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CANCER CHEMOTHERAPY AND CYCLIN DEPENDENT KINASES

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ABSTRACT

Protein kinases play a central role in the regulation of various cell processes including proliferation, cell cycle, differentiation and apoptosis through phosphorylation. Dysregulation of these critical cellular processes, due to the abnormal expression of some of these proteins, is common in many cancers. CDK inhibition can initiate apoptosis and could be particularly useful in treating of various malignancies. In many human cancers CDK/Cyclins are dysregulated, which affect the coordinated cycle of cell growth and proliferation leading contribution to the uncontrolled proliferation characteristic of cancer cells. Therefore the pharmacological inhibition of CDKs can cause cell cycle arrest and induces apoptosis selectively in transformed cells. To date, significant progress has been made in the development of specific CDKs inhibitors. However, the main drawback is to know which CDK or CDK/cyclin should be targeted for selective action so that other vital cellular function can be avoided. After Palbociclib, drugs like dinaciclib, PHA-848125 AC, BAY-1000394 are leading the way. This article reviews about the role and clinical implications of CDKs in cancer chemotherapy, rationale for targeting CDKs and strategies in development of CDK inhibitors.

KEYWORDS: Cyclin dependent kinase, imatinib, cancer chemotherapy.

INTRODUCTION

Since the development of tyrosine kinase inhibitor, imatinib, as a new anticancer drug, protein kinases became clearly validated drug targets for cancer therapy. Protein kinases play a central role in the regulation of various cell processes including proliferation, cell cycle, differentiation and apoptosis through phosphorylation.[1] Cyclin-dependent kinases (CDK) are serine/threonine proline-directed kinases belong to CMGC group play an important role in a number of physiological functions, including the control of the cell cycle and/or proliferation and transcription processes**.** [2] CDKs are inactive as such and forms functional heterodimeric complexes upon binding to a family of regulatory proteins, cyclins - that provide domains essential for enzymatic activity. Dysregulation of these critical cellular processes, due to the abnormal expression of some of these proteins, is common in many cancers. CDK inhibition can initiate apoptosis and could be particularly useful in treating of various malignancies.[3] Over the past decade, a number of pharmacological inhibitors of CDKs (CDKIs) belonging to different chemical substances have been developed, and some of them have been tested in a number of clinical trials. This article reviews about the role and clinical implications of CDKs in cancer chemotherapy, rationale for targeting CDKs and strategies in development of CDK inhibitors.

ROLE OF CDK/CYCLINS IN CELL CYCLE

Over View of Cell Cycle

Cell cycle is a tightly regulated biological system that occurs between the successive divisions of a cell through which a cell duplicates its genome, grows, and divides. Each individual cell receives a number of signals from outside of the cell which are integrated and processed by the cell cycle regulatory system, and the cell makes a decision that it should be divided or is quiescent. There are four phases of cell cycle namely the Gap-1 (G1), DNA Synthesis (S), Gap-2 (G2), and Mitosis (M) phases. In addition to these phases, an extended phase of Gap-1 phase is coined as Gap-0 (G0) phase or resting phase.[4]

All these cell cycle phases are regulated by both the positive as well as negative regulatory proteins i.e. Cyclins, CDKs and other regulatory inhibitor proteins (CKDIs). To date, although 20 different CDKs and same number of cyclins have been identified in mammalian cells but only few of them have defined roles in cell cycle. Based on function the CDKs can be grouped into two groups. First, the CDKs that mediate cell progression (CDK1, CDK2, CDK3, CDK4, CDK6), and the second group, the CDKs that regulate transcription (CDK7,

CDK8, CDK9 and CDK11). All these CