of CDK4/6 inhibitors in tumor treatment.

Keywords: cancer; cell cycle; Rb; CDK4/6 inhibitors; intrinsic resistance; acquired resistance

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INTRODUCTION

Aberrant cell cycle activity is one of central hallmarks of cancer [1]. CDK4 and CDK6 combine with D-type cyclins to control cell cycle progression in the early G1 phases. Early in the G1 phase, cyclins D1, D2, and D3 combine with CDK4 and CDK6 activated by promitotic signaling, and this complex subsequently phosphorylates retinoblastoma (Rb) protein, which in turn releases E2F transcription factors to allow for transcription of E-type cyclins and other proteins, for example, CDK2. Subsequently, the cyclin E-CDK2 complex further phosphorylates Rb, promoting S-phase entry [2]. In this process, the activity of the cyclin D-CDK4/6 complex is negatively regulated by cyclin-dependent kinase inhibitors (CKIs). Some CKIs are members of the inhibitor of CDK4 (INK4) family, which consists of four proteins: $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$ and $p19^{INK4D}$, and these proteins can specifically bind to CDK4 and CDK6 to prevent their association with D-type cyclins, thereby inhibiting the activity of CDK4 and CDK6 [3–5].

The cyclin D-CDK4/6-Rb pathway is commonly mutated in human cancers [6]. In ER^+ breast cancer, multiple oncogenic signals promote cyclin D1 expression and activate CDK4/6 to drive breast cancer progression [7]. Endocrine therapy is the main treatment for ER^+ breast cancer. Previous studies suggested that cyclin D1 and CDK4 remain active against endocrine therapy [8]. Preclinical studies found that ER^+ breast cancers harboring elevated expression of cyclin D1 and Rb protein were sensitive to palbociclib [9]. Palbociclib was the first CDK4/6 inhibitor to demonstrate clinical efficacy in ER^+ breast cancer, and ~60%–70% of all breast cancers respond to palbociclib [10]. Subsequently, two other CDK4/6 inhibitors followed. Ribociclib is very similar in structure to palbociclib and is also used in combination with hormone therapy. While abemaciclib is structurally less similar to both ribociclib and palbociclib, and it can be adopted as a monotherapy [11, 12]. Compared to endocrine therapy, this combination therapy indeed improved the overall survival time, but ~25%–35% of patients did not respond, and almost all patients eventually acquired resistance [13]. Therefore, it is urgent to find the mechanism(s) of drug resistance and choose appropriate strategies against acquired resistance.

This review mainly discusses the mechanisms of resistance to CDK4/6 inhibitors from two aspects: (1) intrinsic resistance mechanisms including amplification or mutation of certain genes in the cyclin D-CDK4/6-Rb pathway; and (2) acquired resistance mechanisms such as activation of other compensatory signaling pathways, tumor metabolism and the tumor immune microenvironment. In addition, this review will summarize the strategies for combating CDK4/6 inhibitors resistance and that are under clinical development in various tumor types.

THE INTRINSIC RESISTANCE MECHANISMS

Currently, the CDK4/6 inhibitors: palbociclib, ribociclib, and abemaciclib have been approved in combination with endocrine therapy as standard treatment for ER^+ breast cancer. In Table 1, we summarized the specific CDK4/6 inhibitors used in combination with endocrine therapy in advanced HR^+HER2^- breast cancer in various clinical settings.

As CDK4/6 inhibitors perform their anti-tumor activities by inhibiting the Rb pathway, Rb loss or CDK4 amplification is

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Table 1. CDK4/6 inhibitors in combination with endocrine therapy in advanced HR^+ $HER2^-$ breast cancer in various clinical settings.

Drug	Combination	Line	Menopausal status	Clinical settings
Palbociclib	Letrozole	1st	Post	PALOMA-2
	Fulvestrant	2nd	Pre and Post	PALOMA-3
Ribociclib	Letrozole	1st	Pre	MONALEESA-7
	Letrozole	1st	Post	MONALEESA-2
	Fulvestrant	1st and 2nd	Post	MONALEESA-3
Abemaciclib	Letrozole	1st	Post	MONARCH 3
	Fulvestrant	2nd	Pre and Post	MONARCH 2
	–	2+	Pre	MONARCH 1

involved in intrinsic resistance. Additionally, other elements that participate in regulating cell cycle or bypassing the cyclin D-CDK6-Rb pathway such as CDK2 overexpression, and other signaling pathways that directly regulate the cell cycle have the potential to contribute to CDK4/6 inhibitors resistance.

