Review

D-type Cyclins in Development and Disease

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Posted Date: 25 June 2023

doi: 10.20944/preprints202306.1703.v1

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Review

D-type Cyclins in Development and Disease

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Abstract: D-type cyclins encode G1/S cell cycle checkpoint proteins, that play a crucial role in defining cell cycle exit and progression. Precise control of cell cycle exit is vital during embryonic development, with defects in the pathways regulating intracellular D-type cyclins resulting in abnormal initiation of stem cell differentiation in a variety of different organ systems. Furthermore, stabilisation of D-type cyclins is observed in a wide range of disorders characterised by cellular over-proliferation, including cancers and overgrowth disorders. In this review, we will summarise and compare the roles played by each D-type cyclin during development and provide examples of how their intracellular dysregulation can be an underlying cause of disease.

Keywords: Cyclin D1; Cyclin D2; Cyclin D3; CDK4; cell cycle; proliferation; cancer; overgrowth

1. Introduction

Progression through the cell cycle is tightly controlled by Cyclin-dependent kinases (CDKs) and their regulatory partners, cyclins [1]. CDKs are activated by binding to their respective Cyclin partner, followed by an activating phosphorylation by a CDK-activating kinase (CAK) [2]. Transition into each phase of the cell cycle is controlled by the kinase activity of a specific cyclin-CDK complex. The 'S-phase' of the cell cycle initiates DNA replication necessary for the forthcoming division [3]. Cyclin D forms active complexes with CDK4 and CDK6 (CDK4/6), which drives the G_1-S phase transition by phosphorylating tumour suppressor 'pocket proteins' Retinoblastoma (Rb), p107 and p130. Phosphorylated pocket proteins are then hyper-phosphorylated by succeeding cyclin E-CDK2 complexes, causing them to dissociate from, and release their E2F transcription factor binding partners, initiating the transcription of several key proteins required to advance the cell cycle and promote cell proliferation [4,5]. Dysfunction of these cell cycle regulators leads to uncontrolled cell proliferation, and genomic instability, contributing to tumorigenesis and overgrowth disorders [1,6–8].

Cyclin D-CDK4/6 also advances the cell cycle by binding to and sequestering the CDK-inhibitors (CKI) p21 and p27, which inhibits succeeding cyclin E-CDK2 complexes [9]. Outside of the Rb family, cyclin D-CDK 4/6 also phosphorylates Smad3, a transcription factor part of the TGF-ß signalling pathway and FOXM1, a transcription factor implicated in promoting cell proliferation and tumorigenesis. Phosphorylation of Smad3 at multiple sites by CDK4 inhibits its anti-proliferative response [10]. Phosphorylation of FOXM1 by CDK4/6 increases its stability, preventing cellular senescence in cancer cells, as well as promoting G_1/S cell cycle entry [11,12].

D-type cyclins D1, D2 and D3 are encoded by separate genes on three chromosomes (CCND1: 11q13.3, CCND2: 12p12.23, CCND3: 6p21.1) [13] and display high homology (~ 57% sequence identity in coding sequence) [1,14]. They can be expressed individually or in combination, typically with one D-type cyclin dominant in progressing the cell cycle in a particular tissue type [14,15]. While functionally interchangeable, loss of one or two D-type cyclins results in focused abnormalities and premature mortality in mice. Highly specialised tissues require a specific D-type cyclin [15]. Therefore, the requirement and dominance of a specific D-type Cyclin in a particular tissue type is likely thought to be a result of extant transcription factors rather than the intrinsic physical properties of the cyclin [15]. Conversely, mice deficient in all three D-type cyclins die in mid-to-late gestation from heart abnormalities, and severe anaemia, suggesting that cyclin D is required for the expansion of haematopoietic stem cells during development [16].

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D-type Cyclins serve as key endpoints of mitogenic signalling, acting as important growth factor sensors. Their transcription and activation are heavily dependent on receiving and integrating mitogenic signals from the Ras/Raf/MAPK (cyclin D synthesis), PI3K-Akt (cyclin D stability) and ß-catenin/TCF-LEF pathways [17–19]. Furthermore, D-type Cyclins are highly labile with proteasomal degradation occurring at rates comparable to their production. Cyclin D-CDK4/6 complexes are deactivated by phosphorylation of a key regulatory C-terminal Threonine ($CCND1^{Thr\text{-}286}$, $CCND2^{Thr\text{-}280}$, $CCND3^{Thr\text{-}283}$) by GSK3-ß via the PI3K/AKT3/mTOR pathways, followed by export out of the nucleus and ubiquitylation in the cytoplasm by SKP1-Cullin1-F-Box (SCF) E3 ubiquitin ligase, and degradation at the proteasome [20–22].

Recent evidence has emerged, however, that challenges this model of cyclin D degradation. $CRL4^{AMBRA1}$ has been identified as the E3 ubiquitin ligase that ubiquitylates cyclin D, with ubiquitylation and degradation likely occurring in the nucleus [23–25]. Further complicating this, is the observation of cyclin D degradation independent of GSK3-ß, suggesting degradation pathways potentially mediated by other kinases [26–28].

Thus, the activity of cyclin D is carefully regulated through its mitogen-activated transcription, binding to CDK4/6, phosphorylation, ubiquitylation, nuclear export, and degradation (Figure 1) [1].

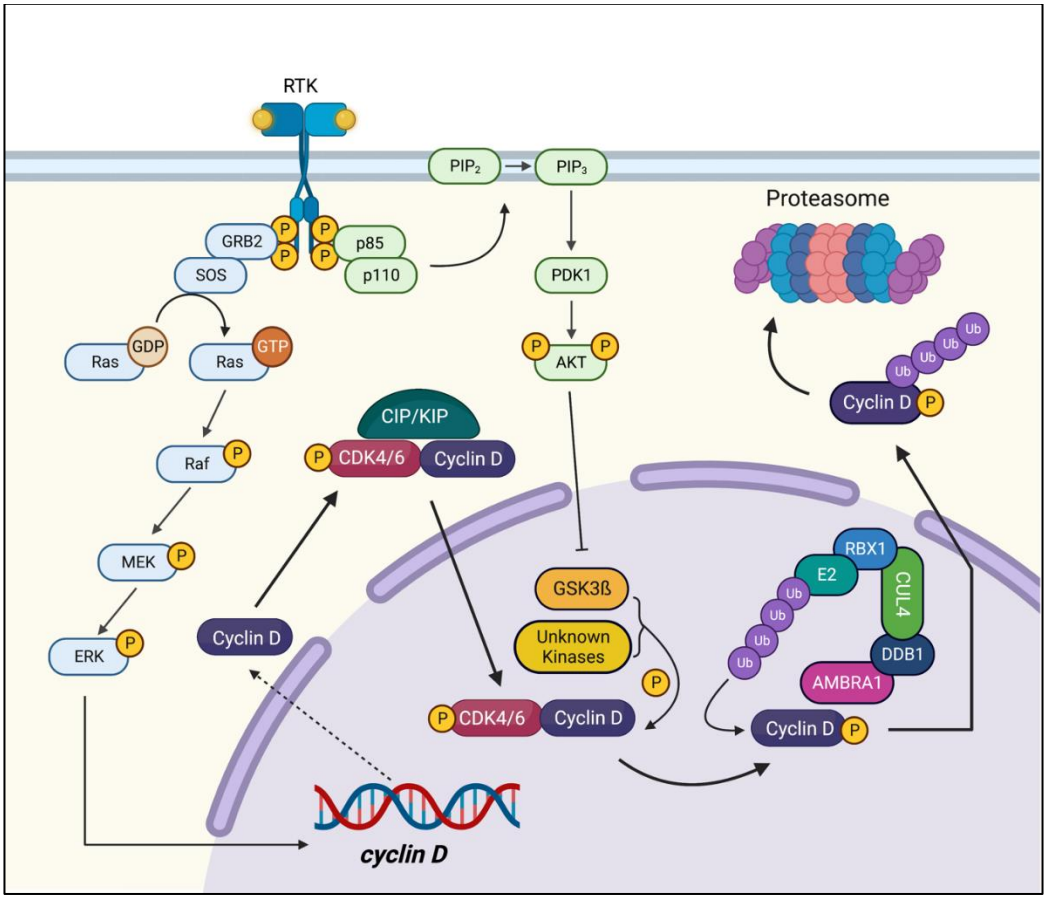

Figure 1. The regulation of Cyclin D – Cyclin D is expressed in response to mitogenic stimulation through the Ras (in light blue) and PI3K-Akt (in light green) pathways stimulate synthesis and promotes stability of Cyclin D respectively. CDK-interacting proteins/kinase inhibitory proteins (CIP/KIP) such as p21 and p27 stabilizes and facilitates the formation of the Cyclin D-CDK4/6 complex. In the nucleus, activity of this complex is terminated by phosphorylation of a C-terminal Threonine by GSK3ß or potentially other unknown kinases. Once phosphorylated, Cyclin D is then polyubiquitylated by the $CRL4^{AMBRA1}$ E3 ubiquitin ligase and is then subsequently degraded.

2. Cyclin D1

Cyclin D1 (CCND1) serves as the general D-type cyclin, expressed in all tissue types except those derived from haematopoietic stem cell lines [13]. *CCND1* is composed of 5 exons which are separated by 4 introns. Alternative splicing at the exon 4-intron 4 boundary results in the cyclin D1b (CCND1b)

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variant, which does not include exon 5, and acquires 33 new amino acids. Both isoforms are identical for the first 240 amino acids but differ in their C-termini. CCND1b is deficient in promoting Rb phosphorylation and lacks the LxxLL motif (aa 251-257) required for ligand-dependent interaction with nuclear receptors, and both the Thr-286 residue, and the PEST sequence (aa 241-290) required for degradation (**Figure 2**). This is postulated to be responsible for the increased nuclear retention of the D1b isoform [29–31]. Furthermore, CCND1b has been observed to have increased oncogenic potential relative to the D1a isoform. CCND1b, but not CCND1, was sufficient to drive transformation of NIH3T3 cells *in vitro* and tumour formation *in vivo* [32,33]. Indeed, the CCND1b isoform is highly expressed in several cancers, including breast cancer [34], prostate cancer [35], and B-lymphoid malignancies [36].

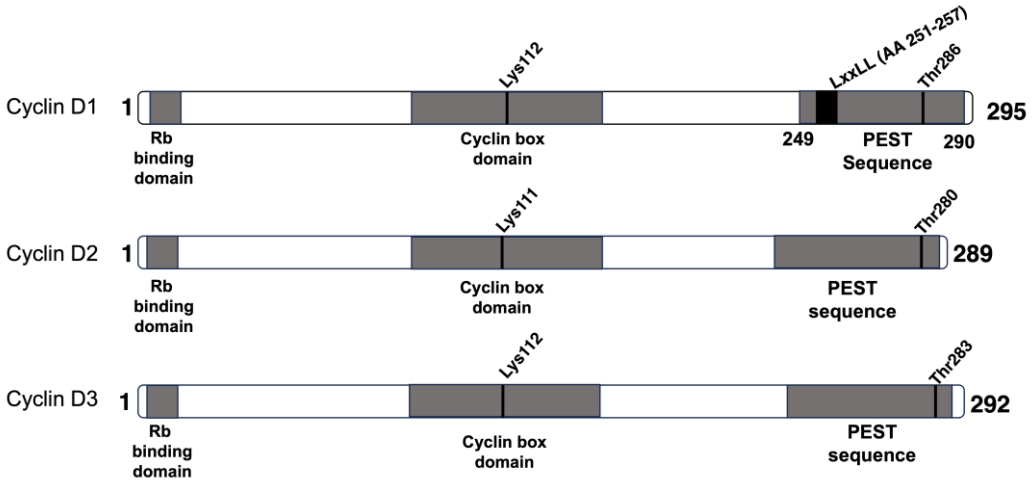

Figure 2 – Comparison of Cyclin D1, D2 and D3. The three D-type cyclins share a number of conserved sequences and domains. The Rb-binding domain, located at the N-terminus, is responsible for binding the C-terminal helix of Rb when Cyclin D is bound to CDK4/6. The cyclin box domain is a heavily conserved region of ~100 amino acids located in the N-terminus of each D-cyclin, which facilitates binding to CDK4/6. Mutagenesis of Lys112 (CCND1 and CCND3) and Lys111 (CCND2) in particular, abolishes binding to CDK4, demonstrating the residue's essential role in mediating CDK-binding. The PEST sequence located at the C-terminus, is required to mediate the degradation of Cyclin D. Mutations at the Threonine phosphodegron site induce stabilisation of Cyclin D, resulting in increased progression of the cell cycle into the S-phase and genomic instability. CCND1 also possess an LxxLL motif which mediates ligand-dependent interaction with nuclear receptors such as ERα.

Ccnd1 deficient mice display developmental abnormalities such as reduced body size, hypoplastic retinas and pregnancy insensitive mammary glands as well as increased premature mortality within the first 3 weeks of life [20,37]. Later studies revealed that the mammary and retinal defects could be rescued following knock-in of a catalytically inactive variant, Ccnd1^{K112E}, indicating that Ccnd1's role in retinal and mammary development occurs independent of CDK4/6 [38]. No human disease has yet been associated with variants in *CCND1* however, a number of studies have identified a common *CCND1* polymorphism (c.870G>A, rs603965) that increase susceptibility to colorectal cancer and multiple myeloma [39–42].

As an oncogene, dysregulation of CCND1 compromises the S-phase checkpoint, inducing forced progression of the cell cycle, disrupting DNA replication, promoting DNA damage and genomic instability, resulting in oncogenesis [43]. CCND1 is more frequently deregulated than CCND2 and CCND3 in both solid and haematological cancers [44], and is over-expressed and upregulated in multiple cancers, including head and neck squamous carcinoma [45], mantle cell lymphoma [46], pancreatic cancer [47], melanoma [48], non-small cell lung cancer [49], gastric cancer [50], colorectal cancer [51], endometrial cancer [52] and over 50% of human breast cancers [53]. The oncogenic activity of CCND1 is strongly tied to its cellular levels, as tumour cells with high CCND1 levels exhibit uncontrolled cell proliferation. Overexpression of CCND1 can be caused by amplification of *CCND1*, chromosomal re-arrangement, or stabilisation of CCND1 protein via impaired degradation, often caused by mutations at or around the Thr-286 phosphorylation site, leading to accumulation in

 doi:10.20944/preprints202306.1703.v1

the nucleus [31]. Additionally, point mutations or deletions around the 3' untranslated region (UTR) of the *CCND1* mRNA transcript results in a shorter, more stable transcript [54].

In addition to its canonical role in the Rb pathway, CCND1 plays a key role in promoting cell proliferation, cell survival, angiogenesis, cell migration and preventing cell senescence [55]. CCND1 can execute some of these functions, independently from its association with CDK4/6. For example, CCND1 binds nuclear receptors such as ERα, and steroid receptor co-factors such as SRC1 and SRC3, enhancing oestrogen receptor-mediated transcription in breast epithelial cells [56,57]. Conversely, CCND1 inhibits the activity of androgen receptor (AR) via binding and preventing the formation of the active AR complex, as well as recruiting histone deacetylases (HDACs) to repress its transcription [58]. Moreover, CCND1 binds histone acetyltransferases such as p300/CREB-binding protein-associated factor (P/CAF) which increases transcriptional activity of ER, and HDACs to enhance transcriptional repression, as seen with AR [58,59]. Indeed, CCND1 acts as a key transcription regulator of several genes through a combination of interactions with transcription factors, coactivators and chromatin altering enzymes.

CCND1 has also been linked to DNA repair. Following DNA damage, CCND1 is recruited by BRCA2 to DNA damage sites, after which CCND1 interacts with Rad51, a critical recombinase involved in homologous recombination and DNA repair and facilitates its recruitment to regions of DNA damage, promoting DNA repair. Decreased expression of CCND1, but not treatment with CDK4/6 inhibitors, decreases Rad51 recruitment to DNA damage sites, confirming that CCND1 promotes DNA repair independently of CDK4/6 [60].

CCND1 can also affect the movement and invasiveness of mantle cell lymphoma cells by localising and accumulating in the cytoplasm. A proteomic analysis of CCND1-interacting proteins showed that many of them are involved in the regulation of cytoskeleton dynamics, migration, and invasion [61]. For example, CCND1 enhances cellular motility by inhibiting the signalling pathways of thrombospondin 1 (TSP-1) and Rho-activated kinase (ROCK) [62]. Additionally, through inhibition of TSP1 and co-expression with the vascular growth factor VEGF, CCND1 can promote angiogenesis, enabling tumour survival, growth and metastasis [63]. Blocking the nuclear export of CCND1 resulted in a significant decrease in migration and invasion, indicating that cytoplasmic CCND1 is essential for chemotaxis and invasion of mantle cell lymphoma cells [61].

However, over-expression of CCND1 is not sufficient to drive oncogenic cell transformation without cooperating mutations [21]. Thr286Ala is a gain-of-function mutation in CCND1 which cannot be phosphorylated by GSK3-ß or AMBRA1, resulting in the stabilisation and accumulation of CCND1 in the nucleus, throughout all stages of the cell cycle. This leads to increased formation of active cyclin D-CDK4/6 complexes, genomic instability, oncogenic cell transformation and neoplastic growth. It has been postulated that degradation of CCND1 is vital to maintaining genomic stability following DNA damage, and therefore dysregulation of its export via stabilisation is a prelude to oncogenesis rather than the underlying cause [64,65].

3. Cyclin D2

CCND2, mapped to chromosome 12p13, consists of 5 exons of which exons 1-4 encode the highly conserved protein Cyclin D2 (CCND2) [13]. Like CCND1, CCND2 is a component of the PI3K-ATK-mTOR pathway, which controls the transition between G1 and S phase of the cell cycle by forming a complex with its cyclin dependent kinase, CDK4 (CCND2-CDK4) and CDK6. The formation of this complex is a rate limiting step for progression through the G1 phase, and heavily depends on availability of serum in cells [66].

Once assembled, CCND2-CDK4 complexes control the phosphorylation of its substrate, Rb [67]. Phosphorylated Rb (pRb) cannot bind to the transcription factor EGF-1 [68], which increases the pool of free, active EGF protein. The accumulation of active EGF activates the expression of many downstream genes needed for S phase initiation and cell proliferation, subsequently triggering S phase entry of the cell cycle [68,69]. A reduction in pRb, and therefore a reduction in the upstream production of CCND2, increases the amount of active (unphosphorylated) Rb. Therefore, Rb-EGF complexes can form and prevent EGF accumulation, leading to cell cycle arrest [68]. It is therefore essential that the components responsible for regulating pRB production, including CCND2, are expressed and regulated precisely to ensure adequate entry through the cell cycle and normal cell growth.

 doi:10.20944/preprints202306.1703.v1

CCND2 is the only D-type cyclin expressed in the adult hippocampus [70] with CCND2 knockout mice (CCND2-KO) displaying reduced hippocampal neuron production [71]. However, these CCND2-KO associated brain abnormalities resulted in limited impact on behavioral phenotypes [72]. Whilst the role of CCND2 in adult neurogenesis is less understood, there is a well-established role of CCND2 in neurodevelopment during embryogenesis [73]. De novo germline CCND2 mutations cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) with mutations clustering at the c-terminus. These mutations occur at and around the phosphodegron residue Thr-280 and result in resistance to proteasomal degradation in vitro, leading to significantly increased CCND2 accumulation in patient cells compared to wildtype [7]. Interestingly, stabilisation of CCND2 is also observed in related neurodevelopmental overgrowth conditions caused by mutations in proteins upstream of CCND2 in the PI3K-ATK-mTOR pathway, such as AKT3, PIK3CA and PIK3R2 [74–76]. CCND2 stabilisation and/or accumulation therefore appears to be a common end point for this group of disorders making it an excellent therapeutic target [7]. Similar results have also been found in mouse models of brain overgrowth, with mice deficient for Dusp16, a negative regulator of MAPK, showing brain overgrowth and stabilised CCND2 [77]. This indicates the important role CCND2 plays in early neurogenesis in regulating cell cycle exit in neural progenitor cells, and how stabilised CCND2 leads to continued entering of the cell cycle into the S phase, resulting in over proliferation and increased brain growth.

Recently, loss of function CCND2 variants resulting in protein truncation were found in patients with microcephaly, the inverse brain phenotype to MPPH [78]. However, loss of function CCND2 was poorly understood with only mice studies indicating CCND2-KO causes a lack of cerebellar stellate interneurons [79], suppressed adult hippocampal neurogenesis leading to learning deficits [80] and severe microcephaly [81]. These recent findings in humans with heterozygous loss of CCND2 confirms the phenotypes observed in mice and further supports a crucial role of CCND2 during neurogenesis and the requirement for careful control of intracellular levels of cyclin D.

In addition to its key role in neurogenesis, CCND2 also regulates the cell cycle in other tissues which may also be affect in patients with stabilising mutations. For example, MPPH patients display 3- or 4- limb postaxial polydactyly indicating a role for CCND2 in early limb bud development [7]. The crucial role of CCND2 in limb development was also identified independently in the developing limb bud of the chick wing [82]. RNA sequencing of the polarizing region of the limb bud and adjacent skeletal progenitor cells revealed Ccnd2 and its inhibitor p27 are the only core cell cycle regulators expressed in these cells [83]. More recently CCND2 has been implicated in pancreatic B-cell proliferation, with MPPH or MCAP patients with CCND2 or PIK3CA variants, respectively, found to have hypoglycemia [84,85]. B-cells of the pancreas are responsible for secretion of insulin, thus over-proliferation of B-cells due to CCND2-stabilising mutations likely results in hyper-insulinaemia and in turn hypoglycemia [86]. Patients with MPPH or MCAP are therefore recommended to undergo regular blood glucose monitoring and those with low blood glucose levels referred for specialist endocrine review.

While the roles of CCND2 in development have only recently been discovered, the role of CCND2 in cancers are more understood. Prior to being identified in patients with MPPH, the same protein-stabilising mutations in and around Thr-280 had been identified somatically in tumors (COSMIC). Recent studies have identified CCND2, and CCND1, mutations as frequent events in myeloid leukemia's, in particular acute myeloid leukemia's [87,88]. These mutations are identical to those seen in MPPH and have the same underlying mechanisms i.e., accumulation of stabilised CCND2, increased phosphorylation of the retinoblastoma protein and uncontrolled cellular proliferation.

While accumulation of CCND2 is the most common disease mechanism associated with CCND2-associated disorders, a number of cancers have been found to have reduced CCND2 due to CCND2 hypermethylation, particularly breast and lung cancers [89]. CCND2 promoter hypermethylation was found at an early stage of breast cancer tumorigenesis and was associated with silencing of CCND2 expression [90]. Administering the demethylating agent antroquinonol D increased CCND2 expression in breast cancer samples and resulted in reduced cancer cell growth through cell cycle arrest [89,91]. It remains unclear why loss of CCND2, and therefore CCND2-associated cell proliferation is observed in cancers but may be due to a compensation effect, leading to up-regulation of another cyclin, e.g., cyclin E. Another explanation may be related to the stage or

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sub-type of cancer. For example, in gastric cancer some studies have CCND2 hypermethylation as an underlying cause of proliferation [92] whereas others have found CCND2 hypomethylation, leading to increased CCND2 expression, in more advanced stage gastric carcinomas [93].

4. Cyclin D3

The major isoform of *cyclin D3 (CCND3)*, located on chromosome 6p21, consists of 5 exons but several alternative isoforms exist with different transcriptional start sites. While each isoform contains the regulatory c-terminal region of CCND3, the alternative start sites affect the CDK4-binding region indicating a CDK4-independent role for these isoforms. In comparison to CCND1 and CCND2, CCND3 is predominantly found in bone marrow and lymphoid tissues, with the highest protein and RNA expression found in the Thymus (proteinatlas.org)[94]. High protein expression is however also observed in cerebellum, duodenum, pancreas, and testis, although RNA levels of CCND3 in these tissues is comparatively low.

CCND3 is an atypical D-type cyclin that is predominantly expressed in differentiated tissues [95–97]. Germline knockout of *CCND3* in mice is viable but homozygous null mice show defects in lymphoid derived cells, such as impaired B- and T- cell differentiation and granulocyte proliferation [98–100]. A similar role is observed in humans, with CCND3 playing a key role during B-cell precursor cell development [101] and at later stages a crucial role in expansion of the germinal centre B-cells [102,103].

In addition to an effect of lymphoid derived cells, $CCND3^{-/-}$ mice also show retarded growth, significant loss of muscle mass and impaired muscle regeneration suggesting an important role for CCND3 in myogenesis [97,99,104] These models suggest that while CCND3 may not have a direct role during development, it does play a key role in differentiation and maturation in lymphoid and musculoskeletal tissues.

Like CCND1, no human disorder has yet been associated with germline mutations in *CCND3*. However, a role for CCND3 in multiple cancers has been observed, with most *CCND3* aberrations being gene amplification rather than single nucleotide variants. Due to the expression profile of *CCND3*, it is no surprise that lymphoid cancers are mostly commonly associated with dysregulation of CCND3. Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin's lymphoma and alterations in the cyclin D/CDK4-6 pathway were found in in 67% of DLBCL cases, with CCND3 overexpression accounting for 53% [105–107]. More significantly, DLBCL patients with high levels of CCND3 had a lower response rate to chemotherapy and a shorter survival time compared to those with low CCND3 expression [108]. Increased expression of CCND3 has also been observed in chronic lymphocytic leukaemia (CLL) cells, with RNA sequencing identifying a 38x increase in *CCND3* in NOTCH1-mutated cells compared to NOTCH1 non-mutated cells [109]. A similar increase was also seen for CDK4 and CDK6 and confirmed a previous study associating Notch signalling with CCND3 [110]. A role for CCND3 in B-cell lymphoblastic leukaemia (B-ALL) has also been described, with CCND3 being found to be indispensable for the growth and survival of B-ALL cells irrespective of the underlying driver mutation [102]. Increased CCND3 expression associated with cells developing resistance to the CDK4 inhibitor Palbociclib suggesting targeting of CCND3, rather than CDK4, may be a more effective therapeutic approach. Altogether this highlights a key role for CCND3 in the regulation of lymphoid-derived cells and the consequences of its dysregulation.

Somatic mutations in CCND3 have also recently been identified in bone tumours. Xie et al found 43/357 (12.04%) bone tumours sequenced had a genomic aberration in *CCND3*, making it the 5th most mutated gene after *TP53*, *NCOR1*, *VEGFA* and *RB1* [111]. *CCND3* amplifications were mainly identified in osteosarcoma. A recurrent fusion gene between *KCNMB4* and *CCND3* has also been identified in a cohort of osteosarcoma, but not in a cohort of 240 other sarcomas, further suggesting CCND3 is specifically associated with osteosarcoma [112]. Functional assessment of the KCNMB4-CCND3 fusion gene showed it promoted cell migration in SAOS-2 cells. By comparing the age of patients, *CCND3* mutations were more frequent in paediatric, adolescent and young adult (P-AYA) osteosarcoma than in adult osteosarcoma [113].

5. Current therapeutic strategies for D-type Cyclin disorders

As D-type cyclins do not possess any enzymatic function alone, a favoured strategy of treating cyclin D-based disorders involves targeting the enzymatic activities of their partners CDK4 and CDK6. As such, three highly specific dual-CDK4/6 inhibitors have been developed and approved by the FDA for use to treat advanced or metastatic breast cancer, Palbociclib, Abemaciclib, and Ribociclib [44,114]. These inhibitors function by binding and blocking the ATP-binding pockets of CDK4 and CDK6, preventing kinase activity, as well as indirect non-catalytic inhibition of CDK2 via displacement of CKI p21 [115]. More recently, these CDK4/6 inhibitors have been used to create IKZF1 and IKZF3, selective imide based CDK4/6 degraders used to reduce cell proliferation in mantle cell lymphoma cell lines [114].

There are caveats, however, potent side effects due to lack of selectivity, the most notable of which is neutropenia, a low neutrophil count [56]. Furthermore, resistance to CDK4/6 inhibitors has been observed in some stabilised cyclin D phenotypes [24]. Loss of AMBRA1 can also reduce sensitivity to CDK4/6 inhibitors by stabilising cyclin D and forming active complexes with CDK2 [25]. This has led to the use of CDK4/6 inhibitors in combination with other therapies such as hormone treatment, chemotherapy, PI3K pathways inhibitors, immunotherapy, and radiotherapy, for increased effectiveness [56,116].

Another therapeutic strategy to treat stabilised D-type cyclin disorders would be disrupt the protein-protein interaction between cyclin D and CDK4. This would prevent the formation of active cyclin D-CDK4 complexes, arresting cell cycle progression and reducing cell proliferation. In addition to also potentially inhibiting formation of cyclin D-CDK2 complexes in some stabilised cyclin D phenotypes. However, no known inhibitors of the cyclin D-CDK4 complex have been identified. This represents an interesting opportunity to explore and investigate novel inhibitors which perturb cyclin D-CDK4 complex formation.

6. Conclusions and Perspectives

Careful regulation of D-type cyclins is crucial to ensure there is sufficient proliferation and cell numbers to develop, maintain and repair tissues throughout life. Dysregulation of D-type cyclins, either by mutation in key regulatory proteins or through hyper-activation of up-stream pathways, results in a range of disorders associated with over- or under-proliferation. Therapies that target Cyclin D may offer exciting possibilities to overcome the effects of any dysregulation, however it will be crucial to ensure that they do not tip the balance from over- to under-proliferation, or vice versa. Further research into Cyclin D regulation and stability is required in order to identify additional possible therapeutic targets that will allow careful modulation of intracellular Cyclin D.

Author Contributions: Conceptualization, J.A.P.; original draft preparation, M.S., E.L.H and J.A.P.; writing—review and editing, M.S., E.L.H and J.A.P.; visualization, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a Future Leaders Fellowship to J.A.P from UK Research and Innovation, grant number MR/T02044X/1, and a University Academic Fellowship to J.A.P from the University of Leeds. The APC was funded by UK Research and Innovation.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the writing of the manuscript or in the decision to publish.

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