CDK4: A Key Player in the Cell Cycle, Development, and Cancer

Stacey J. Baker, and E. Premkumar Reddy

Genes & Cancer
3(11-12) 658-669
© The Author(s) 2013
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1947601913478972
http://ganc.sagepub.com

Abstract

The cell cycle is regulated in part by cyclins and their associated serine/threonine cyclin-dependent kinases, or CDKs. CDK4, in conjunction with the D-type cyclins, mediates progression through the G₁ phase when the cell prepares to initiate DNA synthesis. Although CDK4-null mutant mice are viable and cell proliferation is not significantly affected *in vitro* due to compensatory roles played by other CDKs, this gene plays a key role in mammalian development and cancer. This review discusses the role that CDK4 plays in cell cycle control, normal development, and tumorigenesis as well as how small molecule inhibitors of CDK4 can be used to treat disease.

Keywords: CDK4, cancer, cell cycle, knockout, targeted therapy

Cell Cycle Control and Regulation

Cancer is now believed to result from perturbations in the cell cycle that result in unlimited proliferation and an inability of a cell to undergo differentiation and/or apoptosis.¹⁻⁵ The cell cycle is typically divided into 4 phases, G₁, S, G₂, and M, and within the last 2 decades, there have been a series of discoveries that have provided us with a better understanding of the control mechanisms that regulate cell cycle progression. It is apparent that the order and timing of the cell cycle are critical for accurate transmission of genetic information, and consequently, a number of biochemical pathways have evolved to ensure that initiation of a particular cell cycle event is dependent on the accurate completion of another. These biochemical pathways have been termed “checkpoints.”

CDK4 in Cell Cycle Control and Regulation

Mitogenic growth factors bind to their cognate receptors and initiate a cascade of events that culminate in the expression and assembly of different kinase holoenzymes composed of a regulatory subunit, called a cyclin, and a catalytic subunit, termed a cyclin-dependent

kinase (CDK).^{1-3,5} CDKs are serine/threonine kinases, and a typical CDK protein contains a 300-amino acid catalytic domain that is inactive when it is underphosphorylated and monomeric.⁴ The primary mechanism of CDK activation is the association with a cyclin partner. Unlike CDKs, which are highly homologous, cyclins are a remarkably diverse family of proteins, ranging in size from approximately 35 to 90 kDa.¹⁻⁴ Sequence homology among the cyclins tends to be concentrated in a 100-amino acid domain known as the cyclin box, which is necessary for CDK binding and activation. Complete activation of most CDKs also requires phosphorylation of a conserved threonine residue, located in the T-loop, by CAK1. CAK1 is a cyclin-dependent kinase that has been shown to phosphorylate the catalytic subunit of various CDKs at the equivalent residue Thr¹⁶¹ of CDC2, activating the kinase activity of their holoenzymes. In the case of CDK4, for example, it is Thr¹⁷² that is phosphorylated.⁶ It has been shown that CAK1 associates with cyclin H and has been renamed CDK7. Interestingly, the activity of this protein complex does not change in a cell cycle-dependent manner and is present in quiescent cells.⁷

One of the major breakthroughs in our understanding of cell cycle regulation

was the discovery of the *cdc2+* and *cdc28* genes in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively. Both genes code for related CDKs, and their activities are required during the G₁/S and G₂/M transitions. While a single Cdk triggers the major transitions of the yeast cell division cycle, mammalian cells encode multiple CDC2-related genes. The discovery of more than 10 Cdc2-related proteins in vertebrates initially led to the speculation that regulation of the cell cycle in higher eukaryotes might involve a complex combination of CDKs and cyclins. However, as discussed later, subsequent studies have shown that the majority of these CDKs are not critical regulators of the cell cycle. In mammalian cells, CDK4/6 associate with D-type cyclins and mediate progression through the G₁ phase when the cell prepares to initiate DNA synthesis. Activation of CDK4/6–cyclin D complexes contributes to hyperphosphorylation of the retinoblastoma (RB) protein and its related proteins, p107 and p130. The

Mount Sinai School of Medicine, New York, NY, USA

Corresponding Author:

E. Premkumar Reddy, Department of Oncological Sciences, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1130, New York, NY 10029, USA
(Email: premkumar.reddy@mssm.edu).

hypophosphorylated form of pRB binds to and sequesters several cellular proteins, and its phosphorylation results in the release of these protein factors. One key binding partner is the transcription factor E2F1, which appears to positively activate the transcription of genes whose products are required for S-phase progression. E2F1 and other members of the E2F family are known to bind to pRB and heterodimerize with DP-1 and -2, an interaction that is required for the DNA binding capacity of E2F family proteins.^{1-5,8,9} Once the cell has made the G₁/S transition, cyclin E/CDK2 phosphorylates the remaining residues on the RB family proteins that are critical for E2F activation. Activation of E2F-mediated transcription allows the cell to transit into S phase and initiate DNA replication, which is controlled, in part, through cyclin A/CDK2. Cyclin A/Cdk2 ultimately forces the cell through the G₂ phase prior to the assembly of cyclin B/CDK1 and the initiation of mitosis.⁹

Regulation of CDK4 Activity

A key response to many growth factors in many cell types is the activation of CDK4 or CDK6 by members of the cyclin D family (D1, D2, and D3). Although D-type cyclins are absent in quiescent cells, they are important integrators of mitogenic signaling. Cyclin D expression is stimulated by and is dependent on growth factors, and consequently, if growth factors are removed, cyclin D levels drop immediately, regardless of the stage of the cell cycle.¹⁰ A fully active CDK-cyclin complex can be turned off by at least 2 different mechanisms. Regulatory kinases can phosphorylate the CDK subunit at inhibitory sites near the N-terminus, or cyclin-CDK complexes can be negatively controlled in a tissue-restricted manner by 2 families of cyclin kinase inhibitors (CKIs), the INK4 and CIP/KIP families of proteins.^{1,11,12}

The INK4 family of proteins (p16^{INK4A}, p15^{INK4B}, p18^{INK4C}, p19^{INK4D}) inhibits D-type cyclin activity by specifically associating with CDK4 and CDK6 (Fig. 1) and does not interact with

CDK2 and CDK1 complexes.^{1,11-15} These inhibitory proteins are expressed at low or undetectable levels in proliferating cells and are rapidly induced by growth inhibitory stimuli, such as contact inhibition, senescence, or treatment with TGF- β .¹² Of the 4 INK4 proteins, p16^{INK4A} seems to play a critical role in senescence and tumor suppression in human cells.^{11,15} p16 is composed of 4 ankyrin repeat motifs, which are relatively well conserved motifs of 31 to 34 amino acids that mediate protein-protein interactions. In solution, the 4 ankyrin repeats of p16 are stacked together in a linear fashion to form a helix bundle with a concave surface, which harbors clusters of charged groups that mediate protein-protein binding.^{14,15} The crystal structure of the p16-CDK6 complex has been solved,^{11,15} and these studies show that binding of CDK6 to the charged domain of p16 results in an electrostatic interaction between D84 of p16 and R31 of CDK6 (which corresponds to R24 in CDK4). Because these residues are located in the active site of these 2 CDKs, this interaction diminishes kinase activity. In addition, this interaction appears to impair the binding of CDK4 and CDK6 to cyclin D, as it “shrinks” the cyclin D binding surface. This is finding is consistent with our observation that oncogenic mutations at the R24 residue of CDK4 results in an inhibition of p16 binding, which in turn results in enhanced kinase activity and increased cell proliferation.^{16,17}

The CIP/KIP family (p21^{CIP1}, p27^{KIP1}, p57^{KIP2}) of proteins binds and inactivates the CDK2–cyclin E, CDK2–cyclin A, and CDK1–cyclin B complexes. Structure/function analysis of the p21 and p27 proteins shows that their N-termini contain 2 key domains, one that is required for cyclin binding and another that is required for association with the CDK subunit. The cyclin binding motif appears to be important for providing high-affinity binding and is believed to underlie the specificity of CIP/KIP proteins for all cyclin-containing complexes.¹⁸⁻²² A majority of p27^{KIP1} in proliferating cells is thought to be associated with cyclin D–CDK4 complexes.

These complexes possess kinase activity, suggesting that this interaction does not result in an inhibition of CDK4.^{12,23-}

³⁰ Rather, p27 appears to stabilize cyclin D–CDK4 complexes, as increased expression of p27 has been shown to result in enhanced CDK4 kinase activity. This observation was confirmed using p27^{-/-} mouse embryonic fibroblasts (MEFs), whereby CDK4 enzymatic activity is reduced.^{31,32} Together, these studies suggest that cyclin D–CDK4/6 complexes exhibit a non-catalytic function, whereby their association with p21 and p27 in the G₁ phase sequesters these CKIs and prevents their binding to cyclin E/CDK2 to allow progression through G₁.