The Rb-E2F pathway

Rb loss. Rb loss has been shown to facilitate cell cycle progression, compromising G1/S arrest [14]. Finn et al. detected that breast tumor cell lines with high expression of Rb and cyclin D1 and relatively low expression of p16 were sensitive to PD0332991 (palbociclib) and showed that Rb loss yielded therapeutic failure [9]. Similarly, Konecny et al. demonstrated that cancer cell lines that were sensitive to PD0332991 (palbociclib) exhibited functional Rb and decreased p16 and cyclin E1 expression by testing many ovarian cancer cell lines [15].

In human papilloma virus (HPV)-positive cervical cancer and head and neck cancers, the viral oncoprotein E7 promotes RB protein degradation and disrupt its function, leading to accumulation of $p16^{INK4A}$, which in turn results in resistance to CDK4/6 inhibitors [16, 17]. However, in advanced bladder cancer, regardless of Rb status, palbociclib as a monotherapy or in combination with cisplatin has demonstrated significant efficacy and antitumor effects. Mechanically, palbociclib exerted these antitumor effects by inhibiting FOXM1 phosphorylation [18].

By analyzing circulating tumor DNA (ctDNA) from the breast cancer patients who had been treated with a CDK4/6 inhibitor for several months, Condorelli et al. detected acquired *RB* mutations, and these alterations could lead to Rb functional loss, conferring CDK4/6 inhibitor resistance [19].

E2F. The overexpression of E2F activating transcription factors is capable of bypassing CDK4/6 inhibition, directly driving DNA replication and mitosis, which is involved in the inherent resistance to CDK4/6 inhibitors [20]. In letrozole-resistant ER^+ breast cancers, the activity of E2F4 was increased, and most of the E2F4 target genes were upregulated; in addition, treatment with palbociclib in letrozole-resistance patients before surgery significantly decreased the expression of E2F4 target genes [21]. In BRAF-mutant and NRAS-mutant melanomas, E2F reactivation has been identified as the mechanism by which tumors acquire resistance to combined MEKi and CDK4/6i [22].

CDKs

CDK4. Amplification of CDK4 exists in a number of tumors, for example, approximately 50% of glioblastomas (GBMs) bear CDK4 amplification, and in melanomas, one specific point mutation, R24C in *CDK4*, leads to constitutively activated CDK4, which in turn results in CDK4 being insensitive to inhibition by INK4 family members [23, 24]. In rhabdomyosarcoma (RMS), CDK4 amplification and overexpression could reduce the sensitivity of ribociclib [25]. However, in CDK4-amplified liposarcoma and neuroblastoma, palbociclib and ribociclib have shown significant anti-proliferative activity [26, 27]. Additionally, the T172 phosphorylation of CDK4 was shown to control the cell cycle process, and breast tumors harboring high T172 phosphorylation of CDK4 were more sensitive to palbociclib than those without CDK4 T172 phosphorylation [28].

CDK2. Many tumors show noncanonical cyclin D1-CDK2-mediated S-phase entry. In addition, the noncanonical cyclin D1-CDK2-mediated S-phase transition, in part, can confer resistance to CDK4/6 inhibitors in ER-positive breast cancer [29, 30]. The luminal androgen receptor (LAR) subtype, a subtype of triple-negative breast cancer (TNBC), was sensitive to CDK4/6 inhibitors initially but subsequently developed drug resistance. Compared to that in the sensitive-cells, higher levels of CDK2 activity have been demonstrated in resistant cells. In addition, PI3K inhibition antagonized CDK2 activity and re-sensitized tumors to CDK4/6 inhibitors [31].

Cyclins

Cyclin D1. The CCND1 3′UTR mutation in endometrial cancer has been shown to increase CCND1 expression and can activate D-type cyclins, which enhances the sensitivity to the CDK4/6 inhibitor abemaciclib [32]. In the phase II Paloma 1 study, all enrolled patients harbored cyclin D1 amplification because cyclin D1 overexpression presents the potential for increased CDK4/6 inhibitor sensitivity. The treatment results were not as expected, and these selected patients did not show better therapeutic effects than unselected patients [10].

