Cell cycle regulation and hematologic malignancies

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Abstract

A complex network precisely regulates the cell cycle through the G_1, S, G_2, and M phases and is the basis for cell division under physiological and pathological conditions. On the one hand, the transition from one phase to another as well as the progression within each phase is driven by the specific cyclin-dependent kinases (CDKs; e.g., CDK1, CDK2, CDK4, CDK6, and CDK7), together with their exclusive partner cyclins (e.g., cyclin A1, B1, D1–3, and E1). On the other hand, these phases are negatively regulated by endogenous CDK inhibitors such as $p16^{ink4a}$, $p18^{ink4c}$, $p19^{ink4d}$, $p21^{cip1}$, and $p27^{kip1}$. In addition, several checkpoints control the commitment of cells to replicate DNA and undergo mitosis, thereby avoiding the passage of genomic errors to daughter cells. CDKs are often constitutively activated in cancer, which is characterized by the uncontrolled proliferation of transformed cells, due to genetic and epigenetic abnormalities in the genes involved in the cell cycle. Moreover, several oncogenes and defective tumor suppressors promote malignant changes by stimulating cell cycle entry and progression or disrupting DNA damage responses, including the cell cycle checkpoints, DNA repair mechanisms, and apoptosis. Thus, genes or proteins related to cell cycle regulation remain the main targets of interest in the treatment of various cancer types, including hematologic malignancies. In this context, advances in the understanding of the cell cycle regulatory machinery provide a basis for the development of novel therapeutic approaches. The present article summarizes the pathways as well as their genetic and epigenetic alterations that regulate the cell cycle; moreover, it discusses the various approved or potential therapeutic targets associated with the cell cycle, focusing on hematologic malignancies.

Keywords: Cell cycle, Transcription, Cyclin, Cyclin-dependent kinase, Hematologic malignancy

1. INTRODUCTION

The cell cycle is a defined program that can be divided into four phases—the G_1, S, G_2, and M phases—that result in cell division. The passage of cells through these phases is driven by cyclin-dependent kinases (CDKs) and their partner cyclins. These molecules bind to each other to form several specific active heterodimeric cyclin–CDK complexes that play key roles in the regulation of both cell cycle and gene transcription (Fig. 1). Activated CDKs phosphorylate various proteins, thereby driving the entry into and progression through each phase as well as promoting DNA synthesis and mitosis.[1] The process of cell cycle rigorously follows an empirical rule, that is, the occurrence of an event B is dependent on the completion of the prior event A, to ensure that the cell cycle progresses in an orderly manner. Such control is finely orchestrated through (a) the de novo synthesis (via epigenetic and transcriptional regulation) and turnover (via the ubiquitin–proteasome system, UPS) of cyclins and (b) the phosphorylation (by upstream kinases) and dephosphorylation (by phosphatases) of CDKs, often without a change in their total protein levels. Interestingly, the phosphorylation of certain CDKs could be either activational or inhibitory, which further increases the complexity of cell cycle regulation. A disturbance of cell cycle regulation could lead to or promote the development and progression of cancer. Until the end of the twentieth century, researchers believed that deletion or mutation (gain- or loss-of-function mutations) in tumor-suppressor genes or oncogenes is the sole mechanism via which the "gatekeepers" of the cell cycle could be (in)activated in cancer. Advancements in the understanding of the regulation of gene expression emphasizes a mechanism called epigenetic regulation, which includes several molecular modifications (e.g., DNA methylation and histone methylation and acetylation) that play a key role in the regulation of gene expression; this mechanism is known as (re)programming. In fact, the amplification/overexpression of cyclin and CDK genes or epigenetic silencing/deletion of CDK inhibitor genes is common in nearly all human tumor types,[2] including various hematologic malignancies (Table 1). For instance, the mutations of CDKs, cyclins, and cell cycle-related oncogenic genes were often observed in several hematologic malignancies (Fig. 2). Over the last several decades, research has provided

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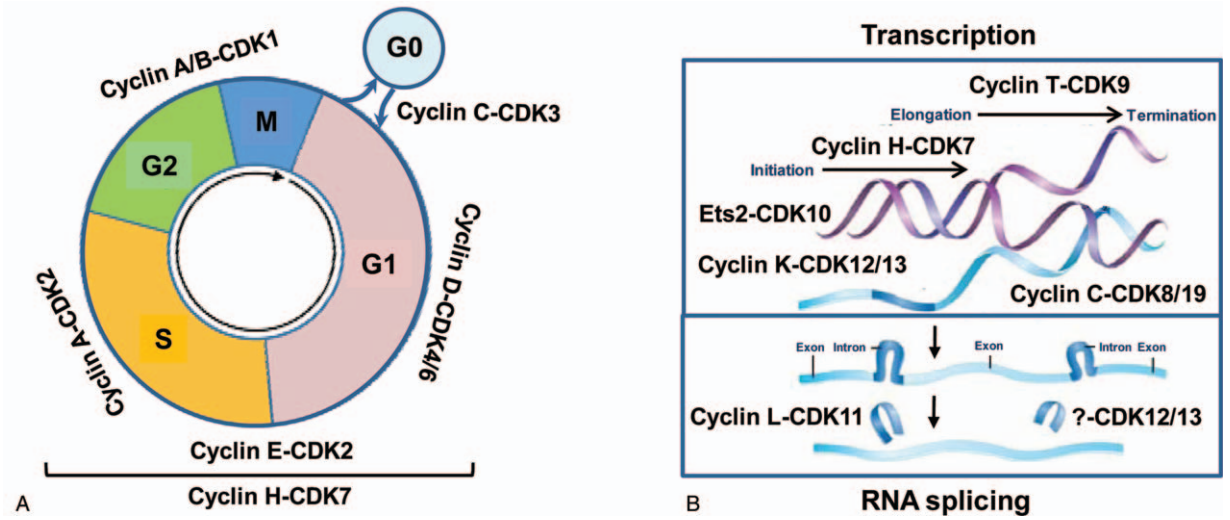

Figure 1. Cyclins and cyclin-dependent kinases (CDKs) involved in the regulation of both the cell cycle and gene transcription. (A) In mammalian cells, cell cycle regulatory CDKs drive intraphase progression and interphase transition during the cell cycle via the formation of complexes with their corresponding cyclins. These complexes include (a) cyclin C–CDK3, which promotes G0–G1 transition (or G0 exit) in an RB-dependent manner; (b) cyclin D–CDK4/6, which primarily functions in the late G1 phase before entry to the S phase via the Rb-E2F pathway, in which the genetic and epigenetic alterations are extremely frequent in cancer; (c) cyclin E–CDK2, which stimulates entry into and progression through the S phase; (d) cyclin A–CDK2, which takes over the function of cyclin E–CDK2 in the late S phase; and (e) cyclin A–CDK1, which is formed before mitosis and is an event likely required for progression through the late G2 phase and entry to the M phase followed by the formation of the cyclin B–CDK1 complex that promotes mitosis. (B) Transcription-regulatory CDKs, also via the formation of complexes with their cyclin partners, govern the entire process of gene transcription, including initiation primarily by the cyclin H–CDK7 complex, elongation almost exclusively and termination largely by the cyclin T–CDK9 complex, and RNA splicing by the cyclin L–CDK11 complex. In addition, the cyclin C–CDK8/19 and cyclin K–CDK12/13 complexes are also involved in transcriptional regulation, although they most likely control the expression of a specific set of genes. Several CDKs (e.g., neural CDK5 and, probably, CDK18) and cyclins (e.g., cyclin F), which bind to other proteins rather than the corresponding cyclins or CDKs, are categorized beyond these two relatively well-defined groups but play important roles in various physiological and pathological processes, particularly cancer, including hematologic malignancies.

tremendous evidence regarding not only the role of cyclins and CDKs in the regulation of cell division but also in numerous other functions of these genes/proteins in transcription, alternate splicing, DNA damage response (DDR), cell death, cell differentiation, and metabolism, among others, under both physiological and pathological conditions, particularly cancer.[3] In this article, we focused on the fields in which the alterations in cell cycle regulation have been investigated in terms of cyclins and CDKs, focusing particularly on hematologic malignancies.

Table 1

Cyclin-CDK complexes and CDK inhibitors in hematologic malignancies.

Cyclin-CDK	Function	Disease	CDK inhibitor	Inhibitor status
B-CDK1	G2-M, mitosis	**MM**: B1↓ (aneuploidy?)		
A-CDK2	S	**AML** (M2, M3): A1↑	Flavopiridol/alvocidib: AML	Phase I/II
E-CDK2	G1-S	**MM**: E2-CDK2↑ (aneuploidy via RB↓)		
C-CDK3	G0-G1			
D-CDK4/6	G1	**MM**: D1↑ (t[11;14]), D2↑ (due to MYC↑), D3↑ (t[6;14])	PD0332991/palbociclib: MM, MCL	Approved
		MCL: D1↑	LEE011/ribociclib: ALL	Approved
		MLL-r AML: CDK6↑	LY2835219/abemaciclib: ?	Approved
?-CDK5		**MM**: CDK5↑	SCH727965/dinaciclib: MM	Phase I
H-CDK7	CAK; Transcription initiation (TFIIH)	**T-ALL**: CDK7↑	THZ1/THZ2: T-ALL	Pre-clinical
			CT7001: AML	Phase I
			SY-1365: ?	Phase I
C-CDK8/19	Transcriptional regulation (Mediator)	?: (β-catenin↑)	BI-1347: AML	Pre-clinical
T-CDK9	Transcription elongation (P-TEFb)	**CLL**: ?	Flavopiridol/alvocidib: CLL, MM	Phase I/II
		MM: P-TEFb↑ (Mcl-1)	SCH727965/dinaciclib: CLL, MM	Phase I
		Lymphoma (?): SEC↑ (MYC)	KL-1/KL-2: ?	Preclinical
M-CDK10	Transcription factor (Ets 2)	**FL**: CDK10↑		
L-CDK11	Splicing; apoptosis/autophagy			
K-CDK12/13	DDR gene transcription			
?-CDK18	S; replication stress			

AML: acute myeloid leukemia; CAK: CDK activating kinase; CDK: cyclin-dependent kinase; CLL: chronic lymphocytic leukemia; DDR: DNA damage response; FL: follicular lymphoma; MCL: mantle cell lymphoma; MLL-r: mixed-lineage leukemia rearrangement; MM: multiple myeloma; P-TEFb: positive transcription elongation factor b; SEC: super elongation complex; T-ALL: T-cell acute lymphoblastic leukemia; TFIIH: transcription factor II human (general transcription factor). G0, G1, S, G2, and M indicate different cell cycle phases.

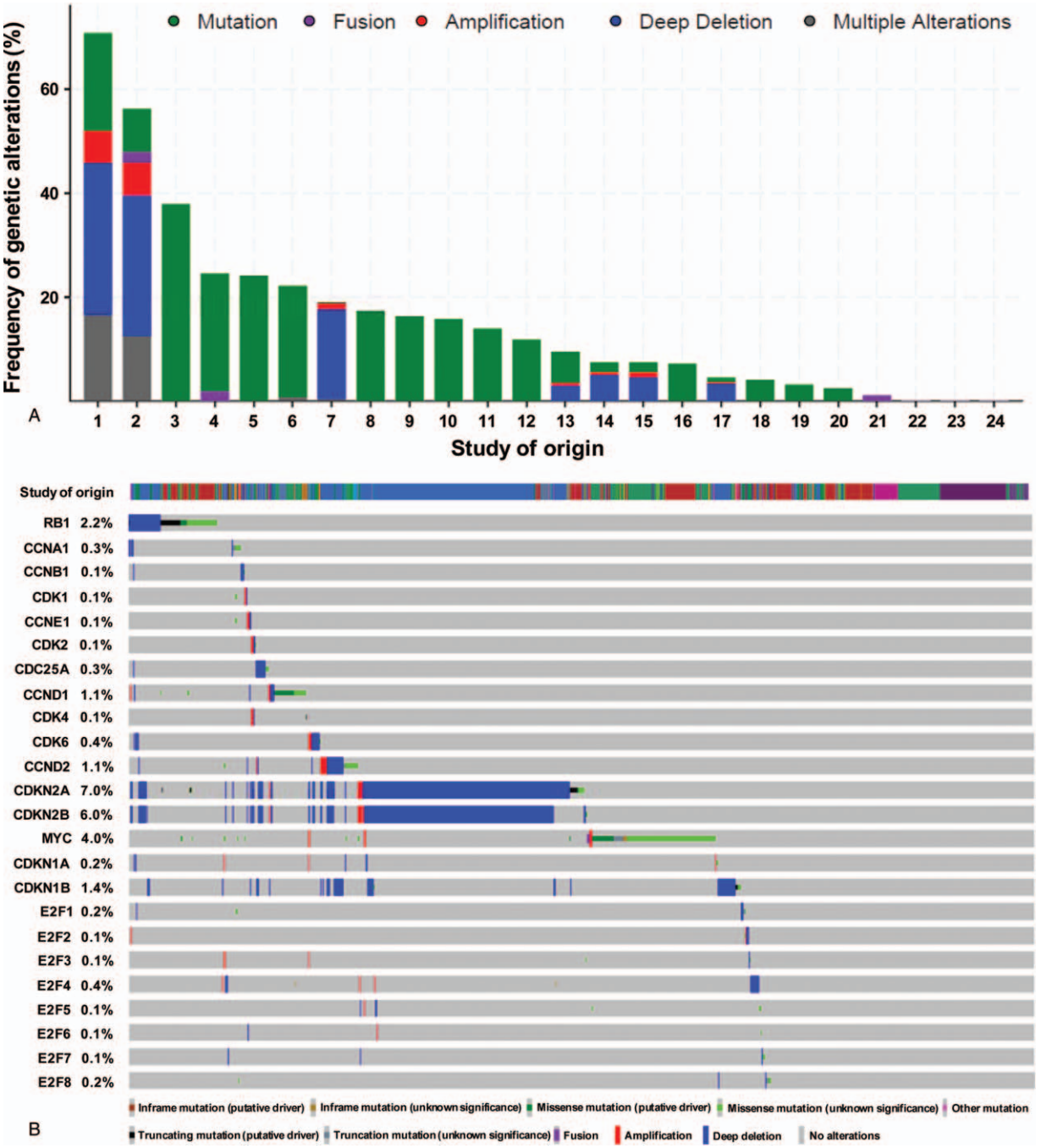

Figure 2. Genetic alterations in cell cycle regulatory genes in hematologic malignancies. Using the cBioPortal for Cancer Genomics platform, genetic abnormalities were analyzed as follows. (A) The frequency of genetic alterations was examined in 24 data sets involving various hematologic malignancies, including (1) diffuse large B-cell lymphoma (DLBC; TGCA); (2) DLBC (TGCA Pan-Cancer); (3) mantle cell lymphoma (MCL; IDIBIPS 2013); (4) non-Hodgkin's lymphoma (NHL; BCGSC 2013); (5) diffuse large B-cell lymphoma (DLBCL; Broad 2012); (6) DLBCL, not otherwise specified (DLBCLNOS; DFCI); (7) acute lymphoblastic leukemia (ALL-phase II; TARGET 2018); (8) DLBCLNOS (Duke 2017); (9) mature B-cell neoplasm (MBN; MDACC 2013); (10) primary central nervous system lymphoma (PCNSL; Mayo Clinic); (11) cutaneous T-cell lymphoma (CTCL; Columbia 2015); (12) multiple myeloma (MM; Broad); (13) acute myeloid leukemia (AML; TCGA Pan-Cancer); (14) AML (TCGA); (15) AML (TCGA pub); (16) NHL (bcgsc 2011); (17) AML (TARGET 2018); (18) ALL (St. Jude, Nat Gen); (19) chronic lymphocytic leukemia (CLL; IUOPA 2015); (20) CLL (Broad 2013); (21) ALL (St. Jude 2015); (22) CLL and small lymphocytic lymphoma (CLLSLL; Nat Genet 2011); (23) histiocytic leukemia (HIST; COBI 2019); and (24) myelodysplastic syndrome (MDS; Tokyo). (B) The frequency of the mutations of each cell cycle regulatory gene in various hematologic malignancies included in all the studies described above, with mutations further functionally categorized as inframe, missense, and truncated, either as putative driver or with unknown significance, as well as other mutations, was assessed. Among all of the cell-regulatory genes considered, the genes that were most frequently altered (>1%) at the genetic level were *CDKN2A* (7.0%), *CDKN2B* (6.0%), *MYC* (4.0%), *RB1* (2.2%), *CDKN1B* (1.4%), *CCND1* (1.1%), and *CCND2* (1.1%).

2. CYCLINS

In mammals, over 20 members of the cyclin family have been identified.[1] All cyclins share a region of approximately 150 amino acids called the *cyclin box*, which interacts with CDKs. G_1 (C, D, and E) and mitotic (A and B) cyclins form distinct categories that are directly involved in cell cycle regulation, whereas cyclins H, K, L (L1 and L2), and T (T1, T2a, and T2b) are categorized beyond these two groups.[4] Cyclin F does not bind to CDKs but to multiple other proteins, including those involved in cell cycle regulation, such as the CDK inhibitor $p27^{kip1}$. As a substrate receptor of 1 of 69 human SCF ubiquitin ligase complexes, cyclin F interacts with all three activators of the E2F transcription factor family and leads to their degradation via UPS during the late S and G2 phases, which prevents premature S-phase entry.[5] Therefore, cyclin F belongs to the family of cell cycle regulatory cyclins, although it acts primarily in an indirect manner.

Cyclins A and B are known as mitotic cyclins as they are upregulated in the late G_2 or G_2/M phase and undergo proteolysis in the M phase. Unlike cyclin B, which interacts with CDK1 during mitosis, the cyclin A–CDK1 complex forms before mitosis and is likely required for progression through the late G_2 phase.[6] Thus, although cyclin A acts at the G_2/M transition, evidence indicates that it binds and activates CDK2 primarily in the S phase. Overexpression of cyclin A in the G1 phase leads to accelerated entry into the S phase,[7] thereby suggesting that this cyclin is involved in transformation. Although cyclin A1 is detected in normal $CD34^+$ hematopoietic progenitor cells, the highest levels of cyclin A1 are observed in acute myeloid leukemia (AML), particularly at the promyelocyte (M3) and myeloblast (M2) stages.[8] Moreover, transgenic cyclin A1-overexpressing mice develop AML, although with low frequency, likely via the regulation of WT1 expression.[9] In patients with AML, high levels of cyclin A1 are associated with poor prognosis,[10] and this cyclin may serve as an immunogenic targetable antigen in AML stem cells.[11] Cyclin A is mandatory for the downregulation of anaphase-promoting complex or cyclosome (APC/C), a complex of at least 11 proteins (including cullin and RING subunits) that constitutes an E3 ubiquitin ligase, which marks cell cycle regulatory proteins for degradation via UPS.[12] Considering the prominent role of APC/C in cell cycle regulation, its gene mutation or dysfunction is speculated to be one of the major reasons for the misregulation of the cell cycle as well as the transformation of cells in certain types of tumors.[13,14]

B-type cyclins associate with CDK1 to form the classical mitotic cyclin–CDK complex.[15] Cyclin B is synthesized in the S phase and accumulates together with CDK2, following which this cyclin is then ubiquitinated and degraded via UPS, thereby allowing the cell to exit from mitosis. Cellular localization of the cyclin B–CDK1 complex is strictly cell cycle-dependent. Although this complex accumulates in the cytoplasm during the G_2 and S phases, it translocates to the nucleus and binds to the mitotic spindle during mitosis.[16] Different family members of the B-type cyclins (i.e., cyclin B1 and B2) have distinct functions.[17] Upon mitotic entry, cyclin B1–CDK1 promotes chromosome condensation, nuclear membrane dissolution, mitotic aster assembly, and Golgi breakdown, whereas cyclin B2–CDK1 only induces Golgi disassembly. Cyclin B1 accumulates in the nucleus at prophase and then translocates to the condensed chromatin, spindle microtubules, centrosomes, and chromatin during prometaphase. Moreover, the localization of cyclin B1 to the chromatin, centrosomes, and kinetochores during mitosis is controlled by distinct sequence elements.[18] Exit from mitosis is characterized by the rapid degradation of cyclin B. Cells with a defect in mitotic cyclin B expression or degradation mechanism easily become aneuploid, which refers to the presence of an abnormal number of chromosomes (e.g., $\leq$45 or $\geq$47 chromosomes instead of the usual 46) in a cell. Aneuploidy originates from the improper separation of chromosomes between the two daughter cells during cell division. The cyclin B/CDK2 checkpoint is often defective in malignant cells, leading to uncontrolled M-phase entry and aneuploidy with either missing or extra chromosomes. In fact, aneuploidy is common in several types of hematologic malignancies. For example, trisomies typically involving odd-numbered chromosomes 5, 7, 9, 11, 13, and 15 represent one of the primary cytogenetic abnormalities in multiple myeloma (MM), leading to a hyperdiploid karyotype.[19] Researchers currently believe that primary genetic events, including aneuploidy and chromosome translocations, are associated with the development of the asymptomatic precursor states of MM (e.g., monoclonal gammopathy of undetermined significance and smoldering MM) and probably with the symptomatic disease as well.[20]

Three cyclin D molecules (i.e., D1, D2, and D3) bind to CDK4 or CDK6 and function mainly in the late G1 phase.[3] The cyclin D–CDK4 or –CDK6 complex phosphorylates RB, thereby restraining the latter's inhibitory effects on E2Fs and other related transcription factors. In turn, RB controls the activity of other cell cycle regulatory elements such as Skp2 [the rate-limiting component of the Skp1/Cul1/F-box protein (SCF) complex, an E3 ubiquitin ligase], which triggers degradation of the CDK inhibitor $p27^{kip1}$ and thereby activates cyclin E-CDK2. Then, activated CDK2 induces RB phosphorylation, followed by E2F-dependent Skp2 expression. Although all three cyclin Ds act in the late G phase, just before entry into the S phase, cyclin D1 represents the major form of D type cyclins in most cell types. Cyclin D1 notably has various cell cycle-independent functions. For example, cyclin D1 regulates micro-RNA biogenesis by the induction of Dicer, a central regulator of miRNA maturation.[21] Moreover, the cyclin D1–CDK4 complex is involved in the regulation of glucose metabolism in post-mitotic cells.[22] Cyclin D1 is highly expressed in several tumor types (e.g., breast cancer), including hematologic malignancies (e.g., mantle cell lymphoma and MM), often without the amplification or mutation of the cyclin D1 gene (*CCND1*) itself. Instead, cyclin D levels are regulated via various other mechanisms. For example, alterations in the RB gene in cancer may secondarily cause the upregulation of cyclin D transcription, thus indicating an RB-dependent feedback loop. As cyclin D dysregulation is mostly prominent in MM carrying IGH rearrangement, cyclin D genes are directly involved in chromosome translocations, resulting in the fusion of these genes to the IGH enhancer on chromosome 14 [e.g., t(11;14) for cyclin D1 and t(6;14) for cyclin D3].[19] Moreover, the overexpression of cyclin D2 is also frequent in MM, mostly likely due to the activation of other transcriptional factors such as MYC.[20]

Cyclin E accumulates at the G_1/S boundary of the cell cycle, where it stimulates functions associated with entry into and progression through the S phase.[3] In normal cells, cyclin E levels are finely regulated to ensure that peak cyclin E–CDK2 kinase activity occurs only for a short interval near the G_1/S boundary. The cyclin E–CDK2 complex becomes active during the S phase, following which it is rapidly ubiquitinated and degraded via UPS.[23] Cells overexpressing cyclin E progress much faster through the G_1 phase and into the S phase, but the time required for DNA synthesis remains normal.[24] Furthermore, cyclin E

levels are regulated by environmental factors, including transforming growth factor-β (TGF-β) and irradiation, which is partly mediated by small proteins known as CDK inhibitors. Cyclin E overexpression delays progression through the early phases of mitosis and causes aberrant mitosis, thereby resulting in the dysregulation of mitotic progression.[25] The poor prognostic implications of cyclin E overexpression, which leads to high cyclin E levels throughout the cell cycle, have been observed in a variety of human cancers.[26] However, the direct association between cyclin E overexpression and tumorigenesis remains poorly understood. In this context, the cyclin E–CDK2 complex phosphorylates and inactivates the RB protein or leads to genomic instability via the generation of aneuploid cells.[27,28]

Although the cyclin E–CDK2 complex controls the progression from the G_1 phase to the S phase, cyclin A, another cyclin that interacts with CDK2, is able to compensate for the loss of cyclin E function.[3] However, the exact time point at which CDK2 "switches" from cyclin E to cyclin A binding during the cell cycle is unclear. In cyclin E-defective cells, cyclin A can take over the function of cyclin E in the S phase. Moreover, cyclin E plays a critical role in the duplication of centrosomes, whereas cyclin A is important for centrosome amplification in G2-arrested cells, irrespective of cyclin E.[29] As a consequence, bifurcations in CDK2 activity determine whether cells immediately commit to the next cell cycle or enter a transient state of quiescence as they exit mitosis.[30]

3. CYCLIN-DEPENDENT KINASES

Early studies on the control of mitosis provided the first evidence about the existence of factors called M- and S-phase-promoting factors.[31] The key element of S-phase-promoting factor was initially believed to be cdc2. Later, a group of cdc2-related kinases were characterized and named CDKs because they all bind to their corresponding cyclins for activation.[32] Similar to cyclins, more than 20 CDKs have been identified in mammalian cells.[1] However, only some cyclin–CDK complexes are known to be involved directly in cell cycle regulation, whereas other similar complexes participate in multiple cell cycle-independent processes such as mRNA transcription and splicing.

Three cdc2-related proteins—cdc2 (i.e., CDK1), CDK2, and CDK3—that were able to replace deficient cdc28 function in budding yeast were originally isolated.[33] CDK1 was initially characterized as an M phase-specific histone H1 kinase[34] and is the only essential member of the CDK subfamily that drives cell cycle progression.[35] The function of this CDK is irreplaceable by its closest homolog, CDK2.[36] Although CDK1 shares several similarities in sequence and structure with CDK2 and CDK4, the structure of cyclin B–CDK1 complex displays a relatively relaxed specificity for residues adjacent to the phosphorylation site, thereby suggesting that its activation segment is relatively more flexible than that of its close relatives.[37] CDK1 is phosphorylated at tyrosine 15 and threonine 14 by Wee1 and Myt1, another key mechanism for the regulation of its activity. Because phosphorylations at these two sites inhibit the kinase activity of CDK1, the dephosphorylation of this CDK by CDC25 phosphatases (A–C), which, in turn, is regulated by Chk1, is required for mitotic initiation.[38] In this context, Wee1 and Chk1, together with their upstream kinases ATM and ATR, are considered as the key cell cycle checkpoint kinases in DDR and believed to be attractive therapeutic targets in cancer, including leukemia and MM.[39–44] As a kinase, CDK1 phosphorylates numerous substrates involved in either cell cycle regulation or non-cell cycle functions. As the master regulator of mitosis, the cyclin B–CDK1 complex directly mediates histone H2B phosphorylation at serine 6 during the early M phase, an event important for accurate chromosome separation that prevents chromosomal instability and aneuploidy, which is common in cancer.[45] During mitosis, CDK1 also substitutes for mTOR and completely phosphorylates the translation initiation factor 4E-binding protein 1 (4E-BP1) at both canonical and non-canonical sites. In contrast to the inhibitory mTOR-mediated 4E-BP1 phosphorylation, the mitotic CDK1-directed phosphorylation at serine 83 of δ-4E-BP1 yields a gain of function, which may be important in mitotic centrosome function as well as tumorigenesis.[46] CDK1 is directly involved in the regulation of DDR, including cell cycle checkpoints and DNA repair mechanisms [particularly homologous recombination (HR)].[38,47] Moreover, CDK1 may contribute to DNA repair via an alternative mechanism in which CDK1 relocates to the mitochondria and enhances ATP generation.[48] Through this communication between the mitochondria and nucleus, CDK1 boosts mitochondrial bioenergetics to fulfill the increased cellular fuel demand for DNA repair.

CDK2 binds to and is activated by cyclin E and cyclin A; these cyclins regulate G1 progression/S-phase entry and acts in the late S phase and mitosis, respectively. Cyclin E–CDK2 activity peaks at the G1–S transition, which is controlled primarily via transcription (de novo synthesis) and degradation by the ubiquitin ligase SCF–Fbw7 complex.[49] Similar to CDK1, CDK2 is phosphorylated at tyrosine 15 and threonine 14 by Wee1 and dephosphorylated by CDC25A. However, the function of these phosphorylations of CDK2 is less clear than those of CDK1. For example, the inhibitory phosphorylation of CDK2 plays a role in S-phase entry and centrosome duplication but may not be required for DDR.[50] In the S phase, tyrosine 15 and threonine 14 phosphorylations of CDK2 directly regulate cyclin E degradation and are essential for the maintenance of genome stability; however, failure of these phosphorylations to inhibit CDK2 during replication stress results in irreparable DNA damage.[51]

Unlike CDK1 and CDK2, which act after cells enter the cell cycle, CDK3 interacts specifically with cyclin C to drive the exit from the G0 phase, a quiescent (resting) state, by phosphorylating RB1 at serine 807/811 during the G0–G1 transition.[52] In addition, CDK3 may promote G1–S transition, probably by involving the activation of E2F1, E2F2, and E2F3 in an RB1-independent manner.

Two other CDKs that bind to cyclin D at the G1 phase are CDK4 and CDK6.[53,54] These two CDKs have recently become the focus of anticancer research, primarily because they form a complex with cyclin D1. Mice lacking cyclin D1 are completely resistant to breast cancer driven by ErbB-2 (HER2).[55] Moreover, the development of mammary tumors induced by ErbB-2 is prohibited by the inactivation of CDK4, thereby underlining the role of the cyclin D1–CDK4 complex in breast cancer.[56] Because aberrations of the p16–cyclin D–CDK4–RB pathway are common in most cancers, the development of selective CDK4 inhibitors has launched promising efforts to target tumors displaying either cyclin D1 overexpression (e.g., breast cancer, mantle cell lymphoma, MM) or CDK4 amplification (e.g., liposarcoma).[57] In addition, cyclin D-dependent CDK4/6 phosphorylates various substrates (e.g., RB1 and its relatives RBL1 and RBL2, SMAD2, SMAD3, FOXM1, and MEP50) that form a central node in a signaling network that governs the overall transcriptional and other biological responses to the activation or inhibition of the kinases.[58] Due to their essential

role in cell cycle progression, cyclin D–CDK4 and cyclin D–CDK6 complexes represent one of the most important therapeutic targets, particularly for the treatment of breast cancers that often overexpress cyclin D1.[59] As a result, CDK4/6 inhibitors (e.g., palbociclib, abemaciclib, and rebociclib), in combination with hormone therapy, have been approved to treat hormone receptor-positive, HER2-negative metastatic breast cancer.[60]

CDK5, an atypical CDK, is predominantly expressed in neurons where it is activated by the non-cyclin proteins p35 and p39 or their truncated forms p25 and p29.[61] Interestingly, although CDK5 was originally considered a neuron-specific CDK, it has also been found to be expressed at high levels in several cancer types,[62] including hematologic malignancies (e.g., MM), thereby representing a potential therapeutic target.[63] Moreover, CDK5 may serve as a prognostic marker to identify patients with MM who are most likely to respond to treatment (e.g., bortezomib).[64]

CDK6 in complex with D-type cyclins plays a redundant role with CDK4 in cell cycle regulation (particularly promoting G1–S transition), and thus, it may be a therapeutic target in cancer treatment.[60] However, CDK6 has a distinct role in tumorigenesis as well under certain circumstances. For example, AML cells carrying mixed-lineage leukemia (*MLL*) rearrangements (e.g., *MLL-AF9*, *MLL-AF4*, and *MLL-AF6*) specifically rely on CDK6, but not on CDK4, to proliferate,[65] thereby suggesting that CDK6 is a target specifically in MLL-driven leukemia.[66] Interestingly, the stabilization of the CDK6 protein by SUMOylation contributes to the progression of some tumors such as glioblastoma.[67] Recent findings suggest that CDK6 has additional substrates and functions beyond cell cycle regulation. For example, CDK6 physically and functionally interacts with p65 (RelA), a key component of the NF-κB transcription factor complex, at defined chromatin regions and transcriptionally activates several NF-κB target genes, including the cytokines and chemokines involved in inflammation and cancer.[68] Interestingly, the expression of $p21^{cip1}$, a specific protein inhibitor of the enzymatic activity of several CDKs, including CDK4/6, is regulated by NF-κB in a p53-independent manner.[69] This event is associated with G1 arrest and differentiation of hematopoietic cells, and its disruption may enhance the antitumor activity of differentiation-inducing agents such as epigenetic HDAC inhibitors.[70,71]

Three CDKs, namely, CDK7 ($p40^{MO15}$), CDK8, and CDK9, have been characterized to control gene transcription. Among these enzymes, CDK7 is the only kinase that plays dual roles in the regulation of both the cell cycle and gene transcription. On the one hand, CDK7 interacts with cyclin H and acts as a CDK-activating kinase to completely activate nearly all cell cycle regulatory CDKs (e.g., CDK1, CDK2, CDK4, and CDK6) by phosphorylating their T loops in a context-specific manner,[72] such as threonine 161 of CDK1.[73] CDK7 is required to determine the cyclin specificity and activation order of CDK1 and CDK2 during the S and G2 phases as well as to maintain the activity of CDK4 as cells exit quiescence and progress to G1 through the restriction (R) point.[74] On the other hand, CDK7 is a key component of the general transcription factor TFIIH (CDK7/cyclin H/Mat1 complex). In this case, CDK7 phosphorylates serine 5 and 7 residues at the carboxy-terminal domain (CTD) of RNA polymerase II, which is responsible for transcription initiation and promoter clearance, a critical step in switching from initiation to elongation during transcription.[75] Pharmacological inhibition of CDK7, as a transcription factor that co-opts the general transcriptional machinery to sustain the oncogenic state, represents a novel approach to treat several cancer types, including T-cell acute lymphoblastic leukemia,[76] triple-negative breast cancer,[77] and glioma,[78] which may be particularly addictive to transcription.

CDK8, or its paralog CDK19, with whom it shares approximately 91% sequence homology, is a subunit of the large Mediator complex (~1.2 MDa), which is composed of 25–30 proteins and acts as a molecular bridge between DNA-binding transcription factors and RNA polymerase II.[79] Although CDK8 and CDK19 interact with different partners due to their diverse C-terminal tails, these two proteins share a particularly high degree of sequence conservation in two critical regions (i.e., the kinase and cyclin-binding domains). CDK8 or CDK19 binds to cyclin C, MED12, and MED13 in a mutually exclusive manner in the Mediator kinase module, which is involved in regulating the gene transcription of nearly all RNA polymerase II-dependent genes.[80] CDK19 may form a Mediator kinase module distinct from the CDK8 module, and therefore, regulate different transcriptional programs.[81] The cyclin C–CDK8 complex facilitates the phosphorylation of both serine 2 and serine 5 at the CTD of RNA polymerase II.[82] However, CDK8 can perform either positive or negative functions in transcriptional regulation during different transcription stages (e.g., preinitiation and elongation), thus providing a mechanism for responding to different promoter contexts (e.g., transcription factors or CDK8 module binding).[79] Unlike CDK7 and CDK9, both of which govern global gene expression, CDK8 appears to promote only gene-specific transcription. In this context, CDK8 and CDK19 can act as drivers or suppressors of tumorigenesis in a context-dependent manner.[83] CDK8 expression has been detected in 70% of colorectal cancers and correlated with β-catenin activation,[84] where CDK8 directly antagonizes the suppression of β-catenin transcription by the transcription factor E2F1.[85] As the suppression of β-catenin by E2F1 contributes to apoptosis, overexpression of CDK8 (and RB) accounts for reduced rates of apoptosis and increased cell growth. In tumor cells with CDK8 depletion, CDK19 may be required for cell proliferation and serve as a regulator of p53 stress response independent of its kinase activity.[86] CDK19 knockdown in these cells reduces the expression of mitotic genes but activates the genes associated with cholesterol metabolism and the p53 pathway.

CDK9 plays an essential role in transcription elongation by RNA polymerase II in eukaryotes.[87] CDK9, as a catalytic subunit with two isoforms (CDK9-p42 and CDK9-p55), partners with the 87-kDa regulatory subunit cyclin T with three isoforms (T1, T2a, and T2b), to form a complex known as positive transcription elongation factor (P-TEFb).[88] However, CDK9 preferentially binds cyclin T1 to form P-TEFb, which hyperphosphorylates the CTD (primarily serine 2) of RNA polymerase II, an event that is essential for transcription elongation. In addition, P-TEFb phosphorylates negative transcription elongation factors (N-TEFs), including DRB-sensitivity inducing factor (DSIF) and negative elongation factor (NELF), to release the transcription block (a pause immediately after transcription initiation) of both N-TEFs present on the hypophosphorylated forms of RNA polymerase II. Other binding partners of CDK9 include inhibitory HEXIM1 (MAQ1) or HEXIM2 and 7SK snRNP, which sequester P-TEFb in an inactive complex to inhibit transcription elongation.[89] After release from the 7SK/HEXIM complex, P-TEFb becomes active in complexes such as BRD4/P-TEFb and super elongation complex (SEC).[89] Moreover, emerging evidence suggests that CDK9 acts as a signaling hub

for transcriptional control and thus plays essential roles in the entire process of gene transcription, including initiation, elongation, and termination.[90] In addition, CDK9 plays a role in rRNA processing via the activation of RNA polymerase II.[91] Interestingly, although mitotic chromosomes have long been considered to be highly compacted, thereby rendering them ineligible for transcription, mitotic transcriptional activation is identified as a key step to control the transcription of genes in mitosis. In contrast, the inhibition of P-TEFb during mitosis results in delays in the progression of cell division.[92] These findings suggest a novel link between the regulation of transcription and the cell cycle, particularly mitosis.

In normal cells, P-TEFb activity is stringently controlled in a functional equilibrium to accommodate transcriptional demands for different biological activities.[93] As a rule in oncogenic transformation, the upregulation of pro-survival genes in transformed cells must be sustained by constitutive RNA polymerase II activity that governs transcription elongation; here, CDK9 is the primary factor responsible for such processivity.[94] In this context, transformed cells are addicted to transcription because of the requirement for the continuous production of anti-apoptotic proteins, particularly those with short half-lives (e.g., Mcl-1). Indeed, abnormal activities in the CDK9-related pathway occur in several human cancers.[95] For example, the high levels of CDK9 and/or cyclin T1 expression are observed in several types of hematologic malignancies, including B- and T-cell precursor-derived lymphomas, anaplastic large cell lymphoma, follicular lymphomas, and MM.[94] Moreover, strong nuclear staining for both proteins is observed in Hodgkin and Reed–Sternberg cells of classical Hodgkin's lymphoma. In addition, the P-TEFb complex interacts with the Tat element of HIV-1 to mediate the latter's transcription, thereby directly linking this CDK to the replication pathway of HIV.[96]

Selective CDK9 inhibitors preferentially target malignant cells in preclinical hematologic tumor models, including leukemia and MM. Mcl-1, a Bcl-2 family anti-apoptotic protein with an estimated half-life of <2–3 hours, represents one of the most important downstream targets for CDK9 inhibition.[97,98] Moreover, CDK9 inhibition disrupts the process of cytoprotective autophagy, for instance, via the downregulation of the adaptor protein SQSTM1/p62, resulting in an "inefficient" form of autophagy due to cargo-loading failure; this event, in turn, triggers apoptosis via the upregulation of the pro-apoptotic BH3-only protein NBK/Bik.[99] CDK inhibitors also induce the upregulation of other BH3-only proteins such as Bim and Noxa.[100] Although whether this event stems from the inhibition of specific CDK(s) is yet to be determined, transcription-regulatory CDKs (e.g., CDK7 and CDK9), in addition to cell cycle-regulatory CDKs (e.g., CDK4 and CDK6), clearly represent another attractive class of therapeutic targets in cancer. The SEC is an alternative target. SEC contains P-TEFb, together with AF4/FMR2 (AFF) family proteins (AFF1–4), the YEATS domain-containing proteins ENL or AF9 (encoded by *MLLT1* and *MLLT3*), the Pol II elongation factor eleven-nineteen lysine-rich leukemia (ELL), and ELL-associated factor 1 (EAF1) or EAF2. Two first-in-class compounds that disrupt the interaction between the SEC scaffolding protein AFF4 and P-TEFb and exhibit promising activity in MYC-driven cancer have been identified.[101]

CDK10 and CDK11 are defined as a novel category of CDKs known as PITSLRE protein kinases. CDK10 was initially suggested to play a role in the G_2–M transition. The CDK10 gene encodes two different CDK-like putative kinases; these two isoforms are present in most human tissues, except in the brain and muscle, and the relative isoform levels do not vary during the cell cycle. In fact, the two CDK10 isoforms exhibit different functions: one isoform is involved in the G2–M transition, whereas the other splicing form interacts with the transcription factor Ets2.[102] In the latter case, CDK10 binds Ets2 and inhibits its activity. Cyclin M is identified as the activating partner of CDK10, and the mutations of the cyclin M gene affecting its interaction with CDK10 results in CDK10 inactivation.[103] The cyclin M–CDK10 complex phosphorylates Ets2, leading to its proteasomal degradation. CDK10 interacts with the N-terminus of Ets2, which contains a highly conserved pointed trans-activation domain. As the pointed domain is implicated in protein–protein interactions, Ets2 requires an intact pointed domain to bind CDK10, thereby inhibiting Ets2 transactivation in mammalian cells.[102] This event may be particularly important for the development of follicular lymphoma because CDK10 is overexpressed in this entity.[104] CDK10 promoters are frequently hypermethylated in cancer, resulting in the low expression levels of CDK10 and impaired cell cycle regulation.[105] CDK10 silencing increases the Ets2-driven transcription of c-RAF, causing the activation of the mitogen-activated protein kinase (MAPK) pathway and loss of tumor cell reliance upon estrogen signaling, which confers resistance to endocrine therapy in breast cancer.[105]

CDK11, in association with cyclin L, is characterized as a member of a large family of p34(cdc2)-related kinases with functions that appear to be linked to cell cycle progression, tumorigenesis, and apoptotic signaling.[106] CDK11 has been reported to be a product of two duplicated genes, *CDK11A* (*CDC2L1*) and *CDK11B* (*CDC2L2*). In addition, CDK11 has two isoforms, CDK11-p110 and CDK11-p58, each of which has different functions. The p58 isoform is involved in centrosome maturation, bipolar spindle formation, and the maintenance of sister chromatid cohesion,[107,108] and the p110 isoform forms a complex with cyclin L to promote pre-RNA splicing.[109] Moreover, CDK11 has roles in the regulation of apoptosis and autophagy,[110] as well as several other functions. For example, CDK11 interacts with the p47 subunit of eukaryotic translational initiation factor 3 (eIF3) during apoptosis and is thus directly involved in cell death mechanisms.[111] Alternatively, CDK11-p110 is cleaved by caspase 8 to generate a p46 fragment that promotes Fas- or TNFα-induced apoptosis. Casein kinase 2 (CK2) phosphorylates the amino-terminal domain of CDK11, which suggests that CDK11 participates in signaling pathways involving CK2 that help coordinate RNA transcriptional regulation and processing events.[106] Moreover, CDK11 stabilizes the microtubule assembly and therefore is mandatory for the maintenance of sister chromatid cohesion.[112] A disruption of this CDK11 function may contribute to the development of cancer.

CDK12 and its paralog CDK13 are unusually large proteins that contain a central kinase domain. These proteins were initially characterized to interact with both isoforms of cyclin L (cyclins L1 and L2), which appear to be involved in the regulation of alterative RNA splicing.[113] However, CDK12 was later identified as another transcription elongation-associated CTD kinase[114] that binds to cyclin K to form the cyclin K–CDK12 complex, which is essential for the phosphorylation of the CTD (preferably serine 2) of RNA polymerase II.[115] Interestingly, the cyclin K–CDK12 complex only regulates the expression of a small subset of genes, predominantly long genes with high exon numbers and DDR genes, including critical regulators of genomic stability, such as *BRCA1*, *ATR*, *FANCI*, and *FANCD2*.[116]

Consistent with these findings, the inhibition of CDK12 in tumor cells results in gene length-dependent elongation defects, including loss of expression of long (>45 kb) genes, most of which participate in DDR.[117] The relatively longer length of DDR genes in comparison with that of other genes provides a rationale for the particular susceptibility of DDR genes to CDK12 inhibition. Moreover, CDK12 and CDK13 associate with a large number of splicing factors and positively regulate their expression; depleting either of the kinases could down-regulate gene expression and result in defects in RNA processing without a marked effect on the global CTD phosphorylation of RNA polymerase II.[118] Similar to CDK1, CDK12 phosphorylates 4E-BP1 (e.g., threonine 37 and 46), thereby promoting the translation of mTOR-dependent mRNAs, including those required for MYC transformation as well as several other subunits of mitotic and centromere/centrosome complexes.[119] This finding suggests the role of CDK12 in maintaining mitotic chromosome stability. Because genomic alterations in CDK12 have been detected in several cancer types, increasing evidence suggests that CDK12 is a potential biomarker and therapeutic target.[120]

CDK18 belongs to the PCTAIRE family of CDKs, which includes human CDK16 and CDK17, all of which share a conserved PCTAIRE amino acid sequence in the region for the binding of cyclins. CDK18 was first described as a neuronal kinase that phosphorylates Tau proteins associated with Alzheimer's disease. However, since then, it has been identified as a regulator of genome stability in other cell types.[121] Depletion of CDK18 leads to the accumulation of cells in the early S phase accompanied by an increase in DNA damage and chromosome abnormalities. CDK18 interacts with multiple DNA repair proteins, such as RAD9, RAD17, and TOPBP1, in response to replication stress. Therefore, CDK18 may play a rate-limiting role in replication stress-triggered signaling.

4. CONCLUSIONS AND PERSPECTIVES

The cell cycle of normal cells is closely monitored and finely regulated by the cell cycle regulatory machinery via a complicated but well-orchestrated signaling network. Although this network primarily involves CDKs (e.g., CDK1-3 and 4/6) and their partner cyclins (A–E), a variety of CDK inhibitors, DDR-related proteins (those associated with cell cycle checkpoints, DNA repair mechanisms, and apoptosis), and several other related molecules have not been described in our review owing to article length considerations. Because uncontrolled cell proliferation via the cell division cycle represents one of the hallmarks for cancer (including hematologic malignancies), it is not surprising that genetic and epigenetic abnormalities occur most frequently in pathways related to cell cycle regulation and involve CDKs, cyclins, CDK inhibitors, checkpoint kinases, and DNA repair genes, among numerous other genes and proteins. Moreover, many of these pathways can be activated in tumor cells in response to various stimuli and stresses, including chemotherapy, radiation therapy, and targeted therapy, most, if not all, of which impair sensitivity or confer resistance to these treatments. Therefore, these pathways have long been recognized as among the most important targets for cancer treatment. Although few successes in the development of small-molecule inhibitors targeting cell cycle regulatory or related pathways have been achieved, recent progress in the use of CDK4 and CDK6 inhibitors for treating breast cancer has reignited the enthusiasm for the research and development of agents targeting various CDKs and DDR signaling components (e.g., p53, Chk1, Wee1, ATM, ATR, Aurora kinases, and Polo-like kinases). Moreover, an increasing understanding of the roles of CDKs (CDK7–9, CDK12, and, probably, CDK13) in the transcription-regulatory machinery has evoked tremendous interest in novel targeted therapy paradigms, including the ongoing development of inhibitors targeting CDK7, CDK9, and BRD4. Certain cancer types (e.g., MYC-driven tumors, including lymphoma and MM with B-cell origin) proposed to be addicted to transcription, also known as transcriptional dependency, would be particularly susceptible to these agents. Although substantial research remains to be conducted, the future of this field appears extremely exciting and promising.

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