Minireview: Cyclin D1: Normal and Abnormal Functions

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Cyclin D1 encodes the regulatory subunit of a holoenzyme that phosphorylates and inactivates the retinoblastoma protein and promotes progression through the G1-S phase of the cell cycle. Amplification or overexpression of cyclin D1 plays pivotal roles in the development of a subset of human cancers including parathyroid adenoma, breast cancer, colon cancer, lymphoma, melanoma, and prostate cancer. Of the three D-type cyclins, each of which binds cyclin-dependent kinase (CDK), it is cyclin D1 overexpression that is predominantly associated with human tumorigenesis and cellular metastases. In recent years accumulating evidence suggests that in addition to its original description as a CDK-dependent regulator of the cell cycle, cyclin D1 also conveys cell cycle or CDK-independent functions. Cyclin D1 associates with, and regulates activity of, transcription factors, coactivators and corepressors that govern histone acetylation and chromatin remodeling proteins. The recent findings that cyclin D1 regulates cellular metabolism, fat cell differentiation and cellular migration have refocused attention on novel functions of cyclin D1 and their possible role in tumorigenesis. In this review, both the classic and novel functions of cyclin D1 are discussed with emphasis on the CDK-independent functions of cyclin D1. (Endocrinology 145: 5439–5447, 2004)

FOR ENDOCRINOLOGISTS AND oncologists, discoveries in the field of cell-cycle regulation have provided key new insights that now impact clinical care. The importance of this field was highlighted through prominent recognition when the Nobel Prize in physiology or medicine was awarded to Hartwell, Nurse, and Hunt in 2001. Since the initial discovery of cdc2 [cyclin-dependent kinase (CDK)1] in yeast, now more than 13 CDKs and 25 proteins with homology in the cyclin box have been identified in the human genome (1). These CDKs heterodimerize with distinct regulatory subunits referred to as cyclins. Among these regulatory subunits, the cyclin D1 gene product has become particularly well known for its prominent role in driving tumorigenesis.

The human cyclin D1 gene was initially cloned as a break point rearrangement in parathyroid adenoma (2) (Fig. 1). In parallel, the murine cyclin D1 homologue was identified as a colony-stimulating factor-1-responsive gene in macrophages (3). It is now known that cyclin D1, when targeted to the parathyroid gland, is sufficient to induce parathyroid adenoma in transgenic mice and regulates Ca\(^{2+}\) sensing (4).

Several findings are consistent with a model in which cyclin D1 serves as a key sensor and integrator of extracellular signals of cells in early to mid-G1 phase, mediating its function through binding both the CDKs and histone acetylase [p300/cAMP response element-binding protein-binding protein (CBP) and P/CAF] and histone deacetylases to modulate local chromatin structure of the genes that are involved in regulation of cell proliferation and differentiation. The abundance of cyclin D1 is induced by growth factors including epithelial growth factor and IGF-I (9) and IGF-II (10); amino acids (11); lysophosphatidic acid (12); and hormones including androgens (13), retinoic acid (14), and peroxisome proliferator-activated receptor (PPAR)\(\gamma\) ligand (15); secreted factors from adipocytes (16) and gastrointestinal hormones such as gastrin each regulate cyclin D1 expression in specific cell types (17, 18). TGF\(\beta\) and PTHrP regulate cyclin D1 in chondrocytes (19), and endothostatin caused G1 arrest of endothelial cells through inhibition of cyclin D1 (20).

Abbreviations: AR, Androgen receptor; bHLH, basic HLH; BRG, Brahma-related gene; CBP, cAMP response element-binding protein-binding protein; CDK, cyclin-dependent kinase; C/EBP, CAAAT-enhancer-binding protein; ER, estrogen receptor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HLH, helix-loop-helix; ILK, integrin-linked kinase; MEF, mouse embryob fibroblast; P/CAF, p300/ CBP-associated factor; PPAR, peroxisome proliferator-activated receptor; pRb, retinoblastoma protein; STAT, signal transducer and activator of transcription.