The SWI/SNF chromatin remodeling protein SMARCA4 (BRG1), is frequently lost in the cells of small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT), a subtype of ovarian cancer. SMARCA4 loss was shown to cause cyclin D1 deficiency in SCCOHT cells, in turn leading to high sensitivity to CDK4/6 inhibitors [33]. In addition, researchers have found that losses of SMARCA4 and SMARCA2 also exist in a subgroup of non-small-cell lung cancer (NSCLC), and these losses restricted *CCND1* mRNA expression, suppressed the activity of c-Jun to reduce cyclin D1 expression and increased the sensitivity to CDK4/6 inhibitors [34].

Cyclin E. Cyclin E overexpression in endometrial cancer and CCNE1 amplification in TNBC were shown to be associated with aggressive tumor grade and poor patient survival [35, 36]. In gastric cancer, cyclin E overexpression conferred resistance to palbociclib [37].

One large-scale clinical trial (NCT01942135) randomly assigned patients to different treatment arms to identify biomarkers associated with the effectiveness of adding palbociclib to fulvestrant in HR^+HER2^- metastatic breast cancer. This study demonstrated that high *CCNE1* mRNA expression was associated with poor anti-proliferative activity of palbociclib, and there were no significant interactions between treatment and the expression levels of CDK4, CDK6, cyclin D1, and Rb. Thus, the level of *CCNE1* mRNA was identified as a potential biomarker to predict

intrinsic resistance to the combination of palbociclib with fulvestrant [38].

CDK4 inhibitors

$p16^{INK4A}$. CDKN2A loss is a frequent event in many tumors [39]. In 143 patients with primary invasive melanoma, 56% of patients possessed hemizygous or homozygous loss of CDKN2A. Further research found that in patients with either CDKN2A methylation or CDKN2A mutation, loss of $p16^{INK4A}$ expression was potentially good for palbociclib sensitivity [40]. In sporadic pancreatic ductal adenocarcinoma (PDAC), loss of function mutations in CDKN2A activated CDK4 or CDK6 to promote cell division, but these $p16^{INK4A}$-deficient PDAC cells showed intrinsic resistance to CDK4/6 inhibitors. Insulin-like growth factor-1 (IGF-1) inhibitors could enhance CDK4/6 inhibitor activity to synergistically inhibit the growth of $p16^{INK4A}$-deficient PDAC cells [41]. In GBM xenograft models, by analyzing mutation or amplification of the components in the $p16^{INK4A}$-CDK4-Rb axis, the study indicated that GBMs harboring p16 deficiency, non-amplified CDK4 and wild-type Rb status could be more susceptible to palbociclib than GBMs without these factors [42].

p53

p53 activation can result in cell cycle arrest and negative regulation of cell cycle genes at the transcription level [43]. The CDK4 inhibitor *p21/CDKN1A* (WAF1, CIP1) was the first transcriptional target identified for p53. p53 loss is associated with lower levels of the p53 target gene p21Cip1, which increases CDK2 activity to promote the cell cycle [44]. In a recent clinical study, p53 mutation was linked with poor clinical response to abemaciclib in breast cancer patients [45]. However, Fernández-Aroca et al. found that CDK4/6 inhibition was effective in many tumor models that had Rb or p53 mutation. Additionally, palbociclib has been reported to be a novel radio-sensitizing agent, and wild-type p53 was strictly required for palbociclib to execute its radio-sensitizing effects [46].

miRNAs

Deregulation of microRNAs (miRNAs) plays an important role in tumor progression [47]. Novel miRNAs that target CDK4/6 and exhibit potential for therapeutic development in multiple tumors have been identified. The microRNA miR-302 suppressed both the cyclin D-CDK4/6 and cyclin E-CDK2 pathways to enhance G1 phase arrest to inhibit the tumorigenicity of human pluripotent stem cells [48]. miRNA-138 can induce cell cycle G1/S arrest to inhibit the growth of GBM cells by directly targeting cell cycle genes such as CDK6, E2F2 and E2F3 [49]. In addition, overexpression of miR-506 in ovarian carcinoma also inhibits cell proliferation by directly targeting CDK4 and CDK6, thereby preventing CDK4/6-FOXM1 signaling [50]. Lulla et al. have demonstrated that a family of miRNAs including miR-6883–5p, miR-149*, miR-6785-5p, and miR-4728-5p directly target the UTRs of CDK4/6 mRNAs, and ectopic expression of miR-6883-5p or miR-149*, two novel miRNAs, downregulated CDK4 and CDK6 levels in human colorectal cancer cells. Restoring expression of miR-6883-5p and miR-149* could block cell growth, leading to colorectal cancer cell apoptosis [51]. CDK6 overexpression has been identified as a key determinant to confer acquired resistance to CDK4/6i, and Cornell et al. have reported that miR-432-5p suppresses the TGF-β pathway to promote CDK6 overexpression in ER^+ breast cancer cells [52].

