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Cyclin D1 in the Liver: Role of Noncanonical Signaling in Liver Steatosis and Hormone Regulation

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Background: Cyclin D1 is an important protein for cell cycle progression; however, functions independent of the cell cycle have been described in the liver. Cyclin D1 is also involved in DNA repair, is overexpressed in many cancers, and functions as a proto-oncogene. The lesser-known roles of Cyclin D1, specifically in hepatocytes, impact liver steatosis and hormone regulation in the liver.
Methods: A comprehensive search of PubMed was conducted using the keywords Cyclin D1, steatosis, lipogenesis, and liver transplantation. In this article, we review the results from this literature search, with a focus on the role of Cyclin D1 in hepatic lipogenesis and gluconeogenesis, as well as the impact and function of this protein in hepatic steatosis.
Results: Cyclin D1 represses carbohydrate response element binding protein (ChREBP) and results in a decrease in transcription of fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC). Cyclin D1 also inhibits peroxisome proliferator-activated receptor gamma (PPARγ) which is involved in hepatic lipogenesis. Cyclin D1 inhibits both hepatocyte nuclear factor 4 alpha (HNF4α) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) and represses transcription of lipogenic genes FAS and liver-type pyruvate kinase (Pklr), along with the gluconeogenic genes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase).
Conclusion: Cyclin D1 represses multiple proteins involved in both lipogenesis and gluconeogenesis in the liver. Targeting Cyclin D1 to decrease hepatic steatosis in patients with nonalcoholic fatty liver disease or alcoholic fatty liver disease may help improve patient health and the quality of the donor liver pool.

Keywords: *Cyclin D1, fatty liver, gluconeogenesis, hormones, lipogenesis, liver, liver transplantation*

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INTRODUCTION

The liver is the major site for nutrient synthesis as well as carbohydrate, lipid, glucose, and drug metabolism. Liver injury resulting from trauma, surgical excision, viral infection, chronic alcoholism, and/or poor diet causes hepatocyte turnover and elicits cellular programs leading to liver regeneration. Viral infections (hepatitis B and C),[1-6] as well as alcoholic fatty liver disease and nonalcoholic fatty liver disease (NAFLD),[7-10] trigger chronic inflammation with persistent activation of liver regeneration pathways that can progress to cirrhosis and fibrosis, requiring curative liver transplantation. Despite advancements in hepatitis C viral (HCV) therapy, patients with HCV may still progress to HCV cirrhosis, requiring liver transplantation. Although the number of HCV patients requiring liver transplantation has stabilized and should decline, the prevalence of NAFLD and nonalcoholic steatohepatitis (NASH) continues to rise. The net effect is a continuous rise in the number of patients on the liver transplant wait list while the donor liver pool remains stable. Although extended criteria donor livers, such as those of advanced age, with liver macrosteatosis, or procured after cardiac arrest, may help address this deficit, these grafts have a higher risk of acute graft dysfunction and graft failure compared to donor livers meeting standard criteria and deemed suitable for transplantation.[11-13] Grafts with prevalent macrosteatosis are particularly at risk for graft failure. The obesity epidemic and the rising prevalence of NAFLD will increase the frequency of steatosis in donor liver procurements. Biomarkers that capture the failure risk associated with donor steatosis will be required, particularly because the percentage of macrosteatosis may not reliably predict failure risk.

The process of organ procurement, preservation, and transplantation causes ischemia/reperfusion injury, leading to inflammation and hepatocyte turnover.[14] In the healthy liver, this process triggers stellate cell activation and hepatocyte proliferation, ultimately stabilizing liver function postsurgery in the transplant recipient.[15-22] Sequential biopsies of a patient undergoing partial orthotopic liver transplantation demonstrated continuous hepatocyte proliferation that eventually restored the patient's original liver mass 14 months after transplantation.[23] Steatotic hepatocytes, containing intracellular lipid droplets, have a lower threshold for ischemic injury

and a dampened proliferative response postinjury that contribute to greater necrotic injury and extended recovery periods in transplanted steatotic donor livers.[24]

Signaling pathways that trigger proliferation in hepatocytes converge upon Cyclin D1, the gatekeeper into the cell cycle. Cyclin D1 plays an instrumental role in liver regeneration, as evidenced by studies of small-for-size vs half-size grafts. Small-for-size grafts resulting in graft failure had limited induction of Cyclin D1, while half-size grafts that regenerated to restore original liver volume contained a nearly 8-fold higher induction of Cyclin D1.[25] Further, impaired liver regeneration in steatotic rats following ischemia/reperfusion injury was attributed to delayed induction of Cyclin D1 compared to healthy controls.[26] Studies have described several cell cycle–independent functions of Cyclin D1, including lipid and carbohydrate metabolism and hormone regulation. Deficits in nonessential amino acids trigger Cyclin D1 repression and impaired hepatocyte regeneration.[27]

In this review, we briefly cover the canonical pathway of Cyclin D1 in cell cycle progression and its overexpression in some cancers. The majority of this article reviews the noncanonical pathways of Cyclin D1 in the liver regarding carbohydrate and lipid metabolism, hormone regulation, and the progression from simple to severe liver steatosis.

CYCLIN D1 PROTEIN STRUCTURE

Cyclin D1 contains multiple domains and motifs that activate and/or repress numerous proteins in the liver involved in steatosis and hormone regulation. Cyclin D1 belongs to the highly conserved D-type cyclin family, originally identified as universal cell cycle regulators.[28] Cyclin D1 is encoded by the gene *CCND1*, located on the long arm of chromosome 11 at position 13.3, and spans 13,388 bases.[29,30] The *CCND1* gene contains 5 exons translated to 295 amino acids with a molecular weight of 33.7 kDa.[31]

The N-terminal consists of a retinoblastoma (Rb) binding domain and an LXCXE motif that some researchers maintain is required for the phosphorylation of Rb. This domain and motif are also found in other D-type cyclins, Cyclin D2 and D3. Reports conflict as to whether the LXCXE motif is required for Rb phosphorylation.[32-35] The cyclin box, located near the center of the protein, functions as the binding location for cyclin-dependent kinase 4 and 6 (CDK4 and CDK6). Point mutation in the 112th residue of Cyclin D1, switching from a lysine to a glutamic acid (K112E mutant), abolishes binding to and subsequent activation of CDK4.[36] The repressor domain, located within amino acids 142-253, is involved in repression of multiple proteins, including androgen receptor and hepatocyte nuclear factor 4 alpha (HNF4α).[37,38] Following the repressor domain is the LLXXXL motif that binds to the steroid receptor coactivator-1 (SRC-1).[39] Last is the PEST sequence, a proline-, glutamate-, serine-, and threonine-rich region through which Cyclin D1 is targeted for degradation through proteolysis.[40] An array of domains and motifs in Cyclin D1 allows complex interactions and the functional diversity required for its important canonical role in cell cycle progression.

THE CANONICAL PATHWAY OF CYCLIN D1 IN THE CELL CYCLE

The mechanisms of the interactions and function of Cyclin D1 and its binding partners in cell cycle progression from the G1 to the S phase, as well as its interactions with CDK4, have been thoroughly characterized and reviewed.[28,41,42] Genes encoding D-type cyclins become activated and are further induced at the beginning of the G1 phase in response to mitogen stimulation.[28,43,44] During this phase, Cyclin D1 translocates to the nucleus and remains as a static protein bound to an immobile complex.[31] In the nucleus, Cyclin D1 assembles with its catalytic partners CDK4 or CDK6[45-50] and targets its primary substrate, the nuclear protein Rb.[31,51,52]

Rb is the principal transcriptional repressor for many of the genes required to progress from G1/S transition through S phase, positioning Rb as a central target for phosphorylation and inactivation downstream of several mitogenic signaling pathways.[53,54] During the G0 phase, Rb binds to and represses E2F transcription factors blocking gene transcription necessary for S phase initiation and DNA replication, thus preventing progression through the cell cycle.[28,53,55] Rb inhibits transcription of the E2F-responsive promoters through enlistment of histone deacetylases (HDACs) to block E2F activity at the Cyclin E promoter.[56-60] Cyclin D1-CDK4 phosphorylates Rb, disrupting the repression of E2F by Cyclin E-CDK2, enabling cell cycle progression.[61] Cyclin D1 activates the kinase of Cyclin E-CDK2 by a second mechanism that also blocks cell cycle progression. This noncatalytic function sequesters Cip/Kip cell cycle inhibitors.[62] Cip/Kip proteins $p21^{Cip1}$ or $p27^{Kip1}$ interact with cyclin/CDK complexes to deter kinase activity and repress cell cycle progression.[28,63] Cyclin D1 accumulates in the nuclei and disappears once the cell enters S phase and DNA replication begins.[64] Cyclin D1 is targeted for degradation by phosphorylation of residue Thr286 by glycogen synthase kinase-3 beta (GSK3β).[65,66] This phosphorylation stimulates association with chromosome maintenance 1 protein and facilitates the nuclear export of Cyclin D1 to the cytoplasm.[65] Once exported, phosphorylated Cyclin D1 is ubiquitinated and degraded by the 26S proteasome.[64-67] Because of the important role of Cyclin D1 during the G1/S cell cycle transition, Cyclin D1 expression and localization are tightly regulated in the cell.

REGULATION OF CYCLIN D1 DURING THE CELL CYCLE

In the G1 phase, Cyclin D1 expression and assembly with CDK4 are regulated by the Ras-Raf1-MEK-ERK kinase cascade.[68-73] Induction of *CCND1* transcription is activated through mitogen-induced Ras signaling and ERK.[74] The *CCND1* promoter can also be transactivated by signal transducer and activator of transcription (STAT) proteins, early growth response protein-1 (Egr-1), nuclear factor-kappa B (NF-κB), cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), β-catenin, and JunB.[75-82] Through this signaling pathway, mitogen-activated protein kinases (MAPKs) induce Cyclin D1-CDK4 assembly, and overexpression of $p41^{MAPK}$ stimulates Cyclin D1 promoter activity.[69] Withdrawal of mitogen signals stops Ras signaling and *CCND1* transcription.[67] Degradation of Cyclin D1 by nuclear exclusion and proteolysis through GSK3β are controlled and prevented by Ras activation (Figure 1). Ras signaling in collaboration with phosphatidylinositol-3-OH (PI3K) downregulates GSK3β through its activity on the c-Akt proto-oncogene product (Akt), inhibiting the rate of Cyclin D1 turnover.[67] The PI3K-Akt-GSK3β

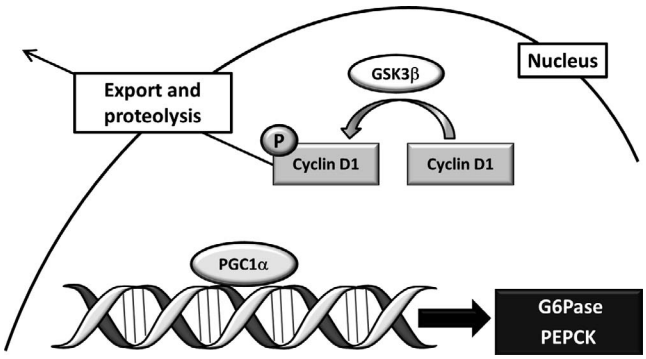

Figure 1. Hepatocytes without glucose. G6Pase, glucose-6-phosphatase; GSK3β, glycogen synthase kinase-3 beta; P, phosphorylated; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha.

cascade affects Cyclin D1 half-life as mutation of Thr286 increases the half-life of Cyclin D1 by 7-fold.[66] Inhibition of PI3K increases Cyclin D1 turnover.[67] Cyclin D1 induction is growth factor–dependent, and its transcription, protein stability, and cellular location are firmly regulated.[46,67,83] Given the importance of Cyclin D1 to promote cell proliferation, aberrant expression of this protein can lead to dysregulated cell division.

ABERRANT EXPRESSION OF CYCLIN D1 IN CANCER

Cyclin D1 is one of the most amplified proteins in the human cancer genome.[84] Clinical studies have identified *CCND1* amplification in 15% of primary breast carcinomas, with overexpression in 30%-50% of cases.[85] Cyclin D1 expression accompanied by increased Rb phosphorylation is associated with poor prognosis, necessitating aggressive therapy.[86] Ectopic expression studies in rodents revealed deregulated cell cycle progression, fibroblast tumor formation after injection into nude mice, and recapitulation of virally induced tumors in transgenic mice, collectively sparking further investigation into Cyclin D1 as a proto-oncogene.[87] Antisense targeting of Cyclin D1 was shown to inhibit growth in cancer cell lines.[88] However, some studies were able to dissociate elevated Cyclin D1 expression from Rb phosphorylation, indicating that these elevations may not be purely the consequence of increased malignant cell proliferation.[89]

Investigations into Cyclin D1 amplification in breast cancer revealed a 3′ truncated mRNA product with a longer half-life relative to the full-length isoform.[90] The truncated cDNA isoform, named Cyclin D1b, was revealed to have a splicing failure event at the 3′ end of exon 4, resulting in exclusion of exon 5 and retaining 150 bases of intron 4.[91,92] Cyclin D1b was frequently associated with an A/G polymorphism located within intron 4 that was associated with decreased event-free survival and greater risk of relapse[93] as well as higher incidence rates in several cancers. Cyclin D1b in cancer has been extensively reviewed elsewhere.[42,94-96] Several drugs have been developed to target Cyclin D1-CDK4 through inhibition of CDK4.[97-101] Studies using forced expression of Cyclin D1 in cancer cell lines revealed resistance to DNA-damaging cancer drugs, implicating new roles of Cyclin D1 in DNA repair.[102,103]

CYCLIN D1 IN THE LIVER

Cyclin D1 is involved in activating hepatocyte proliferation, restoring liver mass after partial hepatectomy. In hepatocytes, Cyclin D1 retains its canonical pathway in cell cycle progression as mentioned above. Hepatocytes account for 70% of the cells in the liver and are normally quiescent. However, upon liver injury, as in the case of liver transplantation or in livers that undergo resection, hepatocytes become primed and enter the cell cycle in efforts to restore liver mass.[20] Increased levels of Cyclin D1 were detected in human liver biopsies following transplantation.[104] *Ccnd1* expression also increased in both mice and rats following 70% partial hepatectomy in efforts to restore liver mass.[104] Cyclin D1 overexpression is sufficient to cause proliferation in hepatocytes both in vitro and in vivo.[105,106] In rat primary hepatocytes stimulated with insulin, the expression of Cyclin D1 coincided with DNA synthesis, confirming the role of Cyclin D1 in hepatocyte cell cycle progression.[107]

Regulation of Lipogenesis and Gluconeogenesis Through Cyclin D1 in the Liver

The liver is the primary site of lipid and glucose metabolism. In recent years, new functions for Cyclin D1 in the liver have emerged, particularly in regulating lipogenesis and gluconeogenesis. Cyclin D1 regulates transcription of genes involved in lipid metabolism and the sensing/processing of carbohydrates and amino acids in mouse hepatocytes.[108] In fasting mice, an overall decrease in both mRNA and protein expression of Cyclin D1 is observed, with levels increasing upon food intake.[109] In primary rat hepatocytes, nonessential amino acid starvation inhibited cell proliferation and resulted in undetectable Cyclin D1; however, overexpression of Cyclin D1 in these starved hepatocytes resulted in proliferation.[27] Additionally, in hepatocytes, minimum essential medium (MEM) amino acids increased *Ccnd1* expression significantly more than nonessential amino acids.[110] These results indicate that nutrient availability can impact the functions of Cyclin D1 in the liver. Nutrient availability is particularly important in donor livers, as time after procurement can dramatically affect nutrient availability which could potentially affect Cyclin D1 levels and have an impact on graft failure. In our laboratory, Cyclin D1 was elevated in the livers of rats with >90% macrosteatosis and resulted in death after partial hepatectomy (unpublished data). The alternatively spliced isoform, Cyclin D1b, was found in nuclear fractions of macrosteatotic livers that showed slow recovery after partial hepatectomy (unpublished data). The exact role of Cyclin D1 in these fatty livers and how it may contribute to liver failure after partial hepatectomy remain under investigation. Further evidence of the importance of Cyclin D1 in liver disease has been validated in animals with increasing steatosis in NAFLD and NASH.[111,112] The drug metformin has been used to treat NAFLD, resulting in inhibition of Cyclin D1 and decreased gluconeogenesis in the liver.[113,114] However, studies of Cyclin D1 in human hepatocytes are lacking, with the majority of experiments having been performed in mice and rats.

Regulation of Lipogenesis by Cyclin D1. Hepatic de novo lipogenesis is the process of generating fatty acids from excess acetyl-coenzyme A (CoA) subunits from the catabo-

lism of carbohydrates.[115] These lipids are stored in hepatocytes and exported as needed via very low density lipoproteins. Glucose is primarily taken up by the liver after feeding, and excess carbohydrates are converted and stored as triglycerides. Glucose is the primary driver for lipogenesis; however, fructose also promotes lipogenesis in the liver as reviewed elsewhere.[116] De novo lipogenesis in hepatocytes is transcriptionally regulated by the carbohydrate response element binding protein (ChREBP).[115,117] ChREBP is phosphorylated and bound to the protein 14-3-3, rendering it inactivate in the cytoplasm. Mediators of glycolysis activate ChREBP via dephosphorylation and dissociation with 14-3-3.[118,119] Once activated, ChREBP interacts with max-like factor x (Mlx) and is translocated to the nucleus to activate transcription of lipogenic genes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC).[38,115,117] Cyclin D1 repressed transcription of ChREBP in a manner that was dependent on the CDK4 binding domain through interactions with the first 492 amino acids of ChREBP.[38] Cyclin D1 also repressed lipogenic gene transcription, particularly FAS and ACC in hepatocytes in the presence of glucose.[38] This repression resulted in decreased lipogenesis and could be beneficial in decreasing fat accumulation in the liver as a treatment for NAFLD when glucose is present. A 2016 report showed that activation of CDK4 induced NAFLD in mice fed a high-fat diet, while inhibiting CDK4 reduced the level of hepatic steatosis in mice fed a high-fat diet.[120] Additionally, elevated levels of CDK4 protein were found in patients with fatty livers.[120] Levels of Cyclin D1 were not evaluated; however, inhibiting CDK4 prevented hepatocyte proliferation,[120] indicating the involvement of Cyclin D1 in conjunction with CDK4.

Peroxisome Proliferator-Activated Receptor Gamma Repression by Cyclin D1. Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-dependent nuclear transcriptional activator that controls fatty acid storage in the liver.[121] Many of the genes activated by PPARγ are involved in lipogenesis.[121] PPARγ is activated by certain ligands such as fatty acids and synthetic ligands from the thiazolidinedione class,[121,122] with activation triggering fatty acid storage. PPARγ is also upregulated in steatotic mice fed a high-fat diet.[123] PPARγ overexpression induces hepatic steatosis in the livers of peroxisome proliferator-activated receptor alpha (PPARα) knockout mice.[124] PPARγ, particularly isoform 2, causes lipid accumulation in hepatocytes, as well as increased expression of ACC and FAS.[125] Additionally, PPARγ-2 activation increases in de novo triacylglycerol synthesis.[125,126] Liver-targeted PPARγ knockout mice are resistant to hepatic steatosis when fed a high-fat diet and have downregulated expression of lipogenesis such as ACC and sterol regulatory element-binding protein-1c.[127] Cyclin D1 has been shown to inhibit PPARγ at residues 143-179 by mechanisms independent of its GSK3β phosphorylation site and CDK4 binding domain.[122] This interaction is likely through its repressor domain. The transcriptional cofactors P300 and CREB interact with PPARγ to enhance transcriptional activity.[128-130] Cyclin D1 represses p300 transactivation at the PPARγ-responsive element.[131] PPARγ was downregulated in fasting mice,[132] and this downregulation is likely independent of Cyclin D1 activity as protein levels of Cyclin D1 also decreased in fasting mice.[109] The drugs rosiglitazone and pioglitazone bind to PPARγ and repress activity and therefore have potential utility in the treatment of NAFLD.[133]

Hepatocyte Nuclear Factor 4 Alpha Repression Through Interactions With Cyclin D1. Cyclin D1 interacts with HNF4α, a master regulator in hepatocytes. HNF4α is required for normal liver development, as HNF4α knockout mice do not express functional levels of hepatocyte-specific gene programs, resulting in incomplete liver maturation.[134] HNF4α also promotes the transcription of the following apolipoproteins: AI, AII, B, CII, and CIII.[134] These proteins form complexes involved in delivering fatty acids into cells. The role of HNF4α in cell proliferation and cancer has been reviewed elsewhere.[135] HNF4α binds to fatty acids; however, evidence suggests this interaction increases HNF4α stability rather than augments transcriptional activity.[136] Cyclin D1, through its repressor domain, interacts with HNF4α to prevent binding to the promoter regions of the lipogenic genes FAS and liver-type pyruvate kinase (Pklr), thereby repressing their transcription in hepatocytes.[38] This observation was reversed in knockdowns of Cyclin D1 in hepatocytes.[38] HNF4α is also involved in the transcription of gluconeogenesis such as glycogen synthase, phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase).[137] These results confirm the involvement of Cyclin D1 in repressing gluconeogenesis in the presence of glucose. Cyclin D1 represses lipogenesis through 2 modes of action: through prevention of PPARγ and through HNF4α inducing lipogenic genes.

Cyclin D1 Repression of Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 Alpha. The hepatic gluconeogenesis regulator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α) interacts with Cyclin D1. PGC1α transcriptionally activates oxidative phosphorylation and regulates carbohydrate metabolism.[138,139] PGC1α also triggers lipogenesis by promoting expression of apolipoproteins AIV, B, CII, and CIII.[140] PGC1α is strongly induced in fasting mice, leading to transcriptional programs promoting gluconeogenesis through G6Pase and PEPCK.[109,141,142] CREB also induces expression of PGC1α in hepatocytes.[142] GSK3β inhibits PGC1α through phosphorylation, targeting PGC1α for proteasomal degradation.[143] GSK3β is also Ras-dependent, as Akt can inactivate GSK3β through phosphorylation.[67] GSK3β is active in fasting animals, but it becomes inactivated upon refeeding through phosphorylation via insulin/Akt signaling,[110] while expression of *Ccnd1* and Cyclin D1 increases after refeeding.[109,110] In the presence of insulin and GSK3β, Cyclin D1 is sequestered in the nucleus.[110] Cyclin D1 represses PGC1α in a manner that is CDK4-dependent.[109] Following Cyclin D1 exportation from the nucleus by GSK3β, PGC1α translocates to the nucleus and activates transcription of G6Pase and PEPCK in the absence of glucose (Figure 1). Because PGC1α regulates its own expression, repression of PGC1α by Cyclin D1 also decreases PGC1α mRNA expression.[109]

In mouse hepatocytes stimulated with insulin, Cyclin D1-CDK4 becomes activated and phosphorylates GCN5.[110] GCN5 is an acetyltransferase and when phosphorylated binds to and acetylates PGC1α (Figure 2), decreasing its promoter binding capabilities and thus decreasing gluconeogenic gene expression through G6Pase and PEPCK repression.[110,144] Lee et al showed that upon refeeding in mice, *Ccnd1* expression increased in the liver.[110] In a study published in 2014, Bhalla et al demonstrated that PEPCK,

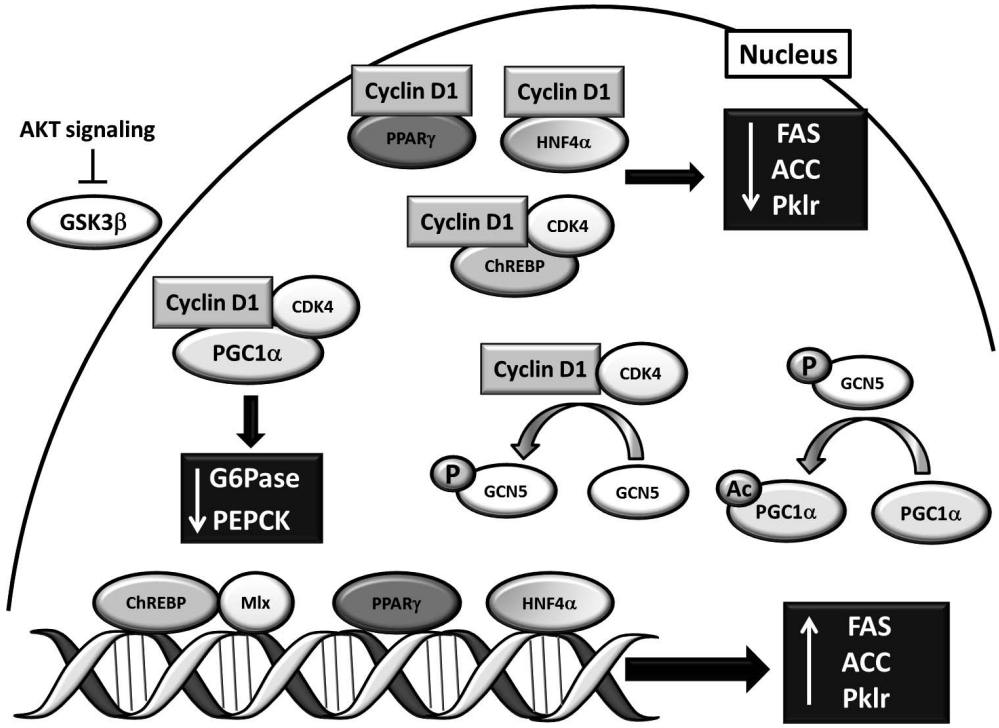

Figure 2. Hepatocytes with glucose. ACC, acetyl-coenzyme A carboxylase; CDK4, cyclin-dependent kinase 4; ChREBP, carbohydrate response element binding protein; FAS, fatty acid synthase; G6Pase, glucose-6-phosphatase; GSK3β, glycogen synthase kinase-3 beta; HNF4α, hepatocyte nuclear factor 4 alpha; Mlx, max-like factor x; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; Pklr, liver-type pyruvate kinase; PPARγ, peroxisome proliferator-activated receptor gamma.

G6Pase, and PGC1α mRNA expression decreased in the livers of refed mice.[109] Chemical inhibition or depletion of CDK4 led to an increase in gluconeogenesis genes (PEPCK and G6Pase) and glucose production in primary hepatocytes,[110] providing evidence that the process is dependent on CDK4. Amino acids, and not insulin, were found to increase *Ccnd1* expression in hepatocytes.[110] Perhaps lower levels of Cyclin D1 may be found in patients with high glucose levels, resulting in increased gluconeogenesis.

Summary. The interaction between PGC1α and HNF4α are required for PEPCK and G6Pase transcription.[137,141] PGC1α and HNF4α both trigger apolipoprotein expression significantly more together than either protein alone.[140] Figure 2 provides an overview of Cyclin D1 in hepatocytes with glucose. Lipogenesis inhibition through PGC1α and ChREBP by Cyclin D1 requires CDK4, while HNF4α inhibition requires the repressor domain located upstream of the CDK4 binding site. Thus, in the presence of glucose and insulin, Cyclin D1 binds to and activates CDK4 in the nucleus. Cyclin D1-CDK4 can then repress PGC1α and through the repressor domain of Cyclin D1, inhibit HNF4α and PPARγ function. As a result, lipogenesis and storage of lipids are inhibited. The question then becomes what turns Cyclin D1-CDK4 to cell cycle progression vs inhibiting lipogenesis? If the cell is trying to regenerate because of liver injury, lipogenesis will be inhibited to convert all processes for cellular division to repair liver tissue. Interestingly, after 70% partial hepatectomy in rats, PPARγ expression increased at 48 hours,[145] while DNA synthesis peaked at 24 hours.[146] Cyclin D1 protein levels increased 24-72 hours after partial hepatectomy in rats,[145] likely revealing that during liver injury, some mechanisms are in place to ensure that hepatocyte proliferation occurs.

Cyclin D1 Activation of Estrogen Receptor and Repression of Androgen Receptor in the Liver

Cyclin D1 controls hormone metabolism in the liver. In cultured hepatocytes, Cyclin D1 is upregulated upon stimulation with epithelial growth factor.[147] Hepatocytes naturally express estrogen receptor alpha (ERα) and androgen receptor. Particularly in the livers of rats, differences in the number of estrogen receptors vary between males and females as well as between prepubescent and adult females.[148] The number of estrogen receptors on the surface of adult female rat livers is estimated to be one-third of the number present in the uterus.[149] For estrogen to activate transcription of genes, estrogen receptors in the livers of adult female rats require high levels of estrogen for nuclear relocationization.[148] In breast cancer cells, Cyclin D1 activates genes containing estrogen receptor elements through estrogen receptors in a manner that is independent of CDK and Rb binding and the presence of estrogen,[39,150,151] as well as independent of phosphorylated ERα.[150] Cyclin D1 directly interacts with ERα and recruits SRC-1 family coactivators to ERα for activation through a leucine-rich motif (Figure 3).[39] Zwijsen et al showed that mutations in the leucine-rich motif abolished direct interactions of Cyclin D1 with SRC-1 and prevented ERα activa-

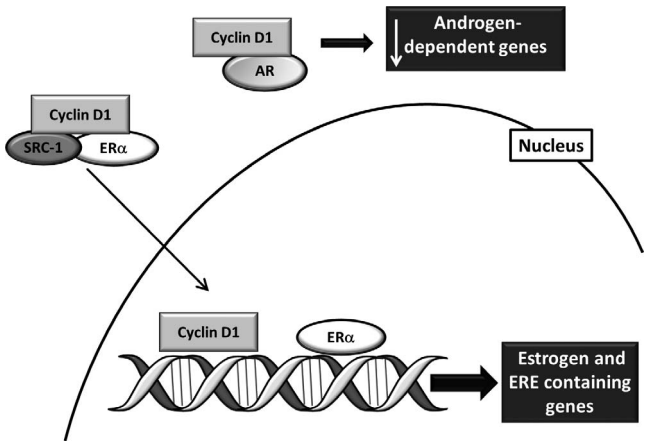

Figure 3. Hormone regulation. AR, androgen receptor**;** ERα, estrogen receptor alpha; ERE, estrogen responsive element; SRC-1, steroid receptor coactivator-1.

tion.[39] Studies conflict on whether antiestrogen compounds affect this interaction, which seems to be cell-type dependent. In the breast cancer cell line T47D, the antiestrogens hydroxytamoxifen and ICI 182780 did not inhibit Cyclin D1 activation of estrogen responsive element (ERE)–containing genes, while sterol carrier protein 2 (SCp2) inhibition was observed.[150,151] The C-terminus is important for Cyclin D1 interaction with ERα, as mutations in amino acids 254 and 255 negate Cyclin D1 interactions with ERα.[39]

Zwijsen et al demonstrated that estrogen could induce proliferation in vitro in hepatocytes.[39] After a partial hepatectomy, antiestrogens have been shown to reduce the number of ERα and proliferation of hepatocytes.[152] Additionally, arsenic-induced hepatocellular carcinoma in mice induced both Cyclin D1 and ERα expression.[153] In the male mouse liver, hepatic Cyclin D1 expression was linked to increases in serum estradiol levels, estrogen-dependent gene expression, and decreased androgen-dependent gene expression.[154] Overexpression of either Cyclin D1 or Cyclin D1b led to the downregulation of steroid 5 β-reductase,[154] an enzyme that converts testosterone into androgen and dihydrotestosterone. Interestingly, overexpression of Cyclin D1b induced the expression of 3β-hydroxysteroid dehydrogenase type 2, a protein involved in steroidogenesis, while Cyclin D1 did not.[154]

Cyclin D1 is also involved in androgen receptor inhibition.[155] Cyclin D1 contains a repressor domain between residues 142-253 that is required for interactions with androgen receptor[37] in the human prostate adenocarcinoma cell line LNCaP. Additionally, the Cyclin D1b isoform reduced androgen receptor regulation, although this isoform retained the repressor domain and the ability to recruit HDACs.[156] Less work has been done looking into the role of Cyclin D1 and sex hormones in hepatocytes. Cyclin D1 may be involved in enhancing estrogen-dependent genes while decreasing androgen-dependent genes. Estrogen has been suggested to play a protective role in the liver by inhibiting apoptosis after ischemia/reperfusion injury.[157] Furthermore, hepatic androgen receptor knockout male mice developed steatosis, while the females did not when fed a high-fat diet.[158] Additionally, men with liver diseases who underwent liver resection developed feminization syndrome,[159] which may be linked to increased Cyclin D1 and estrogen-dependent genes. Although aberrant expression of Cyclin D1 could increase estrogen-dependent gene expression, implications for the development and progression of steatosis remain to be investigated. However, these studies strongly suggest Cyclin D1 expression can influence hormone levels and hormone signaling pathways in the liver.

CONCLUSION

Cyclin D1 has many diverse functions in the liver. Aside from the canonical pathway in cell proliferation, Cyclin D1 is involved in the repression of lipogenesis and gluconeogenesis through the interactions of multiple groups of proteins. Extended criteria donors with hepatic steatosis are on the rise, along with an increase in the number of patients on the wait list. Current methods to improve this imbalance include decreasing hepatic steatosis or preventing damage during ischemia/reperfusion. Reversing hepatic steatosis in patients can improve the quality of the donor liver pool and increase the number of livers used for transplantation. Cyclin D1 may prove to be an excellent target to reduce lipogenesis in fatty livers through its repression of PPARγ and HNF4α in the induction of lipogenic genes. Because of the functions of Cyclin D1 in hepatic proliferation, targeting Cyclin D1 could also improve liver regeneration after liver transplantation. Further studies are necessary to determine how Cyclin D1 functions in steatotic livers.

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REFERENCES

1. Acorn CJ, Millar CC, Hoffman SK, Lyle WM. Viral hepatitis: a review. *Optom Vis Sci*. 1995 Oct;72(10):763-769.
2. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol*. 2006;1:23-61.
3. Memon MI, Memon MA. Hepatitis C: an epidemiological review. *J Viral Hepat*. 2002 Mar;9(2):84-100.
4. Siegl G. Hepatitis A virus infection. A review [in German]. *Praxis (Bern 1994)*. 2003 Oct 1;92(40):1659-1673.
5. Dienstag JL. Hepatitis B virus infection. *N Engl J Med*. 2008 Oct 2;359(14):1486-1500. doi: 10.1056/NEJMra0801644.
6. Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. *Nature*. 2005 Aug 18;436:933-938.
7. Poole LG, Arteel GE. Transitional remodeling of the hepatic extracellular matrix in alcohol-induced liver injury. *Biomed Res Int*. 2016;2016:3162670.
8. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology*. 2011 Nov;141(5): 1572-1585. doi: 10.1053/j.gastro.2011.09.002.
9. Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol*. 2016 Aug;65(2): 425-443. doi: 10.1016/j.jhep.2016.04.005.
10. Katsiki N, Mikhailidis DP, Mantzoros CS. Non-alcoholic fatty liver disease and dyslipidemia: An update. *Metabolism*. 2016 Aug;65(8):1109-1123. doi: 10.1016/j.metabol.2016.05.003.
11. Gastaca M. Extended criteria donors in liver transplantation: adapting donor quality and recipient. *Transplant Proc*. 2009 Apr;41(3):975-979.
12. Chu MJ, Dare AJ, Phillips AR, Bartlett AS. Donor hepatic steatosis and outcome after liver transplantation: a systematic review. *J Gastrointest Surg*. 2015 Sep;19(9):1713-1724. doi: 10.1007/s11605-015-2832-1.

13. Renz JF, Kin C, Kinkhabwala M, et al. Utilization of extended donor criteria liver allografts maximizes donor use and patient access to liver transplantation. *Ann Surg*. 2005 Oct;242(4): 556-563; discussion 563-565.
14. Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation–from bench to bedside. *Nat Rev Gastroenterol Hepatol*. 2013 Feb; 10(2):79-89. doi: 10.1038/nrgastro.2012.225.
15. Fausto N. Liver regeneration. *J Hepatol*. 2000;32(1 Suppl): 19-31.
16. Fausto N. Liver regeneration: from laboratory to clinic. *Liver Transpl*. 2001 Oct;7(10):835-844.
17. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology*. 2004 Jun;39(6): 1477-1487.
18. Fausto N. Involvement of the innate immune system in liver regeneration and injury. *J Hepatol*. 2006 Sep;45(3):347-349.
19. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev*. 2003 Jan; 120(1):117-130.
20. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology*. 2006 Feb;43(2 Suppl 1):S45-S53.
21. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *J Hepatol*. 2012 Sep;57(3):692-694. doi: 10.1016/j.jhep.2012.04.016.
22. Fausto N, Laird AD, Webber EM. Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. *FASEB J*. 1995 Dec;9(15):1527-1536.
23. Fujita M, Furukawa H, Hattori M, Todo S, Ishida Y, Nagashima K. Sequential observation of liver cell regeneration after massive hepatic necrosis in auxiliary partial orthotopic liver transplantation. *Mod Pathol*. 2000 Feb;13(2):152-157.
24. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science*. 2011 Jun 24; 332(6037):1519-1523. doi: 10.1126/science.1204265.
25. Zhong Z, Schwabe RF, Kai Y, et al. Liver regeneration is suppressed in small-for-size liver grafts after transplantation: involvement of c-Jun N-terminal kinase, cyclin D1, and defective energy supply. *Transplantation*. 2006 Jul 27;82(2): 241-250.
26. Kato A, Bamba H, Shinohara M, et al. Relationship between expression of cyclin D1 and impaired liver regeneration observed in fibrotic or cirrhotic rats. *J Gastroenterol Hepatol*. 2005 Aug;20(8):1198-1205.
27. Nelsen CJ, Rickheim DG, Tucker MM, et al. Amino acids regulate hepatocyte proliferation through modulation of cyclin D1 expression. *J Biol Chem*. 2003 Jul 11;278(28): 25853-25858.
28. Coqueret O. Linking cyclins to transcriptional control. *Gene*. 2002 Oct 16;299(1-2):35-55.
29. Motokura T, Arnold A. PRAD1/cyclin D1 proto-oncogene: genomic organization, 5′ DNA sequence, and sequence of a tumor-specific rearrangement breakpoint. *Genes Chromosomes Cancer*. 1993 Jun;7(2):89-95.
30. Arnold A, Kim HG, Gaz RD, et al. Molecular cloning and chromosomal mapping of DNA rearranged with the parathyroid hormone gene in a parathyroid adenoma. *J Clin Invest*. 1989 Jun;83(6):2034-2040.
31. 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 Aug 8;278(32): 30339-30347.
32. Landis MW, Brown NE, Baker GL, et al. The LxCxE pRb interaction domain of cyclin D1 is dispensable for murine development. *Cancer Res*. 2007 Aug 15;67(16):7613-7620.
33. 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 Feb;8(2): 287-301.
34. Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A, Weinberg RA. Physical interaction of the retinoblastoma protein with human D cyclins. *Cell*. 1993 May 7;73(3):499-511.
35. 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 May 7;73(3): 487-497.
36. Baker GL, Landis MW, Hinds PW. Multiple functions of D-type cyclins can antagonize pRb-mediated suppression of proliferation. *Cell Cycle*. 2005 Feb;4(2):330-338.
37. Petre-Draviam CE, Williams EB, Burd CJ, et al. A central domain of cyclin D1 mediates nuclear receptor corepressor activity. *Oncogene*. 2005 Jan 13;24(3):431-444.
38. Hanse EA, Mashek DG, Becker JR, et al. Cyclin D1 inhibits hepatic lipogenesis via repression of carbohydrate response element binding protein and hepatocyte nuclear factor 4α. *Cell Cycle*. 2012 Jul 15;11(14):2681-2690. doi: 10.4161/cc. 21019.
39. Zwijsen RM, Buckle RS, Hijmans EM, Loomans CJ, Bernards R. Ligand-independent recruitment of steroid receptor coactivators to estrogen receptor by cyclin D1. *Genes Dev*. 1998 Nov 15;12(22):3488-3498.
40. Rechsteiner M. PEST sequences are signals for rapid intracellular proteolysis. *Semin Cell Biol*. 1990 Dec;1(6):433-440.
41. Fu M, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: normal and abnormal functions. *Endocrinology*. 2004 Dec; 145(12):5439-5447.
42. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. *Nat Rev Cancer*. 2011 Jul 7;11(8):558-572. doi: 10.1038/nrc3090.
43. Sherr CJ. Mammalian G1 cyclins and cell cycle progression. *Proc Assoc Am Physicians*. 1995 Jul;107(2):181-186.
44. Sherr CJ. Cancer cell cycles. *Science*. 1996 Dec 6;274(5293): 1672-1677.
45. 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 Oct 16;71(2): 323-334.
46. Matsushime H, Roussel MF, Ashmun RA, Sherr CJ. Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell*. 1991 May 17;65(4):701-713.
47. Morgan DO. Principles of CDK regulation. *Nature*. 1995 Mar 9; 374(6518):131-134.
48. Morgan DO. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol*. 1997;13:261-291.
49. Motokura T, Bloom T, Kim HG, et al. A novel cyclin encoded by a bcl1-linked candidate oncogene. *Nature*. 1991 Apr 11; 350(6318):512-515.
50. Xiong Y, Connolly T, Futcher B, Beach D. Human D-type cyclin. *Cell*. 1991 May 17;65(4):691-699.
51. Lukas J, Bartkova J, Rohde M, Strauss M, Bartek J. Cyclin D1 is dispensable for G1 control in retinoblastoma gene-deficient cells independently of cdk4 activity. *Mol Cell Biol*. 1995 May; 15(5):2600-2611.
52. Lukas J, Müller H, Bartkova J, et al. DNA tumor virus oncoproteins and retinoblastoma gene mutations share the ability to relieve the cell's requirement for cyclin D1 function in G1. *J Cell Biol*. 1994 May;125(3):625-638.
53. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. *Cell*. 1999 Sep 17;98(6):859-869.
54. Mittnacht S. Control of pRB phosphorylation. *Curr Opin Genet Dev*. 1998 Feb;8(1):21-27.
55. Dyson N. The regulation of E2F by pRB-family proteins. *Genes Dev*. 1998 Aug 1;12(15):2245-2262.
56. Bremner R, Cohen BL, Sopta M, et al. Direct transcriptional repression by pRB and its reversal by specific cyclins. *Mol Cell Biol*. 1995 Jun;15(6):3256-3265.

57. Strobeck MW, Knudsen KE, Fribourg AF, et al. BRG-1 is required for RB-mediated cell cycle arrest. *Proc Natl Acad Sci U S A*. 2000 Jul 5:97(14):7748-7753.
58. Weintraub SJ, Chow KN, Luo RX, Zhang SH, He S, Dean DC. Mechanism of active transcriptional repression by the retinoblastoma protein. *Nature*. 1995 Jun 29;375(6534): 812-815.
59. Weintraub SJ, Prater CA, Dean DC. Retinoblastoma protein switches the E2F site from positive to negative element. *Nature*. 1992 Jul 16;358(6383):259-261.
60. Zhang HS, Gavin M, Dahiya A, et al. Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. *Cell*. 2000 Mar 31;101(1):79-89.
61. Watanabe G, Albanese C, Lee RJ, et al. Inhibition of cyclin D1 kinase activity is associated with E2F-mediated inhibition of cyclin D1 promoter activity through E2F and Sp1. *Mol Cell Biol*. 1998 Jun;18(6):3212-3222.
62. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev*. 1999 Jun 15; 13(12):1501-1512.
63. Pestell RG. New roles of cyclin D1. *Am J Pathol*. 2013 Jul; 183(1):3-9. doi: 10.1016/j.ajpath.2013.03.001.
64. Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. *Genes Dev*. 1993 May;7(5):812-821.
65. 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 Dec 15;14(24):3102-3114.
66. Diehl JA, Sherr CJ. A dominant-negative cyclin D1 mutant prevents nuclear import of cyclin-dependent kinase 4 (CDK4) and its phosphorylation by CDK-activating kinase. *Mol Cell Biol*. 1997 Dec;17(12):7362-7374.
67. Diehl JA, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev*. 1998 Nov 15;12(22):3499-3511.
68. Aktas H, Cai H, Cooper GM. Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the Cdk inhibitor p27KIP1. *Mol Cell Biol*. 1997 Jul;17(7):3850-3857.
69. Albanese C, Johnson J, Watanabe G, et al. Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. *J Biol Chem*. 1995 Oct 6; 270(40):23589-23597.
70. Cheng M, Sexl V, Sherr CJ, Roussel MF. Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). *Proc Natl Acad Sci U S A*. 1998 Feb 3;95(3):1091-1096.
71. Kerkhoff E, Rapp UR. Induction of cell proliferation in quiescent NIH 3T3 cells by oncogenic c-Raf-1. *Mol Cell Biol*. 1997 May;17(5):2576-2586.
72. Weber JD, Raben DM, Phillips PJ, Baldassare JJ. Sustained activation of extracellular-signal-regulated kinase 1 (ERK1) is required for the continued expression of cyclin D1 in G1 phase. *Biochem J*. 1997 Aug 15;326 (Pt 1):61-68.
73. Winston JT, Coats SR, Wang YZ, Pledger WJ. Regulation of the cell cycle machinery by oncogenic ras. *Oncogene*. 1996 Jan 4; 12(1):127-134.
74. Filmus J, Robles AI, Shi W, Wong MJ, Colombo LL, Conti CJ. Induction of cyclin D1 overexpression by activated ras. *Oncogene*. 1994 Dec;9(12):3627-3633.
75. Bakiri L, Lallemand D, Bossy-Wetzel E, Yaniv M. Cell cycle-dependent variations in c-Jun and JunB phosphorylation: a role in the control of cyclin D1 expression. *EMBO J*. 2000 May 2;19(9):2056-2068.
76. Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. *Cell*. 1999 Aug 6;98(3):295-303.
77. Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol*. 1999 Aug; 19(8):5785-5799.
78. Lee RJ, Albanese C, Stenger R, et al. pp60(v-src) induction of cyclin D1 requires collaborative interactions between the extracellular signal-regulated kinase, p38, and Jun kinase pathways. A role for cAMP response element-binding protein and activating transcription factor-2 in pp60(v-src) signaling in breast cancer cells. *J Biol Chem*. 1999 Mar 12;274(11): 7341-7350.
79. Matsumura I, Kitamura T, Wakao H, et al. Transcriptional regulation of the cyclin D1 promoter by STAT5: its involvement in cytokine-dependent growth of hematopoietic cells. *EMBO J*. 1999 Mar 1;18(5):1367-1377.
80. Shtutman M, Zhurinsky J, Simcha I, et al. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proc Natl Acad Sci U S A*. 1999 May 11;96(10):5522-5527.
81. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature*. 1999 Apr 1; 398(6726):422-426.
82. Yan YX, Nakagawa H, Lee MH, Rustgi AK. Transforming growth factor-alpha enhances cyclin D1 transcription through the binding of early growth response protein to a cis-regulatory element in the cyclin D1 promoter. *J Biol Chem*. 1997 Dec 26; 272(52):33181-33190.
83. Langenfeld J, Kiyokawa H, Sekula D, Boyle J, Dmitrovsky E. Posttranslational regulation of cyclin D1 by retinoic acid: a chemoprevention mechanism. *Proc Natl Acad Sci U S A*. 1997 Oct 28;94(22):12070-12074.
84. Beroukhim R, Mermel CH, Porter D, et al. The landscape of somatic copy-number alteration across human cancers. *Nature*. 2010 Feb 18;463(7283):899-905. doi: 10.1038/ nature08822.
85. Musgrove EA, Hui R, Sweeney KJ, Watts CK, Sutherland RL. Cyclins and breast cancer. *J Mammary Gland Biol Neoplasia*. 1996 Apr;1(2):153-162.
86. McIntosh GG, Anderson JJ, Milton I, et al. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. *Oncogene*. 1995 Sep 7;11(5):885-891.
87. Jiang W, Kahn SM, Zhou P, et al. Overexpression of cyclin D1 in rat fibroblasts causes abnormalities in growth control, cell cycle progression and gene expression. *Oncogene*. 1993 Dec: 8(12):3447-3457.
88. Zhou P, Jiang W, Zhang YJ, et al. Antisense to cyclin D1 inhibits growth and reverses the transformed phenotype of human esophageal cancer cells. *Oncogene*. 1995 Aug 3;11(3): 571-580.
89. Sgambato A, Han EK, Zhang YJ, Moon RC, Santella RM, Weinstein IB. Deregulated expression of cyclin D1 and other cell cycle-related genes in carcinogen-induced rat mammary tumors. *Carcinogenesis*. 1995 Sep;16(9):2193-2198.
90. Lebwohl DE, Muise-Helmericks R, Sepp-Lorenzino L, et al. A truncated cyclin D1 gene encodes a stable mRNA in a human breast cancer cell line. *Oncogene*. 1994 Jul;9(7):1925-1929.
91. Hosokawa Y, Gadd M, Smith AP, Koerner FC, Schmidt EV, Arnold A. Cyclin D1 (PRAD1) alternative transcript b: full-length cDNA cloning and expression in breast cancers. *Cancer Lett*. 1997 Feb 26;113(1-2):123-130.
92. Hosokawa Y, Tu T, Tahara H, Smith AP, Arnold A. Absence of cyclin D1/PRAD1 point mutations in human breast cancers and parathyroid adenomas and identification of a new cyclin D1 gene polymorphism. *Cancer Lett*. 1995 Jul 13;93(2): 165-170.
93. Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. *Oncogene*. 1995 Sep 7;11(5):1005-1011.

94. Knudsen KE, Diehl JA, Haiman CA, Knudsen ES. Cyclin D1: polymorphism, aberrant splicing and cancer risk. *Oncogene*. 2006 Mar 13;25(11):1620-1628.
95. Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Mol Cancer*. 2007 Apr 2;6:24.
96. Kim JK, Diehl JA. Nuclear cyclin D1: an oncogenic driver in human cancer. *J Cell Physiol*. 2009 Aug;220(2):292-296. doi: 10.1002/jcp.21791.
97. DePinto W, Chu XJ, Yin X, et al. In vitro and in vivo activity of R547: a potent and selective cyclin-dependent kinase inhibitor currently in phase I clinical trials. *Mol Cancer Ther*. 2006 Nov;5(11):2644-2658.
98. 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 Nov;3(11):1427-1438.
99. 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 Sep 1;108(5):1744-1750.
100. Siemeister G, Luecking U, Wagner C, Detjen K, Mc Coy C, Bosslet K. Molecular and pharmacodynamic characteristics of the novel multi-target tumor growth inhibitor ZK 304709. *Biomed Pharmacother*. 2006 Jul;60(6):269-272.
101. Scott EN, Thomas AL, Molife LR, et al. A phase I dose escalation study of the pharmacokinetics and tolerability of ZK 304709, an oral multi-targeted growth inhibitor (MTGI), in patients with advanced solid tumours. *Cancer Chemother Pharmacol*. 2009 Jul;64(2):425-429. doi: 10.1007/s00280-009-0968-y.
102. Hosokawa Y, Arnold A. Mechanism of cyclin D1 (CCND1, PRAD1) overexpression in human cancer cells: analysis of allele-specific expression. *Genes Chromosomes Cancer*. 1998 May;22(1):66-71.
103. Jirawatnotai S, Hu Y, Michowski W, et al. A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. *Nature*. 2011 Jun 8;474(7350):230-234. doi: 10.1038/nature10155.
104. Albrecht JH, Hu MY, Cerra FB. Distinct patterns of cyclin D1 regulation in models of liver regeneration and human liver. *Biochem Biophys Res Commun*. 1995 Apr 17;209(2):648-655.
105. Albrecht JH, Hansen LK. Cyclin D1 promotes mitogen-independent cell cycle progression in hepatocytes. *Cell Growth Differ*. 1999 Jun;10(6):397-404.
106. Nelsen CJ, Rickheim DG, Timchenko NA, Stanley MW, Albrecht JH. Transient expression of cyclin D1 is sufficient to promote hepatocyte replication and liver growth in vivo. *Cancer Res*. 2001 Dec 1;61(23):8564-8568.
107. Rickheim DG, Nelsen CJ, Fassett JT, Timchenko NA, Hansen LK, Albrecht JH. Differential regulation of cyclins D1 and D3 in hepatocyte proliferation. *Hepatology*. 2002 Jul;36(1):30-38.
108. Mullany LK, White P, Hanse EA, et al. Distinct proliferative and transcriptional effects of the D-type cyclins in vivo. *Cell Cycle*. 2008 Jul 15;7(14):2215-2224.
109. Bhalla K, Lui WJ, Thompson K, et al. Cyclin D1 represses gluconeogenesis via inhibition of the transcriptional coactivator PGC1α. *Diabetes*. 2014 Oct;63(10):3266-3278. doi: 10.2337/db13-1283.
110. Lee Y, Dominy JE, Choi YJ, et al. Cyclin D1-Cdk4 controls glucose metabolism independently of cell cycle progression. *Nature*. 2014 Jun 26;510(7506):547-551. doi: 10.1038/nature13267.
111. Dorn C, Engelmann JC, Saugspier M, et al. Increased expression of c-Jun in nonalcoholic fatty liver disease. *Lab Invest*. 2014 Apr;94(4):394-408. doi: 10.1038/labinvest.2014.3.
112. Wang Y, Ausman LM, Greenberg AS, Russell RM, Wang XD. Nonalcoholic steatohepatitis induced by a high-fat diet promotes diethylnitrosamine-initiated early hepatocarcinogenesis in rats. *Int J Cancer*. 2009 Feb 1;124(3):540-546. doi: 10.1002/ijc.23995.
113. Mazza A, Fruci B, Garinis GA, Giuliano S, Malaguarnera R, Belfiore A. The role of metformin in the management of NAFLD. *Exp Diabetes Res*. 2012;2012:716404. doi: 10.1155/2012/716404.
114. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1995 Aug 31;333(9):550-554.
115. Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. *Biol Rev Camb Philos Soc*. 2016 May;91(2):452-468. doi: 10.1111/brv.12178.
116. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: a highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis, and the metabolic syndrome. *Am J Physiol Endocrinol Metab*. 2010 Nov;299(5):E685-E694. doi: 10.1152/ajpendo.00283.2010.
117. Ma L, Robinson LN, Towle HC. ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. *J Biol Chem*. 2006 Sep 29;281(39):28721-28730.
118. Dentin R, Tomas-Cobos L, Foufelle F, et al. Glucose 6-phosphate, rather than xylulose 5-phosphate, is required for the activation of ChREBP in response to glucose in the liver. *J Hepatol*. 2012 Jan;56(1):199-209. doi: 10.1016/j.jhep.2011.07.019.
119. Arden C, Tudhope SJ, Petrie JL, et al. Fructose 2,6-bisphosphate is essential for glucose-regulated gene transcription of glucose-6-phosphatase and other ChREBP target genes in hepatocytes. *Biochem J*. 2012 Apr 1;443(1):111-123. doi: 10.1042/BJ20111280.
120. Jin J, Valanejad L, Nguyen TP, et al. Activation of CDK4 triggers development of non-alcoholic fatty liver disease. *Cell Rep*. 2016 Jul 19;16(3):744-756. doi: 10.1016/j.celrep.2016.06.019.
121. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. *Nature*. 2000 May 25;405(6785):421-424.
122. Wang C, Pattabiraman N, Zhou JN, et al. Cyclin D1 repression of peroxisome proliferator-activated receptor gamma expression and transactivation. *Mol Cell Biol*. 2003 Sep;23(17):6159-6173.
123. Inoue M, Ohtake T, Motomura W, et al. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. *Biochem Biophys Res Commun*. 2005 Oct 14;336(1):215-222.
124. Yu S, Matsusue K, Kashireddy P, et al. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. *J Biol Chem*. 2003 Jan 3;278(1):498-505.
125. Schadinger SE, Bucher NL, Schreiber BM, Farmer SR. PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. *Am J Physiol Endocrinol Metab*. 2005 Jun;288(6):E1195-E1205.
126. Gavrilova O, Haluzik M, Matsusue K, et al. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J Biol Chem*. 2003 Sep 5;278(36):34268-34276.
127. Morán-Salvador E, López-Parra M, García-Alonso V, et al. Role for PPARγ in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. *FASEB J*. 2011 Aug;25(8):2538-2550. doi: 10.1096/fj.10-173716.
128. Takahashi N, Kawada T, Yamamoto T, et al. Overexpression and ribozyme-mediated targeting of transcriptional coactivators CREB-binding protein and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of peroxisome proliferator-activated receptor gamma. *J Biol Chem*. 2002 May 10;277(19):16906-16912.
129. Mizukami J, Taniguchi T. The antidiabetic agent thiazolidinedione stimulates the interaction between PPAR gamma and CBP. *Biochem Biophys Res Commun*. 1997 Nov 7;240(1);61-64.

130. Gelman L, Zhou G, Fajas L, Raspé E, Fruchart JC, Auwerx J. p300 interacts with the N- and C-terminal part of PPARgamma2 in a ligand-independent and -dependent manner, respectively. *J Biol Chem*. 1999 Mar 19;274(12):7681-7688.
131. Fu M, Wang C, Rao M, et al. Cyclin D1 represses p300 transactivation through a cyclin-dependent kinase-independent mechanism. *J Biol Chem*. 2005 Aug 19;280(33): 29728-29742.
132. Vidal-Puig A, Jimenez-Liñan M, Lowell BB, et al. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J Clin Invest*. 1996 Jun 1;97(11):2553-2561.
133. Harrison SA. New treatments for nonalcoholic fatty liver disease. *Curr Gastroenterol Rep*. 2006 Feb;8(1):21-29.
134. Li J, Ning G, Duncan SA. Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev*. 2000 Feb 15;14(4):464-474.
135. Walesky C, Apte U. Role of hepatocyte nuclear factor 4α (HNF4α) in cell proliferation and cancer. *Gene Expr*. 2015;16(3): 101-108. doi: 10.3727/105221615X14181438356292.
136. Yuan X, Ta TC, Lin M, et al. Identification of an endogenous ligand bound to a native orphan nuclear receptor. *PLoS One*. 2009;4(5):e5609. doi: 10.1371/journal.pone.0005609.
137. Parviz F, Matullo C, Garrison WD, et al. Hepatocyte nuclear factor 4alpha controls the development of a hepatic epithelium and liver morphogenesis. *Nat Genet*. 2003 Jul;34(3):292-296.
138. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*. 1999 Jul 9;98(1):115-124.
139. Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. *Adv Physiol Educ*. 2006 Dec;30(4):145-151.
140. Rhee J, Ge H, Yang W, et al. Partnership of PGC-1alpha and HNF4alpha in the regulation of lipoprotein metabolism. *J Biol Chem*. 2006 May 26;281(21):14683-14690.
141. Yoon JC, Puigserver P, Chen G, et al. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature*. 2001 Sep 13;413(6852):131-138.
142. Herzig S, Long F, Jhala US, et al. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature*. 2001 Sep 13;413(6852):179-183.
143. Anderson RM, Barger JL, Edwards MG, et al. Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. *Aging Cell*. 2008 Jan;7(1):101-111.
144. Lerin C, Rodgers JT, Kalume DE, Kim SH, Pandey A, Puigserver P. GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1alpha. *Cell Metab*. 2006 Jun;3(6):429-438.
145. Yamamoto Y, Ono T, Dhar DK, et al. Role of peroxisome proliferator-activated receptor-gamma (PPARgamma) during liver regeneration in rats. *J Gastroenterol Hepatol*. 2008 Jun; 23(6):930-937. doi: 10.1111/j.1440-1746.2008.05370.x.
146. Weglarz TC, Sandgren EP. Timing of hepatocyte entry into DNA synthesis after partial hepatectomy is cell autonomous. *Proc Natl Acad Sci U S A*. 2000 Nov 7;97:12595-12600.
147. Loyer P, Cariou S, Glaise D, et al. Growth factor dependence of progression through G1 and S phases of adult rat hepatocytes in vitro. Evidence of a mitogen restriction point in mid-late G1. *J Biol Chem*. 1996 May 10;271(19):11484-11492.
148. Eisenfeld AJ, Aten RF. Estrogen receptors and androgen receptors in the mammalian liver. *J Steroid Biochem*. 1987; 27(4-6):1109-1118.
149. Eisenfeld AJ, Aten R, Weinberger M, Haselbacher G, Halpern K, Krakoff L. Estrogen receptor in the mammalian liver. *Science*. 1976 Feb 27;191(4229):862-865. doi: 10.1126/science.175442.
150. Zwijsen RM, Wientjens E, Klompmaker R, van der Sman J, Bernards R, Michalides RJ. CDK-independent activation of estrogen receptor by cyclin D1. *Cell*. 1997 Feb 7;88(3):405-415.
151. Neuman E, Ladha MH, Lin N, et al. Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. *Mol Cell Biol*. 1997 Sep;17(9):5338-5347.
152. Francavilla A, Polimeno L, DiLeo A, et al. The effect of estrogen and tamoxifen on hepatocyte proliferation in vivo and in vitro. *Hepatology*. 1989 Apr;9(4):614-620.
153. Waalkes MP, Liu J, Chen H, et al. Estrogen signaling in livers of male mice with hepatocellular carcinoma induced by exposure to arsenic in utero. *J Natl Cancer Inst*. 2004 Mar 17; 96(6):466-474.
154. Mullany LK, Hanse EA, Roman A, et al. Cyclin D1 regulates hepatic estrogen and androgen metabolism. *Am J Physiol Gastrointest Liver Physiol*. 2010 Jun;298(6):G884-G895. doi: 10. 1152/ajpgi.00471.2009.
155. Burd CJ, Petre CE, Moghadam H, Wilson EM, Knudsen KE. Cyclin D1 binding to the androgen receptor (AR) NH2-terminal domain inhibits activation function 2 association and reveals dual roles for AR corepression. *Mol Endocrinol*. 2005 Mar;19(3):607-620.
156. Burd CJ, Petre CE, Morey LM, et al. Cyclin D1b variant influences prostate cancer growth through aberrant androgen receptor regulation. *Proc Natl Acad Sci U S A*. 2006 Feb 14;103(7):2190-2195.
157. Lin FS, Shen SQ, Chen ZB, Yan RC. 17β-estradiol attenuates reduced-size hepatic ischemia/reperfusion injury by inhibition apoptosis via mitochondrial pathway in rats. *Shock*. 2012 Feb; 37(2):183-190. doi: 10.1097/SHK.0b013e31823f1918.
158. Lin HY, Yu IC, Wang RS, et al. Increased hepatic steatosis and insulin resistance in mice lacking hepatic androgen receptor. *Hepatology*. 2008 Jun;47(6):1924-1935. doi: 10.1002/hep. 22252.
159. Francavilla A, Gavaler JS, Makowka L, et al. Estradiol and testosterone levels in patients undergoing partial hepatectomy. A possible signal for hepatic regeneration? *Dig Dis Sci*. 1989 Jun;34:818-822.

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