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Review Article
Cyclic Dependent Kinases (CDKs) in Cancer Pathogenesis and Therapeutics

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Abstract

The cell cycle is the process by which mammalian cells regulate proliferation and has S, M, G2 and G1 phase. Loss of cell cycle control and increased resistance to apoptosis represent major hallmarks of cancer. To review the role of cyclic dependent kinase in carcinogenesis and its contribution as a target in drug discovery and development, as well as cancer therapeutics. Reports and publications were found in the peer-reviewed and grey literature through academic search engines and web searches. The studies were reviewed and explored in more depth. Cellular proliferation, driven by CDKs and their cyclin partners is deregulated in cancer, therefore, cancer is regarded as a proliferative disorder and targeting the cell cycle, therefore, seems to be a good strategy for new targeted anticancer agents. The CDKs are a family of serine per threonine kinases, whose activity is tightly associated with specific cyclin co-factors and at least 12 separate genetic loci are known to code for the CDKs. The CDKs are generally classified into two major groups, based on whether they control cell cycle progression (CDK1-CDK6) or regulate gene transcription by RNAPII (CDK 7, CDK8, CDK9 and CDK19). Increases in level of CDKs are observed in cancer. Inhibition of CDKs, which are the key regulators of the cell cycle and RNA transcription, represents an attractive strategy for cancer therapy.

Key words: Cancer, cell cycle, CDK, therapeutic target, drug discovery, cancer therapeutics

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INTRODUCTION

The cell cycle is the process by which mammalian cells regulate proliferation and has 4 functional phases: S phase when DNA replication occurs, M phase when DNA and cellular components are divided to form 2 daughter cells, the G2 phase between S and M when cells prepare for mitosis and the G1 phase after mitosis and before phase S when cells commit and prepare for another round of DNA and cellular replication[1-3]. The two major processes common to all cell cycles are S phase, when chromosomes are replicated and M phase, when the replicated chromosomes are segregated into 2 daughter cells. In most cell cycles, an interval of time G1 phase, separates the previous cell division from the beginning of the next S phase[4]. An orderly progression between these phases is tightly controlled at 'checkpoints' by the interplay of various cyclins and their associated CDKs[3].

Cell division requires staged expression of genes in response to growth factors, which induce cell growth from quiescence or maintain competency for cell cycle progression during periods of active proliferation[5]. The R of G1 was originally described as the point, where cell proliferation becomes independent of mitogens and growth factors and the normal function of the restriction point is essential for maintaining control of cellular proliferation[1,2].

The R is controlled by the retinoblastoma pathway (CDK4/6-cyclin D1-Rb-p16/ink4a). The Rb and p53 are tumor suppressors that inhibit proliferation through binding to and suppressing the activity of the E2F family of transcription factors. In early G1, when conditions are favorable for proliferation, D-type cyclin levels increase through transcriptional and posttranscriptional mechanisms. Sustained proliferative capacity is a hallmark of cancer[1,2,6].

Proliferative disorders such as cancer are associated with somatic mutations and genomic instability, which are generally caused by errors in DNA replication or mitosis. Premature entry into either S or M phase increases the probability of error and hence multiple levels of cell cycle control machinery are dedicated to ensuring that this does not occur[7]. Loss of cell cycle control and increased resistance to apoptosis represent major hallmarks of cancer[8]. The metastatic cascade integrates the invasive growth of cancer cells in primary tumors and their subsequent dissemination to vital organs[9].

Using temperature-sensitive yeast mutants, Lee Hartwell first identified CDC genes as key regulators of cell division some 40 years ago. Paul Nurse subsequently found the human homologues to these genes and named the family CDKs. In the early 1980s Tim Hunt discovered cyclin molecules in his studies of sea urchin egg division. These molecules were named on the basis of their cyclical appearance and were found to play an important role in binding and activating CDK proteins[3]. The CDC is a complex series of events that culminates in the duplication of the genome and segregation of replicated chromosomes into daughter cells. The decision to enter into the cell cycle in eukaryotes is made during G1, a time when cells are poised to transduce growth factor signals that ultimately interface with the basic cell division machinery composed of CDKs[10].

Cell cycle progression, including G1-S phase transition is governed by a complex network of biochemical interactions involving the activity of essential components, the CDKs. Because of its intrinsic complexity, cell cycle progression can be regulated at many molecular levels and distinct CDKs play pivotal roles in G1-S transition, cancer development and metastasis in different neural cell types[11-14]. It is now firmly established that progression of the cell cycle that is transitions between one phase of the cycle and the next are controlled by CDKs[4,15]. Deregulated activity of CDK results in loss of cell cycle checkpoint function and increased expression of antiapoptotic proteins, which has been directly linked to the molecular pathology of cancer[8]. Given the inherent linearity of CDK-cyclin activation during the cell cycle, it was long believed that loss of an individual CDK would have deleterious effects on cellular proliferation and embryonic development. Alterations in the mechanisms governing the cell cycle are considered a 'hallmark of cancer' and result in uncontrolled cellular proliferation[3].

HALLMARKS OF CANCER

The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis[16].

One of the hallmarks of cancer is uncontrolled cell proliferation, leading to malignant tumor development. Dysregulation of cell cycle progression, such as evasion of multiple cell cycle checkpoint can be caused by abnormal activation of two key classes of regulatory molecules, cyclins and CDK[3,8,17-19]. Resistance to apoptosis is the other hallmark of cancer. Oncogenic transformation involves multiple genetic modifications that frequently include up-regulated antiapoptotic and pro-survival components. The Bcl-2 family

proteins consisting of both anti-apoptotic and pro-apoptotic proteins are the main regulators of apoptotic processes[20].

Many human cancers depend on the deregulated expression of MYC family members for their aberrant growth and proliferation, with elevated expression of these oncogenes predicting aggressive disease and a poor clinical outcome. Deactivation of MYC in cell lines and MYC-induced transgenic tumors causes proliferative arrest and tumor regression, suggesting that effective targeting of MYC proteins would have broad therapeutic benefit[21].

Underlying these hallmarks is genome instability, which generates the genetic diversity that expedites their acquisition and inflammation, which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list-reprogramming of energy metabolism and evading immune destruction. Recognition of the widespread applicability of these concepts will increasingly affect the development of new means to treat human cancer[16].

A portrayal of this circuitry that is aligned with individual hallmarks of cancer. Thus, the intracellular integrated circuit can be segmented into distinct subcircuits, each of which is specialized to support a discrete cell-biological property in normal cells and is reprogrammed in order to implement a hallmark capability in cancer cells[16].

Therapeutic targeting: The introduction of mechanism-based targeted therapies to treat human cancers has been heralded as one of the fruits of three decades of remarkable progress of research into the mechanisms of cancer pathogenesis[16]. Cellular proliferation, driven by CDKs and their cyclin partners is deregulated in cancer[18,22,23], therefore, cancer is regarded as a proliferative disorder[20,24] and targeting the cell cycle, therefore, seems to be a good strategy for new targeted anticancer agents[24]. Cancer cells often appear to demonstrate oncogene (ras, cyclin-D, erbB, myc, sis, etc.) addiction for anti-apoptotic proteins in order to maintain their survival advantage and resist apoptosis[25]. Cancer cells appear to depend heavily on antiapoptotic proteins for survival and so targeted inhibition of these proteins has therapeutic potential. One innovative strategy is to inhibit the CDKs responsible for the regulation of RNAPII[26].

The selective cotargeting of multiple cores and emerging hallmark capabilities and enabling characteristics in mechanism-guided combinations will result in more effective and durable therapies for human cancer. The investigational anticancer drugs are being developed to target each of the enabling characteristics and emerging hallmarks, which also hold promise as cancer therapeutics[16].

CYCLIC DEPENDENT KINASES (CDKs)

The human protein kinases set (kinome) is constituted of 518 identified proteins, divided in 7 families. The CDKs are part of the CMGC family named after the members: CDKs, MAPKs, GSKs and CLKs. The CDK sub-family comprises 13 members (CDK1-CDK13). For their discovery, Hartwell, Nurse and Hunt received the Nobel Prize in 2001[27].

The CDKs are a family of serine/threonine kinases, whose activity is tightly associated with specific cyclin co-factors[2,8,18,24,28-31] and at least 12 separate genetic loci are known to code for the CDKs[3]. Over the last decade, more than 20 CDKs have been characterized and are generally classified into two major groups, based on whether they control cell cycle progression or regulate gene transcription by RNAPII[2,3,8,23,27,29]. The CDKs also regulate neuron biology[14,32].

This family includes three interphase CDKs (CDK2, CDK4 and CDK6), one mitotic CDK (CDK1, previously known as CDC2) and a number of regulatory CDKs, such as CDK7 a component of the CDK-activating complex and transcriptional CDKs (CDK8, CDK9)[3]. The mammalian cell cycle is controlled by the periodic association of CDKs with their cyclin partners and kinase inhibitor proteins (for example, p21Waf1/Cip1 and p27Kip1). The primary positive and negative regulation of CDK activity is mediated by the binding of a cyclin and of a CDK1, respectively[2,3,8,23,29]. Unlike CDKs, cyclins are an extremely diverse family of proteins, subdivided into four classes (A-, B-, D- and E-type cyclins) that act as regulatory subunits of the CDKcyclin holoenzyme[3].

The CDKs are activated via binding to their selected cyclins in specific phases of the cell cycle, following which they phosphorylate their target proteins. The CDK1s negatively regulate the activities of CDKs and control the cell cycle. The pRB regulates G1/S progression. The p53 pathway plays a role in DNA damage response as a gatekeeper of the genome. Several lncRNAs control the expression of cyclins-CDKs, CK1s, pRB and p53 and participate in cell cycle regulation. Some of these lncRNAs are induced by DNA damage and inhibit cell cycle progression by regulating these cell cycle regulators[2,28,29].

In mammals, two families of 7 CDK1 have been identified that dimer in both structure and mechanism of action. Members of the CIP/KIP family contain three genes, p21CIP1/Waf1, p27KIP1 and p57KIP2, which inhibit CDK activity by forming a ternary p21-cyclin D-CDK4 complex. The

INK4 family inhibitors which include 4 closely related ankyrin repeat containing genes, p16INK4a, p15INK4b, p18INK4c and p19INK4d, selectively form binary complexes with CDK4 or CDK6 to prevent the CDKs from binding with and becoming activated by D-type cyclins. The main function of CDK1s is believed to couple diversified growth inhibitory signals to the cell cycle clock. In mammalian cells, two CDK enzymes, CDK4 or CDK6 in combination with three D-type cyclins (D1, D2 and D3) and CDK2 in association with cyclin E, play the principle roles in regulating G1 progression[23,28,29].

Multiple CDKs control the cell cycle which includes CDK1-CDK6, while CDK8, CDK9, CDK12 and CDK19 are linked to regulation of transcription. The first group is essential for normal proliferation, development and homeostasis. The CDK4/cyclin D, CDK6/cyclin D and CDK2/cyclin E facilitate the G1-S phase transition by sequentially pRb, while CDK1/cyclin A, CDK2/cyclin A and CDK1/cyclin B are essential for S phase progression and G2-M transition, respectively. The CDK7 and CDK20 act in both cell cycle control and transcription processes[18,20,26,29,31,33]. The G1/S transition is promoted by sequential CDK4/6/cyclin D1-mediated and CDK2/cyclin E-mediated pRb. Phosphorylation of pRb relieves transcriptional repression by the pRb-E2F complex and disrupts the binding of pRb to E2F, allowing E2F activation and transcription of genes necessary for S phase entry and progression. The CDK1/2 inhibition reduced cell proliferation and colony formation irrespective of anti-estrogen sensitivity status[23].