Other signaling pathways

In nonmalignant cells, D-type cyclins can be activated by extracellular mitogens signals through estrogen receptor and human epidermal growth factor receptors [53]. As D-type cyclins accumulate, they combine with CDK4 and CDK6 to phosphorylate Rb, which promotes cell cycle progression [2]. Mitogenic signaling pathways such as the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway were shown to be aberrantly activated by specific molecular events, which may play a role in resistance to CDK4/6 inhibitors. The detailed underlying mechanisms will be discussed in the acquired resistance section.

To better understand the mechanisms responsible for intrinsic resistance to CDK4/6 inhibitors, we summarized the dysregulated expression of the cyclin D-CDK4/6-Rb pathway in different types of tumors and its contribution to the mechanisms of intrinsic resistance to CDK4/6 inhibitors in Table 2a.

MECHANISMS OF ADAPTATION AND ACQUIRED RESISTANCE

To date, the CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) have achieved great success in the treatment of ER^+HER2^- breast cancer. With these successes, acquired resistance to CDK4/6 inhibitors has emerged. Many preclinical studies have suggested that activation of other mitogenic signaling pathways, amplification of specific genes, alteration of the tumor environment and modification of tumor immunity result in the emergence of drug resistance.

Activation of other mitogenic signaling pathways and combination therapies to target them

FGFR1 signaling pathway. In the MONALEESA-2 trial, abnormal fibroblast growth factor receptor (FGFR) signaling was revealed to participate in resistance to fulvestrant ± ribociclib. FGFR1 amplification has been identified to play an important role in acquired resistance in patients with shorter progression-free survival but not in patients with wild-type FGFR1. In addition, combination treatment with the FGFR tyrosine kinase inhibitor (TKI) lucitanib could abrogate this resistance [54].

Similarly, KRAS-mutant NSCLCs are initially sensitive to palbociclib but readily acquire resistance. In the resistant cells, the expression of CDK6, D-type cyclins, and cyclin E increased, and upstream FGFR1 signaling activated by the extracellular secretion of FGF ligands was observed, which in turn drove extracellular signal-regulated kinase (ERK) to activate mTOR; in this setting, combination treatment with a mitogen-activated protein kinase kinase (MEK) inhibitor was able to re-sensitize the resistant cells [55].

MAPK signaling pathway. The mitogen-activated protein kinase (MAPK) signaling pathway was found to be activated in CDK4/6 inhibitor-resistant prostate cancer cells by using integration of RNA sequencing analysis and phosphoproteomics, and studies further found that CDK4/6 inhibitor-resistant models were sensitized by MEK inhibitors. Combination treatments that use MEK inhibition could be adopted as promising therapies to treat or prevent CDK4/6 inhibitor resistance in cancer [56]. In NRAS-mutant melanoma, the strategy of combining MEK and CDK4/6 inhibitors (MEKi + CDK4i) at first demonstrated efficacy, but the patients eventually developed resistance. By whole-exome sequencing, the PIK3CA E545K mutation was detected and identified as the main mechanism of resistance. S6K1, a critical downstream protein of the MAPK pathway, was activated and played a role in PIK3CA E545K-induced drug resistance [57].

PI3K/AKT/mTOR signaling pathway. The AKT or mTOR signaling pathways remain active and drive acquired resistance to CDK4/6 inhibitors in several tumors [58, 59]. In breast cancer, aberrant mTORC1 activation increased cyclin D1 overexpression, participating in CDK4/6 inhibitors resistance. In addition, the upregulation of both cyclin D1 and cyclin E was also observed in pancreatic cancer models that were sensitive to mTOR inhibitors [60]. In ribociclib-resistant ER-positive breast cancer, pyruvate dehydrogenase kinase 1 (PDK1) was activated and served as a key modifier of

Table 2. a Overview of the dysregulated expression and functions of the major cell cycle proteins contribute to the intrinsic resistance to CDK4/6 inhibitors. b Overview of other mitogenic signaling activation to CDK4/6 inhibition and combination strategies.

Gene	Expression	Cancer type	Functions	References
RB1	Rb1 loss	Breast cancer	Resistance to palbociclib	[9]
RB1	Rb1 loss	Ovarian cancer	Resistance to palbociclib	[15]
RB1	Rb1degradation	HPV-positive cervical cancer and head and neck cancer	Resistance to CDK4/6 inhibitors	[16, 17]
CDK4	CDK4-amplification	Liposarcoma and Neuroblastoma	Sensitive to CDK4/6 inhibitors	[26, 27]
CDK4	Activation T172 phosphorylation	Breast cancer	Enhance the sensitivity to palbociclib	[28]
CDK4	CDK4-amplification	Rhabdomyosarcoma	Reduce the sensitivity to ribociclib	[25]
CCND1	Cyclin D1-overexpression	Endometrial cancer	Enhance the sensitivity to abemaciclib	[32]
SMARCA4	SMARCA4 loss	Small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT) cells	Cause cyclin D1 deficiency and enhance the sensitivity to CDK4/6 inhibitors	[33]
]SMARCA2/4	SMARCA2/4 loss	Non-small cancer cell	Cause cyclin D1 deficiency and increase the sensitivity to CDK4/6 inhibitors	[34]
CDKN2A	$p16^{INK4A}$ loss	Melanoma	Good for palbociclib sensitivity	[40]
CDKN2A	$p16^{INK4A}$ loss	Pancreatic ductal adenocarcinoma	Resistance to CDK4/6 inhibitors	[41]
CDKN2A	$p16^{INK4A}$ deficiency	Glioblastoma	More susceptible to palbociclib	[42]
CCNE1	Cyclin E1 overexpression	Gastric cancer	Resistance to palbociclib	[37]
CCNE1	High cyclin E1 expression	HR^+ $HER2^-$ breast cancer	Resistance to palbociclib	[38]
E2F4	E2F4 activation	ER^+ breast cancer	Resistance to palbociclib	[21]
E2F	E2F activation	Mutant BRAF and NRAS melanoma	Resistance to palbociclib	[22]
TP53	p53 loss	Breast cancer	Resistance to abemaciclib	[45]
CDK2	High levels of CDK2 activity	Luminal androgen receptor breast cancer	Resistance to CDK4/6 inhibitors	[31]

Other mitogenic signaling	Cancer type	Functions	Combination with other inhibitors	References
FGFR1 amplification	ER^+ breast cancer	Resistance to CDK4/6 inhibitors	Lucitanib (FGFR inhibitors)	[54]
FGFR1 signaling pathway activation	KRAS-mutant non-small lung cancer	Resistance to CDK4/6 inhibitors	MEK inhibitors	[55]
MAPK signaling pathway activation	prostate cancer	Resistance to CDK4/6 inhibitors	MEK inhibitors	[56]
PIK3CA E545K mutation	NRAS-mutant melanoma	Resistance to CDK4/6 inhibitors	mTOR inhibitors and S6K1 inhibitors	[57]
mTOR activation	Pancreatic cancer	Resistance to CDK4/6 inhibitors	mTOR inhibitors	[60]
PDK1 activation	ER^+ breast cancer	Resistance to ribociclib	PDK1 inhibitors or CDK2 inhibitors	[61]
NF-κB-HGF pathway	Glioblastoma	Resistance to ribociclib	Altiratinib (c-Met/Trk inhibitor)	[62]
Androgen Receptor activation	Breast cancer	Resistance to palbociclib	Androgen receptor inhibitors	[63]
FAT1 loss (Hippo pathway suppression)	ER^+ breast cancer	CDK6 overexpression		[64, 65]
MYC-driven (mTOR activation)	Colorectal carcinoma	Resistance to CDK4/6 inhibitors		[66]
Fbxo4 loss (Gln-addition)	Esophageal squamous cell carcinoma	Resistance to CDK4/6 inhibitors	Glutaminase1plus metformin or phenformin	[67]
IL6/STAT3 activation	ER^+ breast cancer	Resistance to CDK4/6 inhibitors	STAT3 inhibitor plus PARP inhibitor	[68]