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restriction point leads to DNA synthesis (30, 31) (Fig. 2). Overexpression of cyclin D1 is known to correlate with the early onset of cancer and risk of tumor progression and metastasis (6, 32–42). However, a number of studies have shown a surprising lack of correlation between increased cyclin D1 expression and increased DNA synthesis in tumors (43, 44).

pRb is thought to silence specific genes that are active in the S phase of the cell cycle through active repression of E2F transcriptional activity, and this activity is thought to be derepressed by cyclin D1. pRb repression is mediated through recruiting proteins with intrinsic histone deacetylase activities (HDACs) and chromatin remodeling proteins, including Brm/SWI-related gene 1 (BRG1). pRb associates with HDAC1 through the pRb pocket domain and recruits HDACs to E2Fs. pRb cooperates with HDAC1 to repress the E2F activity at the cyclin E promoter. Inhibition of histone deacetylase activity by trichostatin A inhibits pRb-mediated repression of a chromosomally integrated E2F-regulated promoter, suggesting that HDAC activity is necessary for the pRb repressive function (45, 46). The human ortholog of hSWI/SNF, BRG1, also forms a transcriptional repressor complex with pRb that is required for pRb-mediated growth suppression (47). Phosphorylation of the C-terminal region of pRb by cyclin D/CDK4 displaces HDAC but not BRG1 (48), which leads to increased cyclin E expression, S phase progression, and repression of the cyclin A and cdc2 genes by pRb-BRG1. However, it seems cyclin D/CDK4-mediated phosphorylation of pRb does not affect the pRb/BRG1 interaction (48). The role of these cyclin D1-dependent phosphorylation functions identified in cultured cells to human tumorigenesis is, however, unclear.

In addition to its CDK-binding function, a body of evidence now indicates that D-type cyclins have CDK-independent properties (6, 49, 50). As previously proposed (50), the role of these properties in cellular growth, metabolism, and cellular differentiation are substantial. Cyclin D1 forms physical associations with more than 30 transcription factors or transcriptional coregulators (6, 51–53). Several nuclear receptors, including the androgen receptor (AR), estrogen receptor (ER) α, thyroid hormone receptor, and PPARγ bind directly to cyclin D1 within cultured cells. Both basal and ligand-dependent transactivation of nuclear receptors is regulated by cyclin D1 (15, 54–61). Cyclin D1 associates with p300/CBP-associated factor (P/CAF) and potentiates activation of ERα (55). Recent studies of microarray data from tumors overexpressing cyclin D1 identified CCAAT/enhancer binding protein (C/EBP) as a target of cyclin D1 (63). There is evidence that cyclin D1 associates with the TATA-box binding protein-associated factor (II)250 and suppresses pRb-mediated inhibition of TATA-box binding protein-associated factor (II)250 kinase activity (64, 65). Deletion of the N-terminal 20 amino acids of cyclin D1 impaired pRb kinase activity but did not affect its transforming ability (6, 66), suggesting domains other than those involved in pRb inactivation may contribute to the transforming function of cyclin D1.

**Cyclin D1 and Cancer**

Genetic aberrations in the regulatory circuits that govern transit through the G1 phase of the cell cycle occur frequently in human cancer, and overexpression of cyclin D1 is one of the most commonly observed alterations (40). One model suggests that the overexpression of cyclin D1 may serve as a drive oncogene through its cell-cycle regulating function.
Cyclin D1 is amplified and/or overexpressed in a substantial proportion of different human tumors. Increased cyclin D1 abundance occurs relatively early during tumorigenesis (67). Cyclin D1 was initially cloned and recognized as an oncogene in the development of parathyroid tumors (2, 68, 69). A subset of parathyroid adenomas contains a clonal rearrangement that juxtaposed the promoter of the PTH gene in proximity to the cyclin D1 oncogene, resulting in overexpression of cyclin D1 (2, 69). It was subsequently demonstrated that 20–40% of parathyroid adenomas overexpress the cyclin D1 protein (4, 68, 70–73). Overexpression of cyclin D1 protein is not limited to neoplastic proliferation of parathyroid tissue but is also seen in nonneoplastic proliferation of parathyroid gland. However, cyclin D1 protein expression was rarely present in normal parathyroid tissue (71). Transgenic animal models with parathyroid-targeted overexpression of the cyclin D1 oncogene have further confirmed the role of cyclin D1 in driving abnormal parathyroid cell proliferation. Intriguingly, the PTH–cyclin D1 transgenic mice not only developed abnormal growth of parathyroid cells but also developed hyperparathyroidism (4). Thus, cyclin D1 may not only control cellular proliferation but also contributes to abnormal hormonal secretion. However, the molecular mechanism for cyclin D1 in regulating PTH secretion remains to be determined.