In an undamaged cell, progression through G1, S and G2 phase of the cell cycle is dependent on temporal activation of CDK1 and CDK2 in complex with cyclins E, A and B. The CDK1/2 usually exist in a phosphorylated and inactive form that requires dephosphoryation for activation at an appropriate time in the cell cycle. Many anticancer agents damage DNA thereby activating a cell cycle checkpoint that arrests cell cycle progression and permits repair and recovery. The arrest requires activation of Chk1 that inhibits CDC25 and thereby prevents activation of CDK1/2[34]. Inactivation of individual genes encoding members of these complexes (cyclins D1, D2, D3, E1 and E2 and CDK2, CDK4 and CDK6) has revealed that none of these proteins considered to be important for the control of the G1/S transition are essential for viability per second and that their loss causes few cell cycle defects[22].

The formation of two major protein complexes and their associated kinase activities are required for G1-S progression: cyclin D-CDK 4/6 is active in early G1 phase, whereas cyclin E-CDK2 is required for entry into S phase. The CDK1s negatively regulate the activity of these complexes[35]. The expression of dominant negative forms of CDK4 or CDK6 but not CDK2 or CDK3, protects NGF-deprived sympathetic neurons from death. The CDK family, which includes CDK2, CDK3, CDK4, CDK6 and CDC2 among others is an important group of molecules that regulate the proliferation of dividing cells. The CDK2, CDK3, CDK4 and CDK6 control G1 and S phases of the cell cycle, whereas CDC2 is an M phase regulator[36].

In response to DNA damage, cells activate a phosphorylation-based signaling cascade known as the DDR. One of the main outcomes of DDR activation is inhibition of CDK activity to restrain cell cycle progression until lesions are healed. Recent studies have revealed a reverse connection by which CDK activity modulates processing of DNA break ends and DDR activation. The genome maintenance programs of post replicative cells, including DDR are regulated by the overall level of CDK activity and not by specific CDKs. The CDK inhibition in cultured cells results in activation of the DDR[37].

Cell cycle regulation has been identified as an attractive target for targeted drug therapy. Given their kinase activity, the CDKs were pursued as drug targets[3,14]. A large number of drug discovery programs have yielded potent small molecule CDK1s, with several compounds successfully entering preclinical and early clinical trials. In general, CDKI can be broken down into two classes: first generation inhibitors such as flavopiridol, R-roscovitine and UCN-01, which tended to be less specific and broad in their ability to block a number of CDKs (pan-CDK1) and second-generation agents, that are more specific to certain CDKs. The latter group of compounds has now shown more potent activity against their targets and a more favorable safety profile[3].

Inhibition of CDKs, which are the key regulators of the cell cycle and RNA transcription, represents an attractive strategy for cancer therapy. It has been shown that the combined depletion of CDK9, CDK1 and CDK2 resulted in effective induction of apoptosis through both RNAPII CTD and E2F mediated effects[20,29,38]. Among the CDKs inhibitors presented in Table 1 it was found flavopiridol and roscovitine which were the first generation CDK inhibitors to enter clinical trials for use in anticancer therapy[3,27].

CELL CYCLE PROGRESSION REGULATORS

The cell division cycle is controlled by checkpoint mechanisms that arrest further progression if a critical process such as DNA replication or mitotic spindle assembly. Continued defective cell cycle progression could result in tumor development. The balance between cell cycle controls

Table 1: CDKs inhibitors in clinical development

Drug candidate	Company	Administration mode	CDK inhibition profile (IC_{50}, nM)	Clinical trial stage
Flavopiridol	Sanofi-Aventis	Intravenous	CDK1: 30, CDK2: 100 CDK4: 20, CDK6: 60 CDK7: 10, CDK9: 10	II
Roscovitine	Cyclacel	Oral	CDK1: 2700, CDK2: 100, CDK7: 500, CDK9: 800	II
Dinaciclib	Merck	Intravenous	CDK1: 3, CDK2: 1 CDK5: 1, CDK9: 4	III
SNS032	Sunesis	Intravenous	CDK2: 38, CDK7: 62 CDK9: 4	I
AT7519	Astex/Novartis	Intravenous	CDK1: 190, CDK2: 44 CDK4: 67, CDK5: 18 CDK9: <10	I/II
Palbociclib (PD-0332991)	Pfizer, Inc.		CDK4 (Cyclin D1): 11 CDK4 (Cyclin dD3): 9 CDK6 (Cyclin D2): 15	Approved
EM-1421	Erimos	Intravenous	CDK1: N/A	I/II
RGB-286638	Agennix	Intravenous	CDK1: 2, CDK2: 3 CDK3: 5 nM, CDK4: 4 CDK9: 1	I
P276-00	Nicholas piramal	Intravenous	CDK9: 20, CDK1: 79 CDK2: 224, CDK4: 63	II
BAY-1000394	Bayer	Oral	CDK1-4, 7, 9: 5-25	I
TG02/SG1317	S×Bio/Tragara	Oral	CDK9: 3, CDK5: 4, CDK2: 5, CDK3: 8, CDK1: 9	I
PHA-848125 AC	Nerviano	Oral	CDK1: 2, CDK2: 3 CDK4: 5, CDK5: 4	II
Ribociclib (LEE011)	Novartis	Oral	CDK4 (Cyclin D1): 10 CDK6 (Cyclin D2): 40	III
Abemaciclib (LY2835219)	Eli Lilly	Oral	CDK4 (Cyclin D1): 2 CDK6 (Cyclin D1): 9.9	III

and the threshold at which apoptosis is initiated are likely to be critical in determining the cellular response to genomic damage. Initiation of apoptosis in response to many stimuli, including oncogenes, cellular stresses, DNA-damaging agents and many chemotherapeutic drugs, involves a cysteine protease, caspase-9[39]. The CDKs, including three interphase CDKs (CDK2, CDK4 and CDK6) and a mitotic CDK (CDK1) are critical regulators of cell cycle progression in mammalian cells[38].

Entry into the cell cycle is mediated by CDK4/6 activation, followed by CDK2 activation[40]. Typically, repression of cell cycle progression is maintained via sequestration of the E2F family of transcription factors by the pRb and other so-called pocket proteins, including p107 and p130. Newly synthesized cyclin D1 goes on to form activating complexes with CDK4/CDK6, which then initiate phosphorylation of pRb. The process of phosphorylation mediated by the cyclin D1: CDK4/6 complex lifts pRb's transcriptional repression of E2F, resulting in transcription of S-phase-specific target genes[3,22]. Cell cycle dysregulation is prevalent in multiple malignancies. Progression from G1-S phase is an important checkpoint in regulating cell proliferation. Cell cycle progression through the G1 phase is regulated by the action of cyclin D-CDK4, cyclin D-CDK6 and cyclin E-CDK2. This transition is mediated through the Rb, which is regulated through sequential phosphorylations by CDK[22,41-43].

CDK1: The CDK1 (also known at CDC) is the major mitotic serine/threonine kinases that interacts with cyclin B1 to form an active heterodimer, driving progression from G2-M phase and is a key player in cell cycle regulation and particularly mitosis[20,38,44-46]. The CDK1 plays an important role in the maintenance of pluripotency and genomic stability in human pluripotent stem cells. Down regulation of CDK1 led to the loss of typical pluripotent stem cell morphology, down regulation of pluripotency markers and upregulation of a large number of differentiation markers. Thus, CDK1 has a key role in balancing survival and cell death signals to dictate cell fate during mitotic arrest[20,45,46].

The Plk1 is also the major mitotic serine/threonine kinases required for the timely progression through mitosis. The Plk1 co-ordinates a variety of cell division processes including centrosome maturation, recruitment of important mitotic spindle components and cytokinesis. Overexpression of Plk1 has a pro-survival role in tumourigenesis and its depletion leads to apoptosis. The co-operative action of CDK1 and Plk1 towards the novel substrate PTP1B during mitotic arrest is important for mitotic cell death[46]. Down regulation of CDK1 results in accumulation of DSBs and impairment of CHK2 activation. The CDK1 down regulation leads to PARP1 activation but impaired apoptosis in hESC. The CDK1/Cyclin B1 complex is able to interact and phosphorylate both pro and anti-apoptotic proteins such as bad, caspase 9, caspase 8, caspase 2, caspase 3, Bcl-2, Bcl-xl, Mcl-1 and survivin[39,47].

The CDK1 inhibition selectively reduces viability of MYC-dependent cells. The CDK1 inhibitor-induced cell apoptosis is MYC-dependent. Unlike CDK4, CDK6 and CDK2, which are redundant for the mammalian cell cycle, CDK1 is essential for cell division and sufficient for driving the cell cycle in all cell types. The CDK1 regulates chromosome condensation and microtubule dynamics to facilitate the transition from G2-M phase[38]. The function of Nedd1 is regulated by CDK1 and Plk1. The CDK1 phosphorylates Nedd1 at T550 and this phosphorylation enhances the subsequent phosphorylation of Nedd1 at T382, S397, S426 and S637 by Plk1, during mitosis[48].

The CDK1 regulates the expression of HIF-1α, independent of its known regulators. Overexpression of CDK1 and/or cyclin B1 is sufficient to stabilize HIF-1α under normoxic conditions, whereas inhibition of CDK1 enhances the proteasomal degradation of HIF-1α, reducing its half-life and steady-state levels. Inhibition of CDK1 and CDK4 dramatically reduces HIF-1 activity in both normoxic and hypoxic environments. Thus, inhibition of CDK1/4 represents a novel approach for the treatment of cancer cells with constitutively active HIF-1, regardless of whether HIF-1α is overexpressed as a result of intratumoral hypoxia or the deregulation of genetic mechanisms[49,50].

There are several reports which have attempted to establish a direct connection between CDK1 and p53. The p53-mediated transcriptional repression of CDK1 has been shown to occur through the CCAAT-binding NF-Y transcription factor. It is also believed that p53 can bind directly to the CDK1 promoter and inhibit its activity. The loss of p53 might initially induce only a small increase in CDK1 levels but the signal can be amplified in the feedback loop, resulting in even higher levels of CDK1[51].

CDK2/3: The CDK2 controls entry and progression through the DNA synthesis phase of the cell cycle and is altered in many cancer types. Aberrant expression of key regulators of CDK2, such as cyclin E and p27 is associated with a poor prognosis and shorter survival in patients with cancer[52]. Cyclin E-CDK2 kinase activity plays a central role in the regulation of cell cycle progression in mammalian cells, including glia. The time course of regulation of cyclin E-CDK2 activity is consistent with cell cycle withdrawal or arrest in G1 phase. The decrease in cyclin E-CDK2 activity is attributable to inhibition of cyclin E-CDK2 complex formation. Cyclin E and CDK2 levels and cyclin E-CDK2 activity decrease in corpus callosum during development *in vivo*. The CDK2 controls OP cell cycle progression and is down regulated in adult OP cells[12,35].

The CDK2 inactivation accompanies cell cycle arrest by MYC depletion and leads to a subsequent decrease of pRb phosphorylation and E2F1 activity, contributing to MYC RNAi-induced cell cycle arrest. Activation of CDK2, another interphase CDK is involved in MYC regulation of G1-S phase transition. As a major event downstream of MYC activation, CDK2 activation can also suppress MYC-induced senescence, which raised the possibility of CDK2 as a potential therapeutic target for MYC-dependent cancers[38]. Sensitivity to Chk1 inhibition is regulated upstream of CDK2 and correlates with accumulation of CDC25A[34].

Cyclin E, the regulatory cyclin for CDK2 is considered a requisite regulator of G1 progression. Cyclin E is overexpressed in cancer, suggesting that cyclin E/CDK2 deregulation contributes to tumorigenesis. The E type cyclins and their catalytic partner, CDK2, participate in the regulation of Rb inactivation, establishment of the pre-RC and initiation of S phase, their participation in these critical regulatory steps has resulted in the assumption that both cyclin E and CDK2 are indispensable for cell cycle progression. The CDK3 is the closest relative to CDK2 among the 9 mammalian CDK genes identified thus far and its activating cyclin subunit has yet to be identified[28,53].

The cyclin E/CDK2 substrate and cajal body component p220NPAT activates histone transcription through a novel Lish-like domain. The E-, D- and A-type cyclins function together with CDK4 and CDK2 to promote entry into and completion of DNA synthesis. The cyclin E/CDK2 substrate p220NPAT is required for S phase entry, histone gene expression and cajal body maintenance in human somatic cells[5,10,15]. Cyclin E/CDK2, a central regulator of the G1/S transition, coordinates multiple cell cycle events, including DNA replication, centrosome duplication and activation of the E2F transcriptional program. The HiNF-P, a transcriptional regulator of replication-dependent histone H4 genes, interacts directly with p220NPAT, a substrate of cyclin E/CDK2 to coactivate histone genes during S phase. The HiNF-P and p220 are targeted to and colocalize at, subnuclear foci (Cajal bodies) in a cell cycle-dependent manner[5,9,10].

Over expression of CDK2, CDK4, cyclin E and cyclin D1 has been observed in various types of tumors. Concomitant loss of both CDK2 and CDK4 has dramatic effects on the cell cycle. Embryos lacking both CDK2 and CDK4 die during embryogenesis due to heart defects. The loss of CDK2 and CDK4 leads to hypophosphorylation of Rb and therefore to a repression of CDK1 transcription. Loss of CDK4 is believed to prevent tumorigenicity in cells lacking[51] Arf or p53.

CDK4/6: Progression through the cell cycle from G1/G0 to S, G2 and M phases is initiated by CDK4 and the highly homologous enzyme CDK6. They act as master integrators in the G1 phase, coupling with the cell cycle mitogenic and antimitogenic signals as well as with their oncogenic perversions in cancer cells. It is crucial for cortical neural progenitor cell proliferation. In astrocytes, CDK4 is an essential component of cell division. They phosphorylate and inactivate the cell cycle/tumor suppressor proteins of the pRb family (p105Rb, p107 and p130Rb2) and smad3. This leads to both

E2F-dependent transcription of essential cell cycle enzymes and regulators and assembly of the prereplication complex[3,12,19,40,54-56].

The CDK6 gene is located in human chromosome 7 and is translated into a kinase with 326 amino acids. Expression of this gene is up regulated in several types of cancers. The CDK6 is the catalytic subunit of the CDK6-cyclin D complex involved in the G1-S cell cycle progression and negatively regulates cell differentiation[2]. The CDK4 and CDK6 form a complex with one of their activating subunits, which are the cyclins D1, D2 and D3. The activity of CDK4/6 is negatively regulated by the INK4 proteins. Deregulation of the G1 checkpoint is crucial for various oncogenic transformation processes[3,12,19,54-56].

The CDK4 participates in the regulation of apoptosis of nucleated cells by altering transcriptional regulation of genes governing cell proliferation and cell death[56]. Hyperphosphorylation of Rb is mediated by CDK4 and CDK6 in early G1 phase through the interaction with cyclin D. This results in Rb inactivation and release of transcription factors that allows cells progress toward S phase[57]. The CDK4 has been identified as a key MYC target gene in mammals. The CDK4-deficient mice were resistant to skin tumour development induced by MYC, whereas mice lacking cyclin D1 expression and consequently lacking CDK4 activation still developed mammary tumours induced by MYC activation, strongly arguing that the requirement for CDK4 activity in MYC-induced tumorigenesis is affected by cellular context and tissue type[38].

Increased activity of CDK4 is observed in cancer and CDK1 has been shown to induce G1 arrest and apoptosis. It is noteworthy that inhibition of CDK4 reduced the expression of both HIF-1α and HIF-2α[22,56,49]. The efficacy of CDK1 is primarily attributed to altered transcription of antiapoptosis family members, cell cycle regulators as well as p53 and NF-κB-responsive gene targets. On the other hand, CDK4 has been implicated in the regulation of neuronal cell death. Furthermore, CDK4 is a powerful modulator of mitochondrial functions via phosphorylation of mitochondrial targets[56].

The relationship of p27 and cyclin D-CDK4/6 in the cell is complex. Cyclin D-CDK4/6 complexes are mitogen sensors and have at least two main roles in the cell: One catalytic and the other noncatalytic. The catalytic activity involves the phosphorylation of Rb and related family members, p107 and p130. The noncatalytic function of cyclin D-CDK4/6 involves its ability to act as a reservoir for p27 or p21[41]. Cell cycle deregulation is crucial for various oncogenic transformation processes, suggesting that many cancer cells depend on high CDK4/6 activity[19]. The G1 restriction point is critical for regulating the cell cycle and is controlled by the Rb pathway (CDK4/6-cyclin D1-Rb-p16/ink4a). This pathway is important because of its inactivation in a majority of human tumors. Transition through the restriction point requires pRb by CDK4/6, which are highly validated cancer drug targets[1,32].

The CDK4/6 activity is negatively regulated by two families of CKIs, the INK4 (p16, p15, p18 and p19) and CIP/KIP (p21, p27 and p57) protein families. These inhibitors, while largely undetectable in cycling cells are rapidly upregulated in response to inhibitory signals, including transforming growth factor-β, contact inhibition or senescence[3,32]. The CDK4 and CDK6 are related protein kinases that bind D-type cyclins and regulate cell cycle progression. The CDK4/6 inhibitors are currently being used in advanced clinical trials and show great promise against many types of tumors[32].

The interaction of non-phosphorylated p27 with CDK4 also prevents the activating phosphorylation of the T-loop by cyclin H-CDK7, the CAK. Even though the cyclin H-CDK7 complex is present and active in contact arrested cells, p27's association with cyclin D-CDK4 prevents T-loop phosphorylation. The two modes by which p27 inhibits cyclin D-CDK4 are independent and may reinforce one another to inhibit kinase activity in contact-arrested cells, while maintaining a reservoir of preformed complex that can be activated rapidly upon cell cycle reentry[42].

More recently, CDK6 has also been shown to have a transcriptional role in tumor angiogenesis. Up-regulated CDK6 activity is associated with the development of several types of cancers. While CDK6 is over-expressed in cancer cells, it has a low detectable level in non-cancerous cells. Emerging evidence suggests that certain tumor cells require CDK6 for proliferation. Consequently, CDK6 represents a promising target for anti-cancer therapy. In contrast to CDK4, CDK6 is poorly phosphorylated, restricting its activity in a variety of systems[2,55,58].

It has been demonstrated that CDK6 and CDK4 are cyclin D-activated kinases that phosphorylate Rb and its related proteins p107 and p130 in the G1 phase of the cell cycle. The CDK6 phosphorylates the Rb and its related proteins in the G1 phase of the cell cycle, derepressing E2F. The E2F then activates the transcription of genes that encode proteins necessary for DNA replication (S phase entry). Activation of CDK6 requires binding to D-type cyclins and phosphorylation by CAK (CDK7/cyclin H/MAT1). The INK4s deactivate CDK6 and Cip/Kip proteins, acting as negative modulators of the CDK6-cyclin D-complex[2].

Transcription regulators: The CDKs are key cell cycle regulators and some also have regulatory functions in mRNA transcription at the level of RNAPII. The CDKs 1, 2, 7, 8, 9 and 11 have all been implicated in the phosphorylation of the CTD of the largest RNAPII subunit but the most important ones are CDK7-cyclin H and CDK9-cyclin T[6]. Dysregulations of mRNA transcription is common event in human cancers. Inhibition of transcriptional CDKs as an effective anti-cancer strategy has gained considerable attention following the observation that many types of cancer cells rely on the production of short-lived mitotic regulatory kinases and apoptosis regulators such as Mcl-1 for their survival. The CDK7/cyclin H is a component of TFIIH that phosphorylates the serine-5 residues within the heptad repeats of RNAPII CTD to initiate transcription. Moreover, most CDKs are activated through T-loop phosphorylation by a CAK, which in metazoans appears to be uniquely controlled by CDK7. The CDK9/cyclin T, the catalytic subunit of positive transcription elongation factor P-TEFb, phosphorylates two elongation repressors, i.e., the DSIF and the NELF and serine-2 of the CTD heptad repeats to facilitate a productive transcription elongation. The RNAPII-mediated transcription initiation and elongation is regulated by the CDK7 and CDK9, which phosphorylate the CTD of RNAPII[14,25,21,31].

The studies show that specifically targeting individual cell cycle CDKs may not be an optimal therapeutic approach. Inhibition of the transcriptional CDKs is on the other hand, a better strategy because the most sensitive transcripts are those with short half-lives that encode cell cycle regulators, mitotic regulatory kinases and apoptosis regulators such as Mcl-1 and Bcl-2, rendering this group of kinases ideal candidates for blocking MYC-dependent transcriptional amplification[20,21,58]. The CDKIs have shown remarkable activity in cancers, where its efficacy has been linked to inhibition of the transcriptional CDKs (7 and 9) and deregulation of RNA polymerase and short-lived pro-survival proteins such as Mcl-1. Apoptosis preceded inactivation of RNA polymerase and were accompanied by phosphorylation of stress kinases JNK and p38 MAPK. Pharmacologic inhibitors of JNK/p38 MAPK conferred protection from P1446 amediated apoptosis. Inhibition of CDK7/9 results in reduced global transcription and decreased synthesis of short-lived anti-apoptotic proteins such as Mcl1, thus promoting apoptosis[18,58].

The CDK8/cyclin C and CDK11/cyclin L are involved in mRNA splicing. The CDKs 12 and 13 (both activated by cyclin K) regulate, like CDK9, transcription elongation by phosphorylating the RNAPII CTD. Inhibitors of CDKs have been reported to have activities in many types of cancer cells by inhibiting CDK7 and CDK9, which control transcription. Thus, the identification of more selective and potent CDK inhibitors is imperative for the development of effective cancer therapies[13,14].

Over the past decade, the intensive search for pharmacological CDKIs has led to several clinical candidates and the focus on transcriptional CDKs has underlined their antitumor activity. Flavopiridol, the first and currently most promising CDK1 in preclinical and clinical trials has demonstrated marked antitumor activity in many types of cancer. Flavopiridol acts largely through inhibition of CDK9. When RNAPII is repressed after CDK9 inhibition, the result is a blockage of transcriptional elongation, which in turn causes decreases in the cellular levels of short-lived proteins including some antiapoptotic molecules such as Mcl-1 and XIAP and thus promotes the induction of apoptosis[14].

CDK7: Besides its role in CDK activation, CDK7 is also known as the CAK as a component of the GTF TFIIH and is involved in transcription initiation, phosphorylates the CTD of RNAPII and is thus required for its activity which activates other CDKs by phosphorylation of the activation segment. It also participates in the full activation of CDKs by promoting phosphorylation of the conserved threonine residue within the T-loop region of these kinases. The CDK7 kinase activity has been implicated in the regulation of transcription, where it phosphorylates the CTD of RNAPII and CDK9 and the cell cycle, where it functions as the CAK for CDKs1/2/4/6. The CDK7 controls mRNA synthesis by affecting stability of preinitiation complexes, leading to altered gene expression, cell cycle progression and survival of tumor cells[6,7,18,30,55,59-62].

Since the presence, activity and nuclear localization of CAK (CDK7) are generally constitutive and non-regulated during cell cycle or mitogenic stimulations, activating phosphorylation of CDK4/CDK6. Binding of p27 to cyclin D-CDK4/CDK6 also impairs their phosphorylation by CDK7. The CDK7 phosphorylates and activates both CDK4 and CDK6 *in vitro*. However, CDK6, like CDK2 could be efficiently phosphorylated by CDK7 in the absence of a cyclin, whereas CDK4 phosphorylation by CDK7 absolutely requires its binding to a cyclin D[55]. Apart from having a role in RNA transcription, CDK7 is also a CAK, which phosphorylates and activates multiple cell cycle CDKs[31].

The CDK7 binds preferentially to the sumoylation deficient form of SF-1 and that CDK7 inhibition reduces phosphorylation of SF-1. The SF-1 can be phosphorylated on

residue S203 by either ERK1/2 or CDK7. Given that CDK7 is a unique CDK kinase that functions both to facilitate cell cycle progression and to regulate transcriptional activation. The CDK7 kinase binds more avidly to a non-sumoylatable form of SF-1 than to the wild-type (WT) protein. Thus, it appears that the inhibition of SF-1-mediated transcription by sumoylation in adrenocortical cancer cells is mediated through inhibition of CDK7-induced phosphorylation of SF-1[62,63]. The inhibition of CDK7 may induce to block transcription and cell cycle progression. As a member of CDK family, CDK7 shares high structural homology with CDK2. Thus, a deep understanding of molecular mechanism of ligand-specific recognitions towards CDK2 and CDK7, respectively and kinetics of ligand-receptor interactions may be implicated as an important knowledge in the rational design of isoform selective inhibitors[62].

Pharmacological modulation of CDK7 kinase activity may thus provide an approach to identify and treat tumor types exhibiting extreme dependencies on transcription for maintenance of the oncogenic state[63]. The CDK7 inhibition, by selectively targeting the mechanisms that promote global transcriptional amplification in tumor cells, may be useful therapy for cancers that are driven by MYC family oncoproteins[21,55].

CDK9: The CDK9, a member of the CDK family and belongs to a family of 13 protein kinases that share sequence homology and dependence upon the binding of a cyclin subunit for activation, associates with T-type cyclins to form P-TEFb[59,64,65]. The CDK9 is required for cell survival and in complex with T-type cyclins is recruited to promoters, where it stimulates transcriptional elongation by phosphorylating the CTD of RNAPII and NELF. The CDK9 is a key elongation factor for RNA transcription and functions by phosphorylating the CTD of RNAPII[25,31,64].

The CDK9 is the catalytic subunit of the P-TEFb and is critical for stimulation of transcription elongation, which phosphorylates the CTD of RNAPII and NELFs enabling for productive elongation after initiation. The CDK9 associates with T-type cyclins and cyclin K and its activity is tightly regulated in cells at different levels. The CDK9 is also the catalytic subunit of TAK, essential for HIV1 replication. The CDK9 is a potential therapeutic target in cancer, AIDS, inflammation and cardiomyophathy. The nature of the strategy used to inhibit CDK9 profoundly affects the patterns of gene expression resulting from CDK9 inhibition. These results suggest multiple variables that affect outcome, including kinetics of inhibition, potency, off-target effects and selectivity issues. This is particularly important when considering CDK9 as a potential target for therapeutic intervention[18,24,65].

This enzyme is critical for stimulating transcription elongation of most protein coding genes, including key developmental and stimulus-responsive genes, by RNAPII. The RNAPII is paused soon after transcription initiation by DSIF and NELF. The CDK9 is then recruited to the paused transcription complex where it phosphorylates DSIF and NELF as well as the RNAPII CTD and thereby releases RNAPII from the pause site. Activity of CDK9 is dependent on binding to a regulatory cyclin subunit (cyclin T1, T2a or T2b) and is further regulated through association with other macromolecules. These modulators include activators like c-MYC, NF- kB, AR, Brd4 or subunits of the super elongation complex and inhibitory proteins or complexes such as the inhibitory 7SK snRNA containing complex[59].

Unlike other CDKs, CDK9 appears to function exclusively in transcriptional regulation and does not regulate the cell cycle but promotes RNA synthesis in genetic programmes for cell growth, differentiation and viral pathogenesis. It forms complexes with cyclin T1, T2 or K, which participate in the P-TEFb. The CDK9 phosphorylates both Ser-2 and Ser-5 of the CTD heptad, playing a predominant role during transcriptional elongation, in contrast to CDK7, which primarily phosphorylates Ser-5 of RNAPII at the promoter as part of transcriptional initiation[6,24]. The CDK9 was discovered in the context of HIV research because retroviruses hijack host transcription and CDK9 inhibitors might become specific antiretroviral agents, particularly as they might prevent drug resistance. Myocardial hypertrophy is a risk factor in congestive heart failure and is characterised by derepressed CDK9 activity[24].

In addition, CDK9 may have important implication in the Mnk-eIF4E axis, the key determinants of PI3K/Akt/mTOR and Ras/Raf/MAPK-mediated tumorigenic activity. This causes dysregulation of cellular transcription is a fundamental hallmark of cancer. Subsequently, there is a decreased anti-apoptotic proteins Mcl-1 and Bcl-2 and induced apoptosis[25,31]. Apoptosis is preceded by a decrease in the levels of Mcl-1 protein and transcript possibly due to inhibition of CDK9[66]. Specific inhibition of CDK9 activity with dnCDK9 leads to a distinctive pattern of changes in gene expression, with more genes being specifically up regulated than down regulated. Inhibition of CDK9 activity in human cells results in up regulation and down regulation of a distinct set of genes, rather than general down-modulation of genes with short-lived transcripts[64].

The CDK9 has a typical protein kinase fold consisting of an N and a C-terminal kinase lobe and a short C-terminal extension. The ATP-binding site is located between the N and C-terminal lobes and contains highly conserved active site residues that coordinate ATP binding and phosphotransfer. Like other CDKs, CDK9 is activated by the association with a cyclin, cyclin T and phosphorylation of a threonine residue in the activation segment, T186. By compositing the family of inhibitor-bound CDK9 structures that adopt a glycine-rich loop down conformation, it is possible to define the space in the ATP-binding site that can be exploited in the design and further optimization of high affinity CDK9 inhibitors[59].

To date, over 20 potent CDKIs undergo phase I-II clinical trials in patients with different cancers. Although the drugs target several CDKs, it has been proposed that the induction of apoptosis arises primarily due to the inhibition of CDK9. Inhibition of transcription (brought about by inhibiting CDK9) leads to a rapid decrease not only of D-type cyclins, that support uncontrolled proliferation but also of antiapoptotic proteins XIAP and Mcl-1[59].

The targeted inhibition of CDK9 might prove to be a useful therapeutic strategy in cancers in which Mcl-1 is over expressed[25]. The CDKIs are being developed as potential cancer therapeutics based on the promise that they may counteract the unchecked proliferation of cancer cells by targeting the cell cycle regulatory functions of CDKs[26]. Although there are strong signs that CDK9 inhibition would be a useful therapeutic strategy in all cancer, HIV and cardiology indications, the lack of selective inhibitors has so far confounded clinical development[24]. Drugs that target cellular CDK9 kinase activity and down-regulate the RNAPII phosphorylation are considered as CDK9 inhibitors. The CDK9 inhibition as an effective anti-cancer strategy has gained strong support in recognizing that cancer cells rely on the production of short-lived apoptosis regulators and mitotic regulatory kinases for survival. In contrast to CDK7, CDK9 appears to have a minimal effect on cell cycle regulation[31].

The CDK9 inhibitors suppress the expression of anti-apoptotic proteins Mcl-1 and Bcl-2, leading to caspase-3/7 activation a nd PARP cleavage. In addition, MDM2 protein level is reduced and this is accompanied by the up-regulation of p53. The CDK9 inhibitor treated cells decreased Mcl-1, Bcl-2, procaspase-3/7 protein levels and induced cleaved PARP. It also down-regulates the phosphorylation of RNAPII and eIF4E. The CDK9 inhibition causes the down-regulation of Mnk1[31]. The CDK9 inhibitor is consistently the most potent inducer of apoptosis of the MLL-AF9-driven leukemia cells. This apoptosis correlated with suppression of CDK9 enzymatic activity and downregulation of previously identified MLL-AF9-target genes HoxA9 and Meis1. In addition to its crucial role in phosphorylating the CTD of RNA Pol II and regulating gene expression, CDK9 also functionally interacts with other cellular proteins including MyoD, p53, pRb and c-MYC and can affect diverse biological processes including cell differentiation, survival and quiescence. The inhibition of CDK9 enzymatic activity resulted in rapid dephosphorylation of RNA Pol II and induction of apoptosis in AML cells and Mcl-1 is a gene crucial for this response. Proteins with relatively short half-lives such as Mcl-1 appear to be selectively reduced following CDK9 inhibition[10,58,67].

Pharmacotherapy of CDK targeting agents: Personalizing the use of cancer therapeutics is a major focus of current cancer research[68]. Palbo ciclib showed significant hematologic toxicity and dose reduction for hematologic toxicity was required for 24% of patients in clinical trials[31]. Cancer cells develop dependence on other genes and pathways in order to overcome antitumorigenic effects, such as apoptosis and senescence that result from activation of MYC[38]. Resistance can be circumvented by inhibiting Wee1 kinase and thereby directly activating CDK2[34]. Data seemed to suggest that pharmacological inhibition of the cyclin D1: CDK4/6 axis in cancers may be both efficacious and relatively non-toxic. However, the initial clinical experience with broad specificity, first-generation CDK inhibitors proved to be disappointing, yielding poor efficacy and significant toxicity and raising the question of whether these agents failed due to poor pharmacologic characteristics and/or specificities of the compounds or a less essential role of CDK signaling in cancer. Additionally, lack of appropriate patient selection and/or lack of predictive markers of response may have also contributed to these initial clinical failures. Recently, the development of more specific CDK1s has renewed interest in targeting the cell cycle as a novel therapeutic approach in cancer[3,68].

CONCLUSION AND RECOMMENDATIONS

The CDKs are enzymes that control the cell cycle progression and transcription. Deregulation of CDKs results in imbalance in proliferation and apoptosis which is a hall mark of a cancer. Inhibition of CDKs, which are the key regulators of the cell-cycle and RNA transcription, represents an attractive strategy for cancer therapy. There are numerous drug candidates in clinical trial stages but no CDK1 is released yet. Therefore, more study is needed in drug discovery and development to come with CDK1s.

SIGNIFICANCE STATEMENT

This manuscript compiled in detail review of the description of CDK and its discovery, types and contribution of CDKs in cancer pathogenesis; importance of CDK as a drug target in cancer drug discovery and development and also discussed drugs that target CDK under clinical development.

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