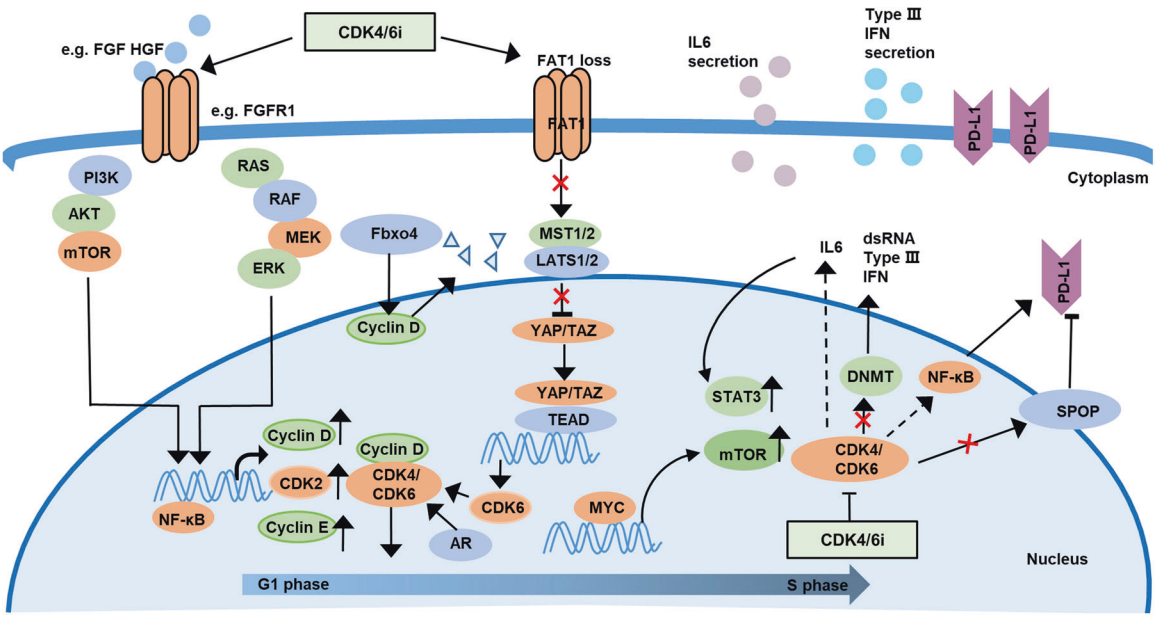

Fig. 1 Mechanisms of resistance to CDK4/6 inhibitors. Upon CDK4/6 inhibition, FGFR1 amplification can drive ERK signaling pathway activation, increasing CDK6, D-type cyclins and cyclin E expression. In addition, the activity of the MAPK signaling pathway or the PI3K/AKT/mTOR pathway is activated or enhanced. The NF-κB pathway can upregulate HGF, which in turn activates the c-Met and TrkA-B pathways. Simultaneously, androgen receptor expression is increased to promote cell cycle progression in palbociclib-resistant breast cancer cells. In CDK4/6 inhibitor-resistant ER$^+$ breast cancer, loss of FAT1 can suppress the Hippo pathway to induce YAP/TAZ nuclear localization, increasing CDK6 expression. Upon inhibition of CDK4/6, MYC overexpression can reprogram tumor metabolism and activate the mTOR pathway. Loss of Foxb4 can increase the accumulation of cyclin D1 in the nucleus, which induces glutamine addiction and promotes tumor progression. In palbociclib-resistant breast cancer cells, the IL-6/STAT3 pathway is activated and promotes SPOP degradation, thereby increasing PD-L1 levels and enhancing the levels of double-stranded RNA, stimulating the production of type III interferon.

CDK4/6 inhibitors, and CDK2 directly phosphorylated AKT at S477/T479 to activate the AKT pathway. Treatment with a PDK1 inhibitor or the CDK2 inhibitor dinaciclib was able to re-sensitize ribociclib-resistant cells to CDK4/6 inhibitors [61].

NF-κB-HGF pathway. Inhibition of CDK4/6 drives NF-κB-mediated upregulation of secreted factors such as hepatocyte growth factor and nerve growth factor, which in turn activate both the c-Met and TrkA-B pathways, eventually leading to development of resistance to abemaciclib in GBM treatment. Combination treatment with a c-Met/Trk inhibitor and altiratinib abrogated this resistance, leading to a significant synergic effect against GBM [62].

Androgen receptor (AR). In a palbociclib-resistant breast cancer cell line (MCF-7pR), ER signaling loss and AR signaling activation have been demonstrated. The AR signaling pathway regulates cell cycle processes, and thereby reduces sensitivity to CDK4/6 inhibitors. Blocking AR could overcome the resistance to palbociclib [63].

Tumor metabolism pathways contribute to acquired resistance to CDK4/6 inhibitors

Hippo pathway. In a genomic analysis of 348 ER$^+$ breast cancers treated with CDK4/6i, a loss-of-function mutation in FAT atypical cadherin 1 (FAT1) was detected in CDK4/6i-resistant patients. FAT1 loss caused Hippo pathway suppression, inducing Yes-associated protein (YAP)/tafazzin (TAZ) nuclear localization to induce CDK6 expression. Neurofibromin 2 (NF2), a Hippo pathway component, was inactivated, which also increased CDK6 expression to reduce sensitivity to CDK4/6i [64].

In radiation-resistant esophageal cancer cells, overexpression of YAP1 increased CDK6 transcription to promote CDK6 expression, and using the YAP1 inhibitor CA3 and the CDK6 inhibitor LEE001 (ribociclib) significantly suppressed esophageal cancer cell growth [65].

MYC-driven combinations. In HCT116 human colorectal carcinoma cells, CDK4/6 inhibition leads to metabolic reprogramming mainly through the MYC transcription factor. Upon inhibition of CDK4/6, MYC overexpression drives glucose, glutamine, and amino acid metabolism, leading to metabolic reprogramming and activation of the mTOR pathway. Hence, MYC-driven adaptions to CDK4/6 inhibition can make cancer cells highly sensitive to inhibitors of MYC, glutaminase or mTOR. The metabolic adaptations caused by anti-proliferative drugs may unveil novel targets to be exploited by treatment combinations to overcome acquired drug resistance [66].

Gln addiction. Aberrations in the Fbxo4-cyclin D1 axis are frequently observed in a number of tumors, and Fbxo4 loss results in hyperactivation of cyclin D1-CDK4/6 to promote esophageal squamous cell carcinoma (ESCC) progression, which drives glutamine addiction (Gln addiction) in ESCC cells. Palbociclib-resistant (PDR) TE7-PDR and TE10-PDR ESCC cells exhibited characteristics of Gln-addiction phenotypically, as well as upregulation of glutaminase 1 (GLS1). In addition, in accordance with these characteristics, with Gln-depletion or GLS1 knockdown, the PDR cells became sensitive to CDK4/6i. Combination treatment with B-839 (glutaminase 1 inhibitor) and metformin/phenformin can increase sensitivity to CDK4/6 inhibitors [67].

Alterations of the tumor immune microenvironment involved in CDK4/6 inhibitor resistance

Whole-exome sequencing and genome-wide expression analysis of palbociclib-resistant MCF-7 and T47D breast cancer cells generated in a stepwise dose-escalating fashion and matched tumor samples showed that the interleukin 6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) pathway was activated. Administration of a specific STAT3 inhibitor combined with a poly ADP-ribose polymerase (PARP) inhibitor effectively treated this acquired resistance to palbociclib [68].

Table 3. Clinical trials studying on CDK4/6 inhibitors in combination with other therapies in other cancer treatment.

Disease	Combination	Phase	Trial
Palbociclib			
KRAS mutant non-small-cell lung cancer, solid tumors	PD-0325901(MEK inhibitor)	I/II	NCT02022982
Squamous cell carcinoma of the head and neck (SCCHN)	Cetuximab	II	NCT02499120
Squamous cell carcinoma of the head and neck	Carboplatin	II	NCT03194373
Recurrent mantle cell lymphoma	Ibrutinib (BTK Inhibitor)	I	NCT02159755
Advanced solid tumors, breast cancer	Taselisib or pictilisib (PI3K inhibitor)	I	NCT02389842
Endometrial cancer	Letrozole	II	NCT02730429
Ovarian epithelial carcinoma		II	NCT01536743
Advanced solid tumor malignancies	5-FU and oxaliplatin	I	NCT01522989
Palbociclib			
Advanced hepatocellular carcinoma,		II	NCT01356628
HCC, liver cancer			
Non-small-cell lung cancer	PF-06747775 and avelumab	II	NCT02349633
Ribociclib			
Glioblastoma glioma		I	NCT02345824
Acute lymphoblastic leukemia ALL	Dexamethasone and everolimus	I	NCT03740334
High grade glioma,		I/II	NCT02607124
Diffuse intrinsic pontine glioma,			
Bithalamic high grade glioma			
Gastrointestinal cancer		II	NCT02420691
Metastatic pancreatic	Everolimus	II	NCT02985125
Ribociclib			
Adenocarcinoma			
Liposarcoma	HDM201	I/II	NCT02343172
Solid tumors harboring	LGX818 and MEK162	I/II	NCT01543698
Abemaciclib			
Glioblastoma		II	NCT02981940
Non-small-cell lung cancer stage IV	Docetaxel	II	NCT02450539
Non-small-cell lung cancer	Erlotinib	III	NCT02152631
Non-small-cell lung cancer	Pembrolizumab and anastrozole	I	NCT02779751
Breast cancer, mantle cell lymphoma		II	NCT01739309

Targeting immune checkpoints, for example, blocking programmed cell death protein 1 (PD-1) and its ligand PD-L1, has been shown to have significant efficacy in several tumors [69]. Zhang et al. found that CDK4/6 inhibitors enhanced PD-L1 stability. Cyclin D-CDK4 could directly phosphorylate speckle-type BTB/POZ protein (SPOP) at Ser6, thereby promoting the physical interaction between 14-3-3γ and SPOP. Inhibition of the interaction of SPOP with 14-3-3γ increased SPOP binding to cadherin 1 (Cdh1), resulting in SPOP poly-ubiquitination, which promoted PD-L1 degradation. Depletion/inhibition of cyclin D-CDK4 directly reduced the interaction of SPOP with 14-3-3γ. Hence, the combination of a CDK4/6 inhibitor with anti-PD-1 immunotherapy could be a promising strategy for tumor treatment [70].

In prostate cancer, the tumor suppressor Rb directly interacts with the NF-κB protein p65 in a p-S249/T252-dependent manner. Rb knockdown or CDK4/6 inhibition decreased Rb phosphorylation at S249/T252 and upregulated the activity of the NF-κB pathway to increase PD-L1 transcription. Administration of an Rb-derived S249/T252 phosphorylation-mimetic peptide suppressed the radiotherapy-induced upregulation of PD-L1 to enhance the efficacy of radiation [71].

Additionally, CDK4/6 inhibitors can remodel the tumor environment by increasing intracellular levels of double-stranded RNA, stimulating type III interferon production and markedly inhibiting the growth of regulatory T cells (Tregs). CDK4/6 inhibitors can also reduce the activity of DNA methyltransferase 1. All these molecular alterations can help cytotoxic T cells clear tumor cells, and combination treatment with immune checkpoint blockade inhibitors further enhanced this effect [72].

In Fig. 1, we summarize the known mechanisms of acquired resistance to CDK4/6 inhibitors, such as activation of other mitogenic signaling pathways and CDK6 gene amplification, and provide rational combination strategies to combat the acquired resistance to CDK4/6 inhibitors in multiple tumor types in Table 2b. Other combination strategies in different types of tumors are being tested in multiple clinical trials, and these are summarized in Table 3.

CONCLUSIONS AND PERSPECTIVES

Targeting the cell-cycle machinery is a potentially promising strategy for cancer therapy, and CDK4/CDK6 inhibitors, palbociclib, ribociclib and abemaciclib have achieved great therapeutic effects in ER^+ breast cancer. However, patients eventually become insensitive to treatment or acquire drug resistance. The potential mechanisms of acquired resistance to CDK4/6 inhibitors include the following: (1) alteration of the cyclin D-CDK4/6-Rb pathway, for instance, CDK6 or CDK4 amplification or cyclin D, cyclin E or E2F overexpression; (2) activation of alternate pathways, including the

FGFR1 signaling pathway and the PI3K/AKT/mTOR pathway; and (3) modulation of the immune microenvironment by the CDK4/6 inhibitors, such as changes in PD-L1 expression. Investigation of the mechanisms of intrinsic resistance and acquired resistance is necessary for choosing the tumors that will respond best and provides potential combinatory strategies with inhibitors of other compensatory signaling pathways to overcome drug resistance.

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AUTHOR CONTRIBUTIONS

XQX drafted the manuscript. XHP, TTW, JW, and BY critically revised the manuscript. LD and QJH designed the article. All authors have read and approved the final manuscript.

ADDITIONAL INFORMATION

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