The cyclin D1 gene, CCND1, is amplified in 15% and overexpressed in 30–50% of primary human breast cancers. In most cancer types, including lung, breast, sarcoma, and colon cancer, cyclin D1 overexpression results from induction by oncopgenic signals, rather than a clonal somatic mutation or rearrangement in the cyclin D1 gene (74).

A common A/G single nucleotide polymorphism (A870G) within exon 4 of the cyclin D1 gene results in two distinct mRNA transcripts (isoforms a and b). The alternately spliced RNA transcripts (isoform b) encodes a protein in which the last 55 amino acids of the C terminus of cyclin D1 are replaced by a shorter sequence encoded by intron 4 (75, 76) (Fig. 1C). This truncated form of cyclin D1 has been linked to higher expression and also resistant to gastrointestinal tumor induction by mutation of the ApcMin gene (98). The possibility that these differences in tissue and oncogene-dependent function of cyclin D1 may relate to a role for cyclin D1 in tissue progenitor cell number being examined by several groups. Recent studies suggest that cyclin D1 may be a potential therapeutic target in gastrointestinal malignancy (1) because transgenic mice with reduced cyclin D1 expression have reduced predisposition to gastrointestinal tumorigenesis (98).

**Cyclin D1 Regulation of Nuclear Hormone Receptor**

Cyclin D1 is a positive regulator of ERα-mediated transcription (58–60, 62, 99). Cyclin D1 enhances transcription of estrogen response element-responsive genes, independent of CDK-binding activity. Unliganded ERα binds to cyclin D1 in vivo and in vitro. ERα activation by cyclin D1 is not inhibited by antiestrogens (99). Cyclin D1 increases the transcriptional activity of ERα through increased binding of both liganded and unliganded receptor to estrogen response element sequences and increases association of ERα with P/CAF (99). P/CAF in turn potentiates cyclin D1 ERα activity, and this effect is largely dependent on the acetyltransferase activity of P/CAF. Together these finding suggest a model in which cyclin D1 triggers ERα activation through the recruitment of P/CAF (58, 62, 99). Cyclin D1 overexpression correlates with a favorable clinical outcome and better response to tamoxifen in ERα-positive human breast cancers (100, 101).
Cyclin D1 selectively inhibits ligand-dependent AR function in several cell types, including breast cancer, bladder cancer, and androgen-independent prostate adenocarcinoma cell lines (54–57). Cyclin D1 forms a specific complex with the AR, requiring the C terminus of cyclin D1 (55). The mechanism by which cyclin D1 inhibits liganded AR appears to be in part dependent on HDACs or histone acetyltransferases (HATs) (55, 57). In keeping with this observation, p300 through its HAT domain and P/CAF, rescued cyclin D1-mediated AR transrepression (55). Both cyclin D1 and the AR bind to similar domains of P/CAF, and cyclin D1 displaced binding of the AR to P/CAF in vitro (55). Together, these studies suggest cyclin D1 binding to the AR may repress ligand-dependent AR activity by competing for AR coactivators or through recruitment of AR corepressors with HDAC activity.

Cyclin D1 Regulates Adipogenic Transcription Factors and Adipogenesis

The transcription program that coordinates cellular differentiation of adipocytes has been mapped in vitro and in vivo (reviewed in Ref. 102). PPARγ is a ligand-activated transcription factor, which is selectively induced by ligands of the thiazolidinedione class. PPARγ plays a critical role in fatty acid metabolism, energy homeostasis, and adipogenesis (61, 103). A second transcription factor playing a key role in adipogenesis is C/EBPβ. C/EBPβ functions upstream of PPARγ in this differentiation cascade (Fig. 3). Two recent studies suggest cyclin D1 may inhibit this differentiation pathway because cyclin D1 was capable of inhibiting the transactivation of C/EBPβ (63, 104) or transactivation and function of PPARγ (15). C/EBPβ is a transcription factor of the basic leucine zipper family that has essential roles in a diversity of physiological processes including cellular differentiation. C/EBPβ is a constitutive repressor of cyclin D1 target genes, and cyclin D1 may act by antagonizing this repressor function (104). In parallel, cyclin D1-deficient mice showed a role for cyclin D1 as an inhibitor of PPARγ function (15). Cyclin D1 inhibited both PPARγ expression and PPARγ-dependent reporter activity, and cyclin D1 deficiency in fibroblast cell lines induced PPARγ expression and adipocyte differentiation. Adipogenesis was reversed by retroviral expression of cyclin D1 in cyclin D1−/− MEFs. Repression of PPARγ transactivation by cyclin D1 was independent of the CDK- or pRb-binding functions but was dependent on a predicted helix-loop-helix (HLH) structure region near the C terminus (15) (Fig. 4). Reduced PPARγ expression was shown during the transition of normal breast epithelium to benign breast disease and subsequently to adenocarcinoma, suggesting the cyclin D1/PPARγ interaction may contribute to normal growth control in breast epithelium (15). The functional interaction between cyclin D1 and either PPARγ or C/EBPβ in tumorigenesis remains speculative but suggests a physiologically relevant role of cyclin D1 to inhibit adipogenesis in vivo.

Cyclin D1 and Transcriptional Factors

Cyclin D1 and myogenic differentiation

Differentiation of skeletal myoblasts is controlled by the basic HLH (bHLH) regulators (105–108). Activation of muscle gene transcription in differentiating skeletal myoblasts requires their withdrawal from the cell cycle. Ectopic ex-
Expression of cyclin D1 inhibited transcriptional activation of muscle gene reporter constructs by myogenic bHLH regulators, and this effect was dependent on the carboxy terminal but not the pRb binding motif of cyclin D1 (109). It has been proposed that G1 cyclin-CDK activity blocks the initiation of skeletal muscle differentiation by both pRb-dependent and pRb-independent mechanisms (110). Cyclin D1 may also regulate myoblast differentiation by interfering with the MyoD-CDK4 interaction, which normally disrupts the ability of MyoD to induce myogenesis (51).

Cyclin D1, BETA2/NeuroD, and enteroendocrine cell differentiation

The mammalian small intestinal epithelium undergoes continuous self-renewal and differentiation along the crypt-villus axis. Cellular proliferation is restricted to the crypt compartment, whereas expression of the hormone secretin in enteroendocrine cells is restricted to the nondividing villus compartment of the intestine. The bHLH protein, BETA2/NeuroD, induces cell cycle withdrawal in addition to increasing secretin gene expression (111, 112). Cyclin D1 may also regulate myoblast differentiation by interfering with the MyoD-CDK4 interaction, which normally disrupts the ability of MyoD to induce myogenesis (51).