Both p21 and p27 have also been shown to inhibit the cyclin D–CDK4/6 complex under certain growth conditions.³³⁻³⁵ p27 levels increase dramatically in response to certain antiproliferative signals, and under these conditions, cyclin D–CDK4 complexes are inactive.²³ These observations suggest that p27 could act both as an inhibitor and activator of CDK4–cyclin D complexes depending on the cellular context (Fig. 1). James *et al.*²⁶ reported that p27 is preferentially tyrosine phosphorylated at positions 88 and 89 in proliferating cells, causing it to bind cyclin D–CDK4 complexes in a noninhibitory fashion. Treatment of tyrosine-phosphorylated p27 protein preparations with a tyrosine phosphatase converted the p27 molecule to its inhibitory form, suggesting that p27 functions as an important molecular switch that discerns between growth-inhibitory and growth-promoting signals. Tyrosine residues 88 and 89 of p27 were shown to be targets of SRC family kinases, such as SRC, YES, ABL, and LYN,^{36,37} and a mutant form of p27 (Y89F) appeared to inhibit both CDK4 and CDK2 complexes, resulting G₁ arrest.²⁶

Downstream Targets of CDK4

As previously stated, one of the most studied G₁ cyclin-CDK substrates is RB, which is phosphorylated in a cell cycle-dependent manner. RB is hypophosphorylated in quiescent cells

Figure 1. CDK4 regulation and activation during cell cycle progression.

and becomes phosphorylated on Ser⁷⁸⁰ and Ser⁷⁹⁵ by CDK4/CDK6 during mid- to late G₁. The hypophosphorylated form of pRB associates with several cellular proteins, and its phosphorylation results in the release/disassociation of RB from its binding partners.³⁸⁻⁴⁰ One such protein is the transcription factor E2F1, which activates the transcription of genes whose products are required for S-phase progression. Most of the E2F-responsive genes identified so far are required for the G₁ to S phase transition of the cell cycle, being transcriptionally activated in a period of G₁ that coincides with passage through the restriction point. The 2 other RB-related genes that encode pocket proteins with similar biochemical activity, p107 and p130, are also substrates of cyclin-CDK complexes. For example,

p107 is phosphorylated by cyclin D-CDK4/6 on Ser⁸⁴².⁴⁰ Studies have shown that hypophosphorylated RB preferentially associates with certain histone deacetylases (HDACs).⁴¹⁻⁴⁴ According to this model of RB-mediated chromatin repression, the RB-E2F1-HDAC complex binds to the promoters of S phase-specific genes, where the HDAC acts on surrounding chromatin and causes it to adopt a closed conformation. Phosphorylation of RB by CDK4/6 appears to result in the dissociation of the repressor complex, which in turn allows the expression of cyclin E.^{9,41} Cyclin D-CDK4/6-mediated phosphorylation of RB not only permits dissociation of the HDACs but also seems to result in the recruitment of the cyclin E-CDK2 complex to the RB pocket. Under these conditions, the

hypoacetylated state of chromatin is no longer maintained, and histone acetylation results in opening of the chromatin structure and the activation of transcription.^{9,41-45}

In addition to these proteins, other CDK4 substrates include, but are not limited to, Smad3, Cdt1, MARCKS, FOXM1, and PRMT5, and several of these proteins have been shown to serve as substrates for other CDKs as well.⁴⁵⁻⁴⁹ Interestingly, CDK4 does not phosphorylate p27 or histone H1, a canonical CDK substrate,^{23,50-52} and when compared to other CDKs, the number of *bona fide* CDK4 substrates is relatively small. Crystal structures of CDK4-cyclin D complexes suggest that the active conformation of CDK4 is highly dependent on binding to both the substrate and cyclin.⁵³

Role of CDK4 and CKIs in Development and Cancer: The Utility of Mouse Models

CKI-null mutant mouse models. The role of CKIs in modulating cyclin-CDK activities in various cells has been studied extensively in tissue culture models and *in vivo* using mutant strains of mice in which the loci of various CKIs have been disrupted. p21-null mice undergo normal development, develop no spontaneous malignancies, but are defective in G₁ checkpoint control in response to DNA damage.⁵⁴ Moreover, primary keratinocytes derived from p21^{-/-} mice are readily transformed by the *ras* oncogene, and *ras*-transformed p21-null cells exhibit a highly metastatic phenotype.⁵⁵ Mice deficient in either p18 or p27 or both develop gigantism and widespread organomegaly. This is most likely due to hyperplasia, which could be attributed to the shortening of the G₁ interval, leading to a faster cell division cycle in certain cell types.⁵⁶⁻⁵⁹ p18^{-/-} mice and p27^{-/-} mice develop intermediate lobe pituitary hyperplasia, which progresses to adenomas by 10 months of age with a nearly complete penetrance, and mice devoid of both p18 and p27 die from pituitary adenomas by 3 months of age, similar to that observed with *Rb* heterozygous mice. Mice lacking the imprinted CDK inhibitor p57^{KIP2} have altered cell proliferation and differentiation, leading to abdominal muscle defects, cleft palate, endochondral bone ossification defects with incomplete differentiation of hypertrophic chondrocytes, renal medullary dysplasia, adrenal cortical hyperplasia and cytomegaly, and lens cell hyperproliferation and apoptosis.^{60,61}

Because the *p16/p19* locus encodes 2 distinct yet overlapping transcripts that are generated due to the usage of differential reading frames and first exons, the first knockout models lacked expression of both genes. Null mutant mice that lack the expression of the entire *p16/p19* locus develop spontaneous tumors at an early stage and are highly sensitive to

carcinogenic agents.⁶² In addition, MEFs derived from *p16/p19*^{-/-} embryos proliferate rapidly, have a high colony-forming efficiency, and readily undergo oncogene-induced neoplastic transformation. The contribution of the *p16* locus, individually, in normal mouse development and to both the induction and suppression of tumor formation has also been demonstrated using 2 different mouse models. One, designated as *p16*^{*/*} by Krimpenfort *et al.*,⁶³ harbors a terminator mutation in exon 2 of p16 that mirrors a mutation frequently present in human tumors. The presence of the mutation at codon 101 gives rise to a truncated, unstable p16 protein that is unable to associate with CDK4/6 and cannot induce cell cycle arrest. In spite of this, *p16*^{*/*} mice fail to show a significant increase in spontaneous tumor formation when compared to their wild-type counterparts. The second line, generated by Sharpless *et al.*,⁶⁴ contains a deletion of exon 1 of the *p16* locus, and these mice developed more tumors of various origins than their wild-type and heterozygous counterparts in response to numerous carcinogens. Mice that have a disruption in the *p19* locus develop a variety of spontaneous tumors at a very young age, and MEFs isolated from *p19*-null embryos fail to undergo senescence.⁶⁵

Knockout mouse models of CDKs. As transition through each phase of the cell cycle is dependent on sequential activation of CDKs, it was believed that unless there are compensatory effects by another CDK that are co-expressed (as seen with CDK4 and CDK6), that loss of a single CDK would have detrimental effects on development or cell cycle progression. Such is the case of CDK1, whereby loss of CDK1 expression results in embryonic lethality at the blastocyst stage of development.⁶⁶ CDK1 is actually sufficient to drive mitosis in the absence of any interphase CDKs and can restore meiosis in oocytes, owing to its ability to bind to all cyclins and phosphorylate RB.^{67,68} Mice lacking either

CDK2, CDK3, CDK4, or CDK6 are viable, and cell proliferation is not significantly affected *in vitro* due to compensatory roles played by other CDKs.^{16,69-73} Nevertheless, these studies do not preclude a role for individual CDKs in mammalian development and disease. Even though *Cdk2/Cdk4*-null MEFs display normal S-phase progression, they eventually become immortalized and exhibit high levels of phosphorylated RB.⁷⁴ However, knockout of these genes in the mouse results in lethality that is likely caused by cardiac failure, a phenotype that is similar to *cyclin D1, D2, and D3* triple knockout mice.^{74,75} Similarly, MEFs isolated from *Cdk4/6* double knockout embryos proliferate *in vitro* with only slight defects in S phase, yet the embryos die *in utero* due to anemia.⁷⁴ Even though it was assumed that CDK4 and CDK6 have compensatory roles, knockout of each of these loci individually has revealed unique roles for both proteins. This is not surprising given that their patterns of expression do not overlap completely. Systemic loss of *Cdk6* in mice only results in a slight impairment of the mature cells that comprise the lymphoid tissues, although recent studies with conditional mouse models show a definitive role in thymocyte proliferation.^{74,76} The phenotype of *Cdk4*-null mutant mice is quite different.

CDK4 knockout mice. Mice that are nullizygous for the *Cdk4* allele exhibit a diabetic-like phenotype, with a 90% reduction in glucose levels, polyuria, polydipsia, and dramatic reductions in the size and number of pancreatic β -islet cells.¹⁶ Both male and female mice are infertile, with males exhibiting testicular atrophy due to meiotic abnormalities and embryos failing to undergo implantation in females that otherwise ovulate normally.^{16,77} Females also display pituitary hypoplasia that is characterized by a reduction in the number of prolactin-producing lactotrophic cells.^{16,77-80} Interestingly, *Cdk4*-null animals are smaller in size as compared to their wild-type

littermates, and it is therefore not surprising that both the size and number of both β -islets and lactotrophs are also reduced in these animals. Although genetic rescue restores proliferation of both cell types (and thereby corrects the defects in glucose levels and female fertility), the number of these specialized cells remains reduced.⁸¹ These data demonstrate that the observed phenotypes are the result of reduced cell numbers as opposed to defects in the functionality of these cell types. That CDK4 plays a key role in homeostasis and cell cycle entry is also revealed through cell cycle experiments using serum-starved CDK4-deficient MEFs that exhibit a considerable delay in reaching S phase and remain in G₁ for prolonged periods of time.^{16,17,72} In addition to these overt phenotypes, *Cdk4*-null mutant mice are also prone to neurological defects such as impaired locomotion, staggering, and hyperactivity; have abnormalities in thymocyte maturation and allergen response; and exhibit impaired adipocyte differentiation and function.^{16,82,83}

While *Cdk4*-null mutant mice underscore a role for the gene in normal cell development, this animal model has also shed light on the role that this kinase plays in the genesis and progression of cancer, particularly that of the mammary gland. As discussed above, a key response to growth factors in many cell types is the activation of CDK4 or CDK6 by D-type cyclins. Approximately 50% of human mammary carcinomas express abnormally high levels of cyclin D1⁸⁴⁻⁸⁸ that are maintained throughout subsequent stages of breast cancer progression from *in situ* carcinoma to invasive carcinomas.^{87,89,90} *CDK4* is also amplified or overexpressed in a variety of tumor types, including sarcomas, gliomas, lymphomas, and those of the breast.¹ Consistent with the oncogenic role of cyclin D1 in mammary epithelium, transgenic mice overexpressing *cyclin D1* in their breast tissue have been found to develop mammary adenocarcinomas,⁹¹ and the loss of

cyclin D1 in mice prevents pregnancy-associated proliferation in the epithelial compartment of the mammary gland.^{92,93} Although these mice are also resistant to mammary tumors induced by the *ras* oncogene, they remain fully sensitive to other oncogenic pathways that drive the expression of cyclin D2 (to compensate for the loss of cyclin D1), such as those driven by *c-Myc* or *Wnt-1*.⁹⁴ The absence of cyclin D1 expression also prevents the onset of *Neu*-induced mammary gland tumors.⁹⁴ While this observation is true in mice that are observed for approximately 1.5 years, these mice do eventually develop tumors over a longer period of time due to the up-regulation of cyclin D3 expression. These differences in experimental outcomes could also be attributed, in part, to the use of mice with different genetic backgrounds.⁹⁵ A requirement for D-type cyclins in cellular transformation *in vitro* has also been shown using *cyclin D* triple knockout MEFs, which are resistant to transformation by *c-Myc* or *Ras* in combination with dn-p53, *E1A*, or *c-Myc*.⁷⁵ Similarly, *Cdk4*-null MEFs have been shown to be refractory to transformation by *ras* and dn-p53.^{17,96}

Like cyclin D1, the absence of CDK4 expression in the mammary gland results in defective mammary gland development. However, while the mammary glands of virgin female CDK4-null mice display defects in ductal outgrowth, a reduction in the number of mammary ducts, and a complete absence of alveoli,⁹⁷ such changes are not evident in nullizygous *cyclin D1* mice at this stage of development. Expression of the MMTV-driven *Neu* oncogene in wild-type animals results in the appearance of infiltrating hyperplastic and dysplastic nodules in the mammary gland that is considerably reduced in the absence of CDK4 expression. *Cdk4*-null females also fail to show any of the proliferative disturbances that are otherwise normally observed as a result of *Neu* expression. As a result, the onset and incidence of mammary carcinoma in MMTV-*Neu-Cdk4*^{-/-} mice are delayed and

substantially reduced, respectively. Interestingly, loss of CDK4 expression does not affect the onset or incidence of mammary tumors that result from *Wnt-1* expression. Although it has been reported that cyclin D2 expression compensates for the loss of cyclin D1 expression in *Wnt*-driven mammary tumors,⁹⁴ neither CDK2 nor CDK6 appears to compensate for the absence of CDK4 expression.⁹⁷ It has also been suggested that WNT and c-MYC communicate with the cell cycle machinery in breast epithelial cells through different targets during tumorigenesis in the mammary gland. In this regard, as previously discussed, cyclin D2 expression is up-regulated in tumors induced by *Wnt-1* and *c-Myc* but not by *Neu* or *Ras*.⁹⁴ Considering that *Cdk4* is also dispensable for *Wnt*-induced tumorigenesis, and that there is no obvious compensation by other G₁ CDKs, it is tempting to speculate that WNT signals downstream of D-type cyclin-CDK4 complexes. This requirement could be for CDK4 kinase activity, or alternatively, for the ability of the cyclin D-Cdk4 complex to sequester p27. Additional studies are required to differentiate between these 2 possibilities.

Studies on MMTV-*Neu-p16* double transgenic mice show that *Neu*-mediated tumorigenesis is blocked by *p16* and that these double transgenic mice develop rare tumors after a long latency.⁹⁸ Because MMTV-*Neu-Cdk4*^{-/-} mice showed decreased levels of ductal branching and lobuloalveolar development of the mammary glands when compared to control animals, it is presumed that CDK4 is required for these proliferative events that are induced by *Neu*. These studies do not rule out the possibility that the observed defects in *Cdk4*-null mammary gland development could be an indirect result of hormonal signaling deficiencies as opposed to an epithelial cell-autonomous defect. Therefore, if the defect in mammary development observed in *Cdk4*-null females is not cell autonomous, then WNT not only bypasses CDK4 function but also any conceivable defects in

hormone signaling resulting from *Cdk4* ablation. Considering previous results indicating that *Neu* acts by inducing *cyclin D1* expression, and the fact that *Cdk4* is required for *Neu*-induced tumorigenesis, it is probable that the cyclin D1-CDK4 complex itself is required for *Neu*-induced tumorigenesis. This is highly likely as mammary gland development in knockin mice expressing a kinase-defective cyclin D1 mutant that does not associate with CDK4/6 complexes proceeds normally, and yet, these animals are resistant to *Neu*-induced tumorigenesis.^{99,100} However, one must be mindful that the oncogenic function of cyclin D1 may be partly independent of its ability to activate CDKs and is perhaps linked to the direct effects of cyclin D1 in controlling the expression of a subset of genes that are co-up-regulated in human tumors with deregulated cyclin D1.¹⁰¹ In spite of the fact that the mechanism is not 100% defined, the lessons learned from *cyclin D1* and *Cdk4*-null mouse models have important implications with respect to therapeutic modalities that might be effective in the treatment of breast cancers that are NEU positive.

Although *Cdk4*-null mutant mice highlight the importance of the CDK4-cyclin D1 complex in breast tumors and provide evidence to suggest that small molecule inhibitors of CDK4 kinase activity could be effective in the treatment of human disease, the importance of mutations in the *CDK4* locus in human cancer was first underscored by discoveries showing that germline mutations in this gene, which abolish the ability of the encoded protein to bind to p16^{INK4A}, result in a predisposition of individuals to the development of melanoma.^{102,103} The CDK4-Arg24Cys (R24C) mutation was also detected in sporadic melanomas, suggesting that a *CDK4* gene containing this mutation could act as a dominant oncogene that is resistant to normal physiological inhibition by p16^{INK4A}. At the time, the only previous example of a dominant oncogene transmitted in the human germline

was the *RET* gene that gives rise to MEN2A and MEN2B.¹⁰⁴

CDK4^{R24C} knockin mice: a role for CDK4 and cancer. Because the R24C mutation abolishes the ability of CDK4 to interact with p16, it was thought that the phenotype of both CDK4^{R24C} mutant mice and *p16*-null mutant mice would be identical. However, this is not the case,^{16,17,105} and their phenotype more closely resembles that of *p16/p19* double knockout animals.⁶² Although CDK4^{R24C} mice develop a variety of spontaneous primary and metastatic tumors,^{16,17,105} the major pathological abnormality observed in these mice is the onset of pancreatic islet cell hyperplasia during the first 3 months of life. These islets are primarily composed of insulin-secreting β cells, and interestingly, as stated above, mice that are null for CDK4 expression develop insulin-deficient diabetes. Together, both models illustrate a critical and highly specific role for *Cdk4* in the development and proliferation of this particular cell type. As expected, the CDK4^{R24C} protein isolated from MEFs does not associate with p16^{INK4A} and is therefore not subject to its negative regulatory effects as is evidenced by the increased expression of hyperphosphorylated members of the RB family. CDK4^{R24C}-expressing MEFs exhibit decreased doubling times, with a slightly higher percentage of cells in the S and G₂M phases, and fail to undergo senescence. The fact that long-term cultured cells (20 passages or more) spontaneously form foci, and that the MEFs themselves are highly susceptible to *H-ras*, *E1A*, and *v-myc* oncogene-driven transformation, suggests that the *Cdk4*^{R24C} mutation serves as a primary event in the progression towards a fully transformed phenotype.^{16,17} CDK4^{R24C} mice are also susceptible to an increase in the development of pituitary tumors arising either in the pars intermedia or the pars distalis with characteristic angiomatous areas or dilated "blood-filled lakes" of various sizes. In many cases, the pituitary tumors compressed

adjacent nontumorous tissues, such as the hypothalamus, pons, and brain. Interestingly, mice that are heterozygous at the *Rb* loci and those that have disruptions in *p27^{Kip1}* and *p18^{Ink4c}* also develop pituitary tumors.⁵⁶⁻⁵⁹ While the germline R24C mutation predisposes humans to hereditary melanoma, in general, there is a low level of spontaneous melanoma occurrence in CDK4^{R24C} mice.^{17,106-108} This observation suggests that other mutagenic events, such as exposure to ultraviolet radiation or other carcinogens, could play a major role in this process and is consistent with reports demonstrating that melanoma development in *p16/p19* double knockout mice is dependent on the expression of the *H-RasV12G* transgene.¹⁰⁹

Studies using the 2-step model of skin carcinogenesis that involves sequential treatment with the mutagen 9,10-dimethyl-1,2-benz[*a*]anthracene (DMBA) and tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and the effects on skin tumor formation in CDK4^{R24C} mice have also been reported, but with slightly different results. Our group has shown that CDK4^{R24C} heterozygous and homozygous mice develop papillomas with regions of hyperplasia in the epidermis with a very short latency period. No invasion into the underlying dermis was observed, and there was also a reduced incidence of benign epidermal tumors (classified as keratoacanthomas), consisting of large keratin-filled cystic structures surrounded by a very well differentiated squamous epithelium.¹⁷ These results differed from what was reported by Sotillo *et al.*,¹⁰⁶ who treated the animals with the same carcinogen and tumor promoter but showed that the mice developed both papillomas and melanomas. (The main differences between these 2 studies were the age at which the mice were treated and the dose of the DMBA/TPA. Mice that received larger doses of TPA starting at a younger age also developed melanomas.) In general, treatment with DMBA/TPA results in the development of

papillomas at the site of initiation and promotion with a characteristic oncogenic mutation in the 61st codon of the *Ha-ras* gene,¹¹⁰ and examination of the skin papillomas in the DMBA/TPA-treated mice contained this mutation. However, as only 10% of the melanomas contained mutations in the *H-ras* and *N-ras* genes in the Sotillo *et al.* study,¹⁰⁶ these results suggest that other genes are targets of DMBA in these animals and/or that *Ras* is not necessarily the gene that “drives” melanoma initiation in response to the R24C mutation, at least in response to DMBA/TPA.

Nevertheless, these studies do not preclude a role for *Ras* genes in CDK4^{R24C}-mediated melanoma development. Several studies using tyrosinase-*Hras* (Tyr-*HRas*)/CDK4^{R24C} mice report that these compound mice develop melanomas in response to DMBA/TPA and ultraviolet radiation.¹⁰⁶⁻¹⁰⁸ These mice also develop spontaneous melanomas, albeit at a lesser frequency, and all spontaneous melanomas tested in our study showed activation of the *Ras* pathway (measured as high levels of ERK phosphorylation).¹⁰⁷ While these results indicate that additional changes at the genetic level are required for maximal penetrance and tumor incidence, CDK4 deficiency in mice inhibits the development of DMBA/TPA-induced skin tumors, even though the proliferation of keratinocytes and wound healing proceed normally in these animals. In normal keratinocytes, CDK6, and to a lesser extent CDK2, appear to compensate for the loss of CDK4 activity.¹¹¹ It is therefore likely that in the case of CDK4, the R24C mutation contributes to tumor progression and aggressiveness in melanomas that are initiated by *H-ras* activation or other changes in gene expression.¹¹²⁻¹¹⁴ This is also likely the case with other tumor types, such as those that arise in the colons of *Apc*^{+/*Min*}*Cdk4*^{R24C} mice.¹¹⁵

In addition to melanomas, CDK4^{R24C} female mice develop severe mammary duct dilation and a high incidence of mammary tumors, which are often very aggressive with a large tumor burden.¹⁷ The majority of the mammary tumors

analyzed were adenosquamous carcinomas with papillary and cribriform elements or adenocarcinomas and adenocanthomas with squamous differentiation. The tumor cells lining the cavities undergo squamous differentiation or metaplasia with keratinization and formation of laminated horny pearls. This is consistent with the observation that MMTV-*cyclin D1* transgenic mice are prone to a high frequency of adenocarcinomas and adenocanthomas.⁹¹ As CDK4 is required for *vHa-ras*-driven mammary tumorigenesis,¹¹⁶ and given that CDK4^{R24C} females have an increased incidence of spontaneous mammary tumors and that the CDK4^{R24C} protein cooperates with *Ras* to drive melanoma formation, it is surprising that co-expression of CDK4^{R24C} and *vHa-ras* in mammary epithelial cells delays the onset of tumorigenesis. This is not due to reduced proliferation as both control and CDK4^{R24C}-expressing tumors express comparable levels of proliferative markers. Rather, expression of *RAS* and CDK4^{R24C} leads to the activation of senescence pathways that are followed by the onset of apoptosis and induction of DNA damage pathways.¹¹⁶ Loss of CDK4 expression also results in the senescence of preneoplastic cells in the lung and blocks the development of lung tumors in mouse models.¹¹⁷ Although the *KRAS-G12V* transgene is expressed in multiple tissues in this model, tumor induction and subsequent senescence were only detected in the lung. It is at present unclear as to why only cells of the lung are dramatically affected, although it is possible that there are additional changes in gene expression that occur only in lung tumor tissue. In humans, the *RAS-G12V* mutation is present in multiple tumor types, including those of the lung, pancreas, and colon. Given that oncogenic mutations can result in a tumor cell's dependence on CDK4, these studies suggest that the development of CDK4-specific inhibitors may be beneficial in the treatment of cancer types that rely predominantly on CDK4 expression.

Role of CDK4, CDK6, and D-Type Cyclins in Human Cancer

Proliferative abnormalities invariably manifest themselves as defects in cell cycle control. It is now believed that a vast majority of human tumors exhibit deregulation of the CDK4/6-cyclin D-INK4-RB pathway by multiple mechanisms.^{1,118,119} For example, CDK4/6 is hyperactivated in a number of human cancers as a result of overexpression of positive regulators such as cyclin D, inactivation of INK4 and CIP/KIP inhibitors, or deletion and/or epigenetic alterations of substrates such as RB.^{1,118,119} Hyperactive CDK4 has been reported in epithelial malignancies in the endocrine tissues and mucosa, while CDK6 activation was reported in certain mesenchymal tumors such as sarcomas and leukemias.¹ Mutations and chromosomal translocations in the *Cdk4* and *Cdk6* loci have also been described. One of the best examples is the CDK4^{R24C} mutation that results in insensitivity to INK4 family inhibitors and was first described in patients with familial melanoma.^{102,103} An analogous point mutation in *Cdk6* that blocks the interaction of p16^{INK4a} with CDK6 has also been reported in a human neuroblastoma cell line.¹²⁰ Chromosomal translocations within the *Cdk6* promoter that lead to CDK6 overexpression were also described in splenic marginal zone lymphomas and B-cell lymphocytic leukemias.^{121,122} Finally, *Cdk4/6* amplification or overexpression has also been observed in a wide spectrum of tumors, including gliomas, sarcomas, lymphomas, melanomas, carcinomas of the breast, squamous cell carcinomas, and leukemias.¹²³

Genomic Alterations of D-Type Cyclins in Human Cancer

Genomic alterations leading to aberrant cyclin D1 expression have been described in a variety of tumors.^{124,125} Chromosomal translocation is a common genetic event in the pathogenesis of B-cell lymphomas,¹²⁶ especially mantle cell lymphoma (MCL), a mature B-cell

malignancy that constitutes 5% to 10% of non-Hodgkin lymphomas. This disease is characterized by the t(11;14)(q13;q32) translocation that juxtaposes the cyclin D1 gene (*CCND1*) to the immunoglobulin (*Ig*) heavy chain gene¹²⁷ and results in constitutive overexpression of cyclin D1 under the control of an active *Ig* locus. In a similar manner, the t(6;14)(p21.1;q32.3) chromosomal translocation has been reported in multiple myeloma¹²⁸ and in several subtypes of mature B-cell malignancies including diffuse large B-cell lymphoma.¹²⁹ Overexpression of cyclin D1 resulting from the amplification of the 11q13 locus has been described in a number of cancers such as head and neck carcinoma,¹³⁰ pituitary tumors,¹³¹ esophageal squamous cell carcinoma,¹³² and breast cancer.^{84,85} In addition to these chromosomal alterations, single nucleotide polymorphisms are also thought to contribute to increased levels of cyclin D1 via production of an alternatively spliced isoform. Among more than 100 polymorphisms identified in the *cyclin D1* locus, the G/A870 polymorphism has been extensively studied as it is thought to hinder normal splicing at the exon 4/5 boundary. An absence of splicing at this junction causes read-through into intron 4 and results in the expression of a truncated cyclin D1 protein.¹³³ Although this truncated variant of cyclin D1 (cyclin D1b) forms an active complex with CDK4,^{134,135} it lacks the C-terminal threonine residue whose phosphorylation is critical for nuclear export and subsequent cytoplasmic degradation,¹³⁶ thereby increasing its oncogenic potency.^{132,137} In normal cells, cyclin D1 turnover is tightly regulated by a defined mechanism involving GSK3 β -mediated phosphorylation of Thr286^{136,138} nuclear export by CRM1,¹³⁹ and subsequent ubiquitination by the SCF^{Fbx4- α B Crystallin} ligase that targets cyclin D1 to the 26S proteasome.¹⁴⁰

A number of recent studies have shown that mutations in this pathway can lead to aberrant accumulation of cyclin D1. This is particularly true for

those tumors, such as breast and esophageal cancers, that express high levels of cyclin D1 in the absence of its gene amplification or chromosomal translocation.^{89,132,141} Consistent with this notion, mutations targeting the nuclear export or proteolysis of cyclin D1 have been documented in esophageal cancers and cell lines.¹⁴² Similarly, in endometrial cancer, the *cyclin D1* gene also possesses mutations or deletions that are expected to affect Thr²⁸⁶ phosphorylation and CRM1 binding.¹⁴³ Finally, esophageal tumors have been found to contain a number of mutations targeting *Fbx4*, the factor that dictates substrate specificity for the SCF E3 ligase, resulting in decreased proteolysis of cyclin D1.¹⁴⁴

Targeting CDK4 for Cancer Therapy

Oncoproteins, especially serine/threonine and tyrosine kinases, are attractive therapeutic targets as their activity is intimately associated with tumor cell proliferation. Early evidence that cyclin D and CDK4 and CDK6 activities are up-regulated in certain tumor cell types led to concerted efforts to develop small molecule inhibitors for these kinases. Experimental evidence indicating that CDK4 is dispensable for development but is required for *Neu*- and *Ras*-induced breast cancers^{16,72,97,116} suggested that inhibitors of this kinase may turn out to be nontoxic and effective therapeutic agents for the treatment of cancers that are dependent on CDK4 activity for proliferation. The first generation of CDK inhibitors developed, for example, flavopiridol and roscovitine (CYC202), were potent CDK4 inhibitors but were nonselective, inhibited multiple kinases (including CDK1 and CDK7) and caused severe toxic side effects when these molecules entered clinical trials.^{19,125} Several other pan-CDK inhibitors have since entered clinical trials, but the therapeutic efficacy of these first-generation pan-CDK inhibitors was modest due to dose-limiting

toxicity and poor pharmacokinetics. Several early trials have since been discontinued.^{145,146}

In an attempt to overcome the toxicity profile of pan-CDK inhibitors, several groups have undertaken the development of next-generation CDK inhibitors that are specific for individual CDKs. Some of these compounds exhibited a high degree of selectivity towards CDK4/6 by targeting the ATP binding site of CDK4/6–cyclin D complexes. Structures and IC₅₀ values of potent CDK4/6 selective compounds, members of chemical classes of oxindoles, triaminopyrimidines, diarylureas, thioacridones, benzothiadiazines, indolocarbazoles, and pyrido[2,3-*d*]pyrimidines, have been summarized by Lee and Sicinski.¹⁴⁷ Of these, one CDK4/6 selective compound, PD-0332991, has entered phase I clinical trials as a cancer therapeutic (<http://clinicaltrials.gov>). This compound, which is a pyrido[2,3-*d*]pyrimidine derivative, is exquisitely specific for CDK4 and CDK6, inhibiting these 2 kinases with IC₅₀ values of 0.011 and 0.015 μ M against these 2 enzymes, respectively, with little or no inhibitory activity against a panel of 350 kinases that include other CDKs and a wide variety of serine, threonine, and tyrosine kinases (our unpublished results).¹⁴⁸ This compound has been extensively studied for its efficacy in tissue culture model systems as well as in mouse xenograft models of colorectal cancer, MCL, and disseminated myeloma, where it has shown excellent efficacy.¹⁴⁸⁻¹⁵¹ PD-0332991 causes G₁ arrest in cultured tumor cell lines and inhibits tumor growth in xenograft models of RB-positive human tumor cell lines derived from the breast, ovary, lung, colon, prostate, brain, and blood, such as multiple myeloma and MCL.¹⁴⁸⁻¹⁵⁷ Therapeutic doses of PD-0332991 resulted in a marked reduction of both phosphorylated RB and the proliferative marker Ki-67 in the tumor tissue and the down-regulation of E2F target genes. Based on these very promising results, this compound entered clinical trials in 2004, and early results from phase I

trials indicate that the side effects are tolerable.¹⁵⁸⁻¹⁶¹ However, the efficacy profile of this compound has been somewhat disappointing. Two dosing regimens were tested in a phase I trial of oral PD-0332991 that enrolled 57 patients with advanced solid tumors. In one dosing regimen, PD-0332991 was administered daily for 21 days, in 28-day cycles, at doses ranging from 25 to 100 mg (3/1). In the second dosing regimen, the drug was administered for 14 days, in 21-day cycles (2/1). In the first dosing regimen (3/1), 6 patients experienced stable disease over 10 treatment cycles. In the second dosing regimen (2/1), one patient's disease stabilized for about 10 cycles. In addition to these trials, a pharmacodynamic study was conducted in 17 patients with relapsed MCL using 2-deoxy-2-[18F]fluoro-D-glucose (FDG) and 3'-deoxy-3'-[18F]fluorothymidine (FLT) positron emission tomography (PET) to study tumor metabolism and proliferation, respectively.¹⁶² RB phosphorylation and markers of proliferation (Ki-67) and apoptosis were also measured both before and during treatment in lymph node biopsies. Substantial reductions in the summed FLT-PET maximal standard uptake value (SUV_{max}), RB phosphorylation, and Ki-67 expression were observed after 3 weeks in most patients.¹⁶² Five patients also achieved progression-free survival time of >1 year, with 1 complete and 2 partial responses. Although these patients exhibited reductions in the summed FLT SUV_{max} as well as lower levels of RB phosphorylation and Ki-67 expression, control of the disease on a long-term basis is unlikely.¹⁶²

The results from combination therapy with PD-0332991 have been more encouraging. In a phase I portion of a randomized open-label phase I/II trial (A5481003) in postmenopausal women with metastatic ErbB2-negative breast cancer, PD-0332991 was administered daily (125 mg dose) for 3 to 4 weeks along with repeated cycles of letrozole (2.5 mg daily) on a continuous regimen. In this study of 6 evaluable patients, 2

achieved a partial response, and 4 achieved stable disease. Similar combination therapeutic strategies with other drugs such as the proteasome inhibitor bortezomib or dexamethasone in multiple myeloma^{163,164} are currently being tested in phase I and phase II clinical trials. In addition, other phase I/II studies aimed at testing PD-0332991 in combination with oxaliplatin and fluorouracil, or in combination with bortezomib, are being conducted in patients with colorectal cancer and MCL, respectively. The success of these combination therapies should provide an answer as to whether selective inhibitors of CDK4/6 can provide a therapeutic benefit in cancer patients.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the National Cancer Institute (5P01CA130821-05) to E.P.R.

References

- Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer*. 2009;9:153-66.
- Graña X, Reddy EP. Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). *Oncogene*. 1995;11:211-9.
- Hunter T. Oncoprotein networks. *Cell*. 1997;88:333-46.
- Morgan DO. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Ann Rev Cell Dev Biol*. 1997;13:261-91.
- Malumbres M, Barbacid M. To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer*. 2001;1:222-35.
- Kato JY, Matsuo M, Strom DK, Sherr CJ. Regulation of cyclin D-dependent kinase 4 (cdk4) by cdk4-activating kinase. *Mol Cell Biol*. 1994;14:2713-21.
- Larochelle S, Pandur J, Fisher RP, Salz HK, Suter B. Cdk7 is essential for mitosis and for in vivo Cdk-activating kinase activity. *Genes Dev*. 1998;12:370-81.
- Dyson N. The regulation of E2F by pRB-family proteins. *Genes Dev*. 1998;12:2245-62.
- Harbour JW, Dean DC. The Rb/E2F pathway: expanding roles and emerging paradigms. *Genes Dev*. 2000;14:2393-409.
- Yang K, Hitomi M, Stacey DW. Variations in cyclin D1 levels through the cell cycle determine the proliferative fate of a cell. *Cell Div*. 2006;1:32.
- Gil J, Peters G. Regulation of the INK4b-Arf-INK4a tumor suppressor locus: all for one and one for all. *Nat Rev Mol Cell Biol*. 2006;7:667-77.
- Blain SW. Switching cyclin D-Cdk4 kinase activity on and off. *Cell Cycle*. 2008;7:892-8.
- Canepa ET, Scassa ME, Ceruti JM, et al. INK4 proteins, a family of mammalian CDK inhibitors with novel biological functions. *Life*. 2007;59:419-26.
- Li J, Poi MJ, Tsai MD. Regulatory mechanisms of tumor suppressor p16INK4A and their relevance to cancer. *Biochemistry*. 2011;50:5566-82.
- Endicott J, Noble ME, Tucker JA. Cyclin-dependent kinases: inhibition and substrate recognition. *Curr Opin Struct Biol*. 1999;9:738-44.
- Rane SG, Dubus PD, Mettus RV, et al. Loss of cyclin-dependent kinase 4 expression causes infertility and insulin-dependent diabetes while its activation results in pancreatic islet hyperplasia. *Nat Gen*. 1999;22:44-52.
- Rane SG, Cosenza SC, Mettus RV, Reddy EP. Germline transmission of the Cdk4R24C mutation facilitates tumorigenesis and escape from cellular senescence. *Mol Cell Biol*. 2002;22:644-56.
- Denicourt C, Dowdy SF. Cip/Kip proteins: more than just CDKs inhibitors. *Genes Dev*. 2004;18:851-5.
- Ullah Z, Lee CY, Depamphilis ML. Cip/Kip cyclin-dependent protein kinase inhibitors and the road to polyploidy. *Cell Div*. 2009;4:10.
- LaBaer J, Garrett MD, Stevenson LF, et al. New functional activities for the p21 family of CDK inhibitors. *Genes Dev*. 1997;11:847-62.
- Cheng M, Oliver P, Diehl JA, Fero M, Roussel MF, Roberts JM. The p21 (cip1) and p27 (kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *EMBO J*. 1999;18:1571-83.
- Besson A, Dowdy SF, Roberts JM. CDK inhibitors: cell cycle regulators and beyond. *Dev Cell*. 2008;14:159-69.
- Blain SW, Montalvo E, Massague J. Differential interaction of the cyclin-dependent kinase (cdk) inhibitor p27 with cyclin A-cdk2 and cyclin D2-cdk4. *J Biol Chem*. 1997;272:25863-72.
- Soos TJ, Kiyokawa H, Yan JS, et al. Formation of p27-CDK complexes during the human mitotic cell cycle. *Cell Grow Diff*. 1996;7:135-46.
- Mahony D, Parrry DA, Lees E. Active cdk6 complexes are predominantly nuclear and represent only a minority of the cdk6 in T cells. *Oncogene*. 1998;16:603-11.
- James M, Ray A, Leznova D, Blain SW. Differential modification of p27Kip1 controls its cyclin D-cdk4 inhibitory activity. *Mol Cell Biol*. 2007;28:498-510.
- Sugimoto M, Martin N, Wilks DP, et al. Activation of cyclin D1-kinase in murine fibroblasts lacking both p21(Cip1) and p27(Kip1). *Oncogene*. 2002;21:8067-74.
- Bagui TK, Mohapatra S, Haura E, Pledger WJ. P27Kip1 and p21Cip1 are not required for the

- formation of active D cyclin-cdk4 complexes. *Mol Cell Biol.* 2003;23:7285-90.
29. Toyoshima H, Hunter T. p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. *Cell.* 1994;78:67-74.
 30. Polyak K, Lee MH, Erdjument-Bromage H, *et al.* Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell.* 1994;78:59-66.
 31. Larrea MD, Liang J, Da Silva T, *et al.* Phosphorylation of p27Kip1 regulates assembly and activation of cyclin D1-Cdk4. *Mol Cell Biol.* 2008;28:6462-72.
 32. Bagui TK, Jackson RJ, Agrawal D, Pledger WJ. Analysis of cyclin D3-cdk4 complexes in fibroblasts expressing and lacking p27(kip1) and p21(cip1). *Mol Cell Biol.* 2000;20:8748-57.
 33. Ciarallo S, Subramaniam V, Hung W, *et al.* Altered p27(Kip1) phosphorylation, localization, and function in human epithelial cells resistant to transforming growth factor beta-mediated G(1) arrest. *Mol Cell Biol.* 2002;22:2993-3002.
 34. Kato A, Takahashi H, Takahashi Y, Matsushime H. Inactivation of the cyclin D-dependent kinase in the rat fibroblast cell line, 3Y1, induced by contact inhibition. *J Biol Chem.* 1997;272:8065-70.
 35. Bockstaele L, Kookan H, Libert F, *et al.* Regulated activating Thr172 phosphorylation of cyclin-dependent kinase 4 (CDK4): its relationship with cyclins and CDK "inhibitors". *Mol Cell Biol.* 2006;26:5070-85.
 36. Chu I, Sun J, Arnaout A, *et al.* p27 phosphorylation by Src regulates inhibition of cyclin E-Cdk2. *Cell.* 2007;128:281-94.
 37. Grimm M, Wang Y, Mund T, *et al.* Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. *Cell.* 2007;128:269-80.
 38. Kitagawa M, Higashi H, Jung HK, *et al.* The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2. *EMBO J.* 1996;15:7060-9.
 39. Connell-Crowley L, Harper JW, Goodrich DW. Cyclin D1/Cdk4 regulates retinoblastoma protein-mediated cell cycle arrest by site-specific phosphorylation. *Mol Biol Cell.* 1997;8:287-301.
 40. Grafstrom RH, Pan W, Hoess RH. Defining the substrate specificity of cdk4 kinase-cyclin D1 complex. *Carcinogenesis.* 1999;20:193-8.
 41. Nigg EA. Targets of cyclin-dependent protein kinases. *Curr Opin Cell Biol.* 1993;5:187-93.
 42. Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T. Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature.* 1998;391:597-601.
 43. Luo RX, Postigo AA, Dean DC. Rb interacts with histone deacetylase to repress transcription. *Cell.* 1998;92:464-73.
 44. Managhi JL, Groisman R, Naguibneva I, *et al.* Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature.* 1998;391:601-5.
 45. Matsuura I, Denisova NG, Wang G, He D, Long J, Liu F. Cyclin-dependent kinases regulate the antiproliferative function of Smads. *Nature.* 2004;430:226-31.
 46. Liu E, Li X, Yan F, Zhao Q, Wu X. Cyclin-dependent kinases phosphorylate human Cdt1 and induce its degradation. *J Biol Chem.* 2004;279:17283-8.
 47. Manenti S, Yamauchi E, Sorokine O, *et al.* Phosphorylation of the myristoylated protein kinase C substrate MARCKS by the cyclin E-cyclin-dependent kinase 2 complex in vitro. *Biochem J.* 1999;340:775-82.
 48. Anders L, Ke N, Hydring P, *et al.* A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. *Cancer Cell.* 2011;20:620-34.
 49. Aggarwal P, Vaites LP, Kim JK, *et al.* Nuclear cyclin D1/CDK4 kinase regulates CUL4 expression and triggers neoplastic growth via activation of the PRMT5 methyltransferase. *Cancer Cell.* 2010;18:329-40.
 50. Matsushime H, Ewen ME, Strom DK, *et al.* Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. *Cell.* 1992;71:323-34.
 51. Ewen ME, Sluss HK, Sherr CJ, Matsushime H, Kato J, Livingston DM. Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell.* 1993;73:487-97.
 52. Kato J, Matsushime H, Hiebert SW, Ewen ME, Sherr CJ. Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. *Genes Dev.* 1993;7:331-42.
 53. Takaki T, Echalié A, Brown NR, Hunt T, Endicott JA, Noble ME. The structure of CDK4/cyclin D3 has implications for models of CDK activation. *Proc Natl Acad Sci U S A.* 2009;106:4171-6.
 54. Deng C, Zhang P, Harper JW, Elledge SJ, Leder P. Mice lacking p21Cip1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell.* 1995;82:675-84.
 55. Missero C, Di Cunto F, Kiyokawa H, Koff A, Dotto GP. The absence of p21Cip1/WAF1 alters keratinocyte growth and differentiation and promotes ras-tumor progression. *Genes Dev.* 1996;10:3065-75.
 56. Franklin DS, Godfrey VL, Lee H, *et al.* CDK inhibitors p18(INK4c) and p27(Kip1) mediate two separate pathways to collaboratively suppress pituitary tumorigenesis. *Genes Dev.* 1998;12:2899-911.
 57. Fero ML, Rivkin M, Tasch M, *et al.* A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. *Cell.* 1996;85:733-44.
 58. Kiyokawa H, Kineman RD, Manova-Todorova KO, *et al.* Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). *Cell.* 1996;85:721-32.
 59. Nakayama K, Ishida N, Shirane M, *et al.* Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell.* 1996;85:707-20.
 60. Yan Y, Frisen J, Lee MH, Massague J, Barbacid M. Ablation of the CDK inhibitor p57Kip2 results in increased apoptosis and delayed differentiation during mouse development. *Genes Dev.* 1997;11:973-83.
 61. Zhang P, Liegeois NJ, Wong C, *et al.* Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature.* 1997;387:151-8.
 62. Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D, DePinho RA. Role of the INK4a locus in tumor suppression and cell mortality. *Cell.* 1996;85:27-37.
 63. Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature.* 2001;413:83-6.
 64. Sharpless NE, Bardeesy N, Lee KH, *et al.* Loss of p16INK4a with retention of p19Arf predisposes mice to tumorigenesis. *Nature.* 2001;413:86-91.
 65. Kamijo T, Zindy F, Roussel MF, *et al.* Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell.* 1997;91:649-59.
 66. Diril MK, Ratnacaram CK, Padmakumar VC, *et al.* Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. *Proc Natl Acad Sci U S A.* 2012;109:3826-31.
 67. Santamaria D, Barrière C, Cerqueira A, *et al.* Cdk1 is sufficient to drive the mammalian cell cycle. *Nature.* 2007;448:811-5.
 68. Adhikari D, Zheng W, Shen Y, *et al.* Cdk1, but not Cdk2, is the sole Cdk that is essential and sufficient to drive resumption of meiosis in mouse oocytes. *Hum Mol Genet.* 2012;21:2476-84.
 69. Ortega S, Prieto I, Odajima J, *et al.* Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat Genet.* 2003;35:25-31.
 70. Berthet C, Aleem E, Coppola V, Tassarollo L, Kaldi P. Cdk2 knockout mice are viable. *Curr Biol.* 2003;13:1775-85.
 71. Ye X, Zhu C, Harper JW. A premature-termination mutation in the *Mus musculus* cyclin-dependent kinase 3 gene. *Proc Natl Acad Sci U S A.* 2001;98:1682-6.
 72. Tsutsui T, Hesabi B, Moons DS, *et al.* Targeted disruption of CDK4 delays cell cycle entry with enhanced p27(Kip1) activity. *Mol Cell Biol.* 1999;19:7011-9.
 73. Malumbres M, Sotillo R, Santamaria D, *et al.* Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. *Cell.* 2004;118:493-504.
 74. Barrière C, Santamaria D, Cerqueira A, *et al.* Mice thrive without Cdk4 and Cdk2. *Mol Oncol.* 2007;1:72-83.
 75. Kozar K, Ciernerych MA, Rebel VI, *et al.* Mouse development and cell proliferation in the absence of D-cyclins. *Cell.* 2004;118:477-91.
 76. Hu MG, Deshpande A, Schlichting N, *et al.* CDK6 kinase activity is required for thymocyte development. *Blood.* 2011;117:6120-31.
 77. Moons DS, Jirawatnotai S, Parlow AF, Gibori G, Kineman RD, Kiyokawa H. Pituitary hypoplasia and lactotroph dysfunction in mice deficient for cyclin-dependent kinase-4. *Endocrinology.* 2002;143:3001-8.
 78. Moons DS, Jirawatnotai S, Tsutsui T, *et al.* Intact follicular maturation and defective luteal

- function in mice deficient for cyclin-dependent kinase-4. *Endocrinology*. 2002;143:647-54.
79. Jirawatnotai S, Aziyu A, Osmundson EC, *et al*. Cdk4 is indispensable for postnatal proliferation of the anterior pituitary. *J Biol Chem*. 2004;279:51100-6.
 80. Mettus RV, Rane SG. Characterization of the abnormal pancreatic development, reduced growth and infertility in Cdk4 mutant mice. *Oncogene*. 2003;22:8413-21.
 81. Martin J, Hunt SL, Dubus P, *et al*. Genetic rescue of Cdk4 null mice restores pancreatic beta-cell proliferation but not homeostatic cell number. *Oncogene*. 2003;22:5261-9.
 82. Abella A, Dubus P, Malumbres M, *et al*. Cdk4 promotes adipogenesis through PPARgamma activation. *Cell Metab*. 2005;2:239-49.
 83. Chow YH, Zhu XD, Liu L, *et al*. Role of Cdk4 in lymphocyte function and allergen response. *Cell Cycle*. 2010;9:4922-30.
 84. Buckley MF, Sweeney KJ, Hamilton JA, *et al*. Expression and amplification of cyclin genes in human breast cancer. *Oncogene*. 1993;8:2127-33.
 85. Dickson C, Fantl V, Gillett C, *et al*. Amplification of chromosome band 11q13 and a role for cyclin D1 in human breast cancer. *Cancer Lett*. 1995;90:43-50.
 86. Lammie GA, Fantl V, Smith R, *et al*. D11S287, a putative oncogene on chromosome 11q13, is better expressed in squamous cell and mammary carcinomas and linked to BCL-1. *Oncogene*. 1991;6:439-44.
 87. Gillett C, Smith P, Gregory W, *et al*. Cyclin D1 and prognosis in human breast cancer. *Int J Cancer*. 1996;69:92-9.
 88. McIntosh GG, Anderson JJ, Milton I, *et al*. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. *Oncogene*. 1995;11:885-91.
 89. Bartkova J, Lukas J, Müller H, Lützhøft D, Strauss M, Bartek J. Cyclin D1 protein expression and function in human breast cancer. *Int J Cancer*. 1994;57:353-61.
 90. Weinstat-Saslow D, Merino MJ, Manrow RE, *et al*. Overexpression of cyclin D mRNA distinguishes invasive and in situ breast carcinomas from non-malignant lesions. *Nat Med*. 1995;1:1257-60.
 91. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature*. 1994;369:669-71.
 92. Sicinski P, Donaher JL, Parker SB, *et al*. Cyclin D1 provides a link between development and oncogenesis in the retina and breast. *Cell*. 1995;82:621-30.
 93. Fantl V, Stamp G, Andrews A, Rosewell I, Dickson C. Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. *Genes Dev*. 1995;9:2364-72.
 94. Yu Q, Geng Y, Sicinski P. Specific protection against breast cancers by cyclin D1 ablation. *Nature*. 2001;411:1017-21.
 95. Zhang Q, Sakamoto K, Liu C, *et al*. Cyclin D3 compensates for the loss of cyclin D1 during ErbB2-induced mammary tumor initiation and progression. *Cancer Res*. 2011;71:7513-24.
 96. Zou X, Ray D, Aziyu A, *et al*. Cdk4 disruption renders primary mouse cells resistant to oncogenic transformation, leading to Arf/p53-independent senescence. *Genes Dev*. 2002;16:2923-34.
 97. Reddy HK, Mettus RV, Rane SG, Graña X, Litvin J, Reddy EP. Cyclin-dependent kinase 4 expression is essential for neu-induced breast tumorigenesis. *Cancer Res*. 2005;65:10174-8.
 98. Yang C, Ionescu-Tiba V, Burns K, *et al*. The role of the cyclin D1-dependent kinases in ErbB2-mediated breast cancer. *Am J Pathol*. 2004;164:1031-8.
 99. Landis MW, Pawlyk BS, Li T, Sicinski P, Hinds PW. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. *Cancer Cell*. 2006;9:13-22.
 100. Yu Q, Sicinska E, Geng Y, *et al*. Requirement for CDK4 kinase function in breast cancer. *Cancer Cell*. 2006;9:23-32.
 101. Lamb J, Ramaswamy S, Ford HL, *et al*. A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer. *Cell*. 2003;114:323-34.
 102. Wolfel T, Hauer M, Schneider J, *et al*. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science*. 1995;269:1281-4.
 103. Zuo L, Weger J, Yang Q, *et al*. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet*. 1996;12:97-9.
 104. Romeo G, Ronchetto P, Luo Y, *et al*. Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. *Nature*. 1994;367:377-8.
 105. Sotillo R, Dubus P, Martin J, *et al*. Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors. *EMBO J*. 2001;20:6637-47.
 106. Sotillo R, Garcia JF, Ortega S, *et al*. Invasive melanoma in Cdk4-targeted mice. *Proc Natl Acad Sci U S A*. 2001;98:13312-7.
 107. Chawla R, Procknow JA, Tantravahi RV, Khurana JS, Litvin J, Reddy EP. Cooperativity of Cdk4R24C and Ras in melanoma development. *Cell Cycle*. 2010;9:3305-14.
 108. Hacker E, Muller HK, Irwin N, *et al*. Spontaneous and UV radiation-induced multiple metastatic melanomas in Cdk4R24C/R24C/TPras mice. *Cancer Res*. 2006;66:2946-52.
 109. Chin LA, Tam J, Pomerantz M, *et al*. Essential role for oncogenic Ras in tumour maintenance. *Nature*. 1999;400:468-72.
 110. Finch JS, Albino HE, Bowden GT. Quantitation of early clonal expansion of two mutant 61st codon c-Ha-ras alleles in DMBA/TPA treated mouse skin by nested PCR/RFLP. *Carcinogenesis*. 1996;17:2551-7.
 111. Rodriguez-Puebla ML, Miliani de, Marval PL, LaCava M, Moons DS, Kiyokawa H, Conti CJ. Cdk4 deficiency inhibits skin tumor development but does not affect normal keratinocyte proliferation. *Am J Pathol*. 2002;161:405-11.
 112. Tormo D, Ferrer A, Gaffal E, *et al*. Rapid growth of invasive metastatic melanoma in carcinogen-treated hepatocyte growth factor/scatter factor-transgenic mice carrying an oncogenic CDK4 mutation. *Am J Pathol*. 2006;169:665-72.
 113. Gaffal E, Landsberg J, Bald T, Sporleder A, Kohlmeyer J, Tüting T. Neonatal UVB exposure accelerates melanoma growth and enhances distant metastases in Hgf-Cdk4(R24C) C57BL/6 mice. *Int J Cancer*. 2011;129:285-94.
 114. Hyter S, Bajaj G, Liang X, Barbacid M, Ganguli-Indra G, Indra AK. Loss of nuclear receptor RXRα in epidermal keratinocytes promotes the formation of Cdk4-activated invasive melanomas. *Pigment Cell Melanoma Res*. 2010;23:635-48.
 115. Abedin ZR, Ma Z, Reddy EP, Litvin J. Increased angiogenesis in Cdk4(R24C/R24C):Apc(+/Min) intestinal tumors. *Cell Cycle*. 2010;9:2456-63.
 116. Reddy HK, Graña X, Dhanasekaran DN, Litvin J, Reddy EP. Requirement of Cdk4 for v-Ha-ras-induced breast tumorigenesis and activation of the v-ras-induced senescence program by the R24C mutation. *Genes Cancer*. 2010;1:69-80.
 117. Puyol M, Martín A, Dubus P, *et al*. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell*. 2010;18:63-73.
 118. Deshpande A, Sicinski P, Hinds PW. Cyclins and cdk4s in development and cancer: a perspective. *Oncogene*. 2005;24:2909-15.
 119. Graf F, Mosch B, Koehler L, Bergmann R, Wuest F, Pietzsch J. Cyclin-dependent kinase 4/6 (Cdk4/6) inhibitors: perspectives in cancer therapy and imaging. *Mini Rev Med Chem*. 2010;10:527-39.
 120. Easton J, Wei T, Lahti JM, Kidd VJ. Disruption of the cyclin D/cyclin-dependent kinase/INK4/retinoblastoma protein regulatory pathway in human neuroblastoma. *Cancer Res*. 1998;58:2624-32.
 121. Corcoran MM, Mould SJ, Orchard JA, *et al*. Dysregulation of cyclin dependent kinase 6 expression in splenic marginal zone lymphoma through chromosome 7q translocations. *Oncogene*. 1999;18:6271-7.
 122. Hayette S, Tigaud I, Callet-Bauchu E, *et al*. In B-cell chronic lymphocytic leukemias, 7q21 translocations lead to overexpression of the CDK6 gene. *Blood*. 2003;102:1549-50.
 123. Ortega S, Malumbres M, Barbacid M. Cyclin D-dependent kinases, INK4 inhibitors and cancer. *Biochim Biophys Acta*. 2002;1602:73-87.
 124. Kim JK, Diehl JA. Nuclear cyclin D: an oncogenic driver in human cancer. *J Cell Physiol*. 2009;220:292-6.
 125. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer*. 2011;11:558-72.
 126. Kupperts R. Mechanisms of B-cell lymphoma pathogenesis. *Nat Rev Cancer*. 2005;5:251-62.
 127. Bertoni F, Rinaldi A, Zucca E, Cavalli F. Update on the molecular biology of mantle cell lymphoma. *Hematol Oncol*. 2006;24:22-7.
 128. Bergsagel PL, Kuehl WM. Chromosometranslocations in multiple myeloma. *Oncogene*. 2001;20:5611-22.

129. Sonoki T, Harder L, Horsman DE, *et al.* Cyclin D3 is a target gene of t(6;14)(p21.1;q32.3) of mature B-cell malignancies. *Blood*. 2001;98:2837-44.
130. Izzo JG, Papadimitrakopoulou VA, Li XQ, *et al.* Dysregulated cyclin D1 expression early in head and neck tumorigenesis: in vivo evidence for an association with subsequent gene amplification. *Oncogene*. 1998;17:2313-22.
131. Hibberts NA, Simpson DJ, Bicknell JE, *et al.* Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. *Clin Cancer Res*. 1999;5:2133-9.
132. Shinozaki H, Ozawa S, Ando N, *et al.* Cyclin D1 amplification as a new predictive classification for squamous cell carcinoma of the esophagus, adding gene information. *Clin Cancer Res*. 1996;2:1155-61.
133. Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. *Oncogene*. 1995;11:1005-11.
134. Lu F, Gladden AB, Diehl JA. An alternatively spliced cyclin D1 isoform, cyclin D1b, is a nuclear oncogene. *Cancer Res*. 2003;63:7056-61.
135. Solomon DA, Wang Y, Fox SR, *et al.* Cyclin D1 splice variants: differential effects on localization, RB phosphorylation, and cellular transformation. *J Biol Chem*. 2003;278:30339-47.
136. Diehl JA, Zindy F, Sherr CJ. Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev*. 1997;11:957-72.
137. Alt JR, Cleveland JL, Hannink M, Diehl JA. Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. *Genes Dev*. 2000;14:3102-14.
138. Diehl JA, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3 β regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev*. 1998;12:3499-511.
139. Benzeno S, Diehl JA. C-terminal sequences direct cyclin D1-CRM1 binding. *J Biol Chem*. 2004;279:56061-6.
140. Lin DI, Barbash O, Kumar KG, *et al.* Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(Fbx4- α crystallin) complex. *Mol Cell*. 2006;24:355-66.
141. Barnes DM, Gillett CE. Cyclin D1 in breast cancer. *Breast Cancer Res Treat*. 1998;52:1-15.
142. Benzeno S, Lu F, Guo M, *et al.* Identification of mutations that disrupt phosphorylation-dependent nuclear export of cyclin D1. *Oncogene*. 2006;25:6291-303.
143. Moreno-Bueno G, Rodríguez-Perales S, Sanchez-Estevez C, *et al.* Cyclin D1 gene (CCND1) mutations in endometrial cancer. *Oncogene*. 2003;22:6115-8.
144. Barbash O, Zamfirova P, Lin DI, *et al.* Mutations in Fbx4 inhibit dimerization of the SCF(Fbx4) ligase and contribute to cyclin D1 overexpression in human cancer. *Cancer Cell*. 2008;14:68-78.
145. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. *Nature Rev Drug Discov*. 2009;8:547-66.
146. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol*. 2006;24:1770-83.
147. Lee YM, Sicinski P. Targeting cyclins and cyclin-dependent kinases in cancer: lessons from mice, hopes for therapeutic applications in human. *Cell Cycle*. 2006;5:2110-4.
148. Fry DW, Harvey PJ, Keller PR, *et al.* Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol Cancer Ther*. 2004;3:1427-38.
149. Toogood PL, Harvey PJ, Repine JT, *et al.* Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6. *J Med Chem*. 2005;48:2388-406.
150. Saab R, Bills JL, Miceli AP, *et al.* Pharmacologic inhibition of cyclin-dependent kinase 4/6 activity arrests proliferation in myoblasts and rhabdomyosarcoma-derived cells. *Mol Cancer Ther*. 2006;5:1299-308.
151. Baughn LB, Di Liberto M, Wu K, *et al.* A novel orally active small molecule potently induces G1 arrest in primary myeloma cells and prevents tumor growth by specific inhibition of cyclin-dependent kinase 4/6. *Cancer Res*. 2006;66:7661-7.
152. Fry DW, Bedford DC, Harvey PH, *et al.* Cell cycle and biochemical effects of PD 0183812: a potent inhibitor of the cyclin D-dependent kinases CDK4 and CDK6. *J Biol Chem*. 2001;276:16617-23.
153. Marzec M, Kasprzycka M, Lai R, *et al.* Mantle cell lymphoma cells express predominantly cyclin D1a isoform and are highly sensitive to selective inhibition of CDK4 kinase activity. *Blood*. 2006;108:1744-50.
154. Finn RS, Dering J, Conklin D, *et al.* PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res*. 2009;11:R77.
155. Michaud K, Solomon DA, Oermann E, *et al.* Pharmacologic inhibition of cyclin-dependent kinases 4 and 6 arrests the growth of glioblastoma multiforme intracranial xenografts. *Cancer Res*. 2010;70:3228-38.
156. Wiedemeyer WR, Dunn IF, Quayle SN, *et al.* Pattern of retinoblastoma pathway inactivation dictates response to CDK4/6 inhibition in GBM. *Proc Natl Acad Sci U S A*. 2010;107:11501-6.
157. Konecny GE, Winterhoff B, Kolarova T, *et al.* Expression of p16 and retinoblastoma determines response to CDK4/6 inhibition in ovarian cancer. *Clin Cancer Res*. 2011;17:1591-602.
158. Diab S, Eckhardt S, Tan A, *et al.* A phase I study of R547, a novel, selective inhibitor of cell cycle and transcriptional cyclin dependent kinases (CDKs) *J Clin Oncol* (Meeting Abstracts) 2007; 25: suppl 3528.
159. Schwartz GK, LoRusso PM, Dickson MA, *et al.* Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (schedule 2/1). *Br J Cancer*. 2011;104:1862-8.
160. O'Dwyer PJ, LoRusso P, DeMichele A, *et al.* A phase I dose escalation trial of a daily oral CDK 4/6 inhibitor PD-0332991. *J Clin Oncol* (Meeting Abstracts) 2007; 25: suppl 3550.
161. Schwartz GK, LoRusso PM, Dickson MA, *et al.* Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. *Clin Cancer Res*. 2012;18:568-76.
162. Leonard JP, LaCasce AS, Smith MR, *et al.* Selective CDK4/6 inhibition with tumor responses by PD0332991 in patients with mantle cell lymphoma. *Blood*. 2012;119:4597-607.
163. Niesvizky R, Ely S, Jayabalan DS, *et al.* A Phase I Trial of PD 0332991, a Novel, Orally-Bioavailable CDK4/6-Specific Inhibitor Administered in Combination with Bortezomib and Dexamethasone to Patients with Relapsed and Refractory Multiple Myeloma. *Blood* (ASH Annual Meeting Abstracts) 2009; Vol. 114: 8503.
164. Chen-Kiang S, Di Liberto M, Louie T, *et al.* Targeting Cdk4/6 in combination therapy of chemoresistant multiple myeloma. *J Clin Oncol* (Meeting Abstracts) 2008; 26: suppl 8503.