Repression of STAT3 by Cyclin D1

STAT3 transcription factors are important transcriptional regulators in the Janus kinase 1/STAT pathways, which are activated by various growth factors and cytokines such as epidermal growth factor and IL-6. Binding of these cytokines to their receptors activates the Janus kinase tyrosine kinases, followed by tyrosine phosphorylation of the receptors. This leads to activation and homo- or heterodimerization and translocation of the STAT1/3 transcription factors into the nucleus in which the downstream target genes are activated (113, 114). Cyclin D1 is an important target of the STAT signaling pathway in several cell types (26, 28, 115–118). Recent studies demonstrated that cyclin D1 inhibits STAT3 activation (114). In coimmunoprecipitation and pull-down assays, cyclin D1 was found to associate with the activation domain of STAT3 upon IL-6 stimulation. Overexpression of cyclin D1 inhibited transcriptional activation by STAT3 proteins, and this effect was again independent of CDK4 kinase activity (114). It was hypothesized that binding of cyclin D1 with the activation domain of STAT3 could occlude the interactions of STAT3 with the RNA polymerase II transcriptional machinery or with its essential cofactors such as CBP/p300; alternatively, STAT3 nuclear localization could be regulated by cyclin D1 in a cell-cycle-dependent manner (114).

Regulation of B-Myb Activity by Cyclin D1

B-Myb, a conserved member of the Myb transcription factor family, plays a role in the G1/S transition of the cell cycle. Constitutive expression of a human B-myb cDNA in BALB/c 3T3 fibroblasts reduced its growth factor requirements and induced a transformed phenotype (119). Evidence obtained during recent years suggests that cyclin D1, in contrast to cyclin A, inhibits the activity of B-Myb through formation of a B-Myb-cyclin D1 complex (52). Cyclin D1 associates with the central domain of B-Myb in vitro and in cultured cells. The cyclin D1 inhibitory effect on B-Myb was CDK-independent (52). The transcriptional activity of another Myb-like protein, DMP1, is also antagonized by D-type cyclins through a CDK-independent mechanism (53).

Cyclin D1 and HAT

Cyclin D1 associates with HATs, HDACs, and chromatin remodeling proteins. p300 and P/CAF are transcriptional coactivators that contain intrinsic HAT enzyme activities. They are components of a complex of multiple proteins that participate in transcription by supplying HAT activity and linking the general transcriptional machinery to specific activator-responsive promoters. Cyclin D1 physically interacts with p300/CBP and P/CAF (55, 62). Consistent with these findings, the abundance of cyclin D1 affects local histone acetylation and methylation of specific promoters in chromatin immunoprecipitation as-
says (98). P/CAF and p300 directly associate with cyclin D1 in culture cells (112) and can be recruited into a complex with the ERα (62) or AR (55) by cyclin D1. Zwijsen et al. (99) reported an interaction between cyclin D1 and the coactivators SRC1 (steroid receptor coactivator-1) and AIB1 (amplified in breast cancer 1) and showed that cyclin D1 could bring about an estrogen-independent recruitment of SRC1 to the ERα.

Cyclin D1 repressed p300 activity in cultured cells in a trichostatin A-dependent manner, suggesting that HDACs are involved in p300 repression (Fu, M., and R. G. Pestell, unpublished data). In line with this observation, cyclin D1 repressed thyroid hormone receptor (TR) by recruiting HDAC3 to form ternary complexes (120). Although cyclin D1 abundance regulates histone acetylation in the context of local chromatin at specific promoter sites (98), it remains to be determined whether cyclin D1 directly affects the enzyme activities of histone acetylase or HDACs (Fig. 5).

The function of pRb is regulated both by phosphorylation and acetylation. p300 and P/CAF were found to acetylate pRb, and acetylation of pRb is under cell-cycle control. Acetylation of pRb prevents efficient phosphorylation by cyclin E/CDK2 but facilitates its binding to the MDM2 (mouse double minute-2) protein (121). Intriguingly, acetylation does not affect pRb-dependent growth arrest or the repression of E2F transcriptional activity. Instead, acetylation is required for pRb-mediated terminal cell cycle exit and the induction of late myogenic gene expression. It was proposed that acetylation of pRb by p300 together with the resulting obstruction to cyclin/CDK phosphorylation renders the cell in a growth-arrested state, allowing it to respond to differentiation-inducing signals (121). In view of the physical association between cyclin D1 and HATs, the known binding of cyclin D1 to pRb, and recent finding that pRb is acetylated, future studies will be important to determine the relative role of cyclin D1 in regulating phosphorylation vs. acetylation of pRb.

Cyclin D1, Cellular Adhesion, and Mobility

Integrin-mediated cell adhesion to the extracellular matrix, which is required for normal cell growth, transduced at least in part through cyclin D1 via focal adhesion kinase and integrin-linked kinase (ILK). Integrin signaling through focal adhesion kinase regulates cell cycle progression and cyclin D1 abundance at the transcriptional level dependent on integrin-mediated cell adhesion and ERK signaling (122). Cyclin D1 is also induced by the ankyrin repeat containing serine-threonine protein kinase, ILK (123), which elevates cyclin D1 protein levels and transcription through the PI3 kinase and AKT/PKB pathway. Wnt-1 induces both ILK and cyclin D1 mRNA, consistent with a growing evidence that both mitogen signaling and cytoskeletal integrity are required for cell cycle progression through cyclin D1 (124).

Cyclin D1 is essential in cellular adhesion, motility, and guided migration of primary bone marrow macrophages (5). Compared with cyclin D1 wild-type macrophages, cyclin D1−/− macrophages are constitutively well spread and attached, yielding a flattened, circular morphology with reduced membrane ruffles. The attachment is mediated via increased numbers of circumferentially arrayed focal complexes rich in phospho-Y118 paxillin. The circumferential arrangement of the adhesion sites in the cyclin D1-deficient cells is associated with a closely aligned distribution of multiple cortical F-actin cables. Migration in response to wound-healing, cytokine-mediated chemotaxis, and transendothelial cell migration of cyclin D1−/− bone marrow-derived macrophages were all substantially reduced (5). The fact that cyclin D1 abundance regulates the dynamics of cellular adhesion suggest that cyclin D1 may also contribute to cellular growth properties through regulating cellular substrate interactions and therefore contribute to the invasiveness and/or metastatic phenotype, independently of its effects on cell cycle progression (5).

Conclusions

Growing evidence suggests that cyclin D1 physically associates with transcriptional factors or coactivators including HATs and HDACs to regulate transcription and epigenetic changes. The finding that cyclin D1 null mice have failed differentiation of mammary epithelium and hepatic steatosis is consistent with a role for cyclin D1 as a regulator of cellular differentiation and metabolism. Understanding the mechanisms by which cyclin D1 abundance couples the metabolic environment to regulate fat cell formation and metabolism in...
vivo may provide important insight into the role of metab-
olism in human cancer.

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References


3. Sherr CJ, Rettemer CW, Sacca R, Roussel MF, Look AT, Stanley ER 1985 The c-fms proto-oncogene product is related to the receptor for the mono-
nuclear phagocyte growth factor. Cell 41:665–674


16. Motokura T, Albanese C, Arnold A 1993 PRAD1/cyclin D1 proto-oncogene: genomic organization, 5′ DNA sequence, and sequence of a tumor-specific rearrange-
ment breakpoint. Genes Chromosomes Cancer 7:89–95


30. Shoker BS, Jarvis C, Davies MP, Iqbal M, Sibson DR, Sissons JG 2003 Immunodetectable cyclin D1 is associated with oestrogen receptor but not with cyclin D2 in primary breast cancers. Br J Cancer 89:1064–1069

Endocrinology, December 2004, 145(12):5439–5447


Ligand-independent recruitment of steroid receptor coactivators to estrogen receptor by cyclin D1. Genes Dev 12:3488–3498


103. Koeffler HP

104. Lassar AB, Davis RL, Wright WE, Kadesch T, Murre C, Voronova A, Bal-...skep SX, Rhee J, Kim PS, Novitch BG, Lassar AB

105. Rao SS, Chu C, Kohtz DS


126. Bohmer RM, Scharf E, Asoian RK 1996 Cytoskeletal integrity is required throughout the mitogen stimulation phase of the cell cycle and mediates the anchorage-dependent expression of cyclin D1. Mol Biol Cell 7:101–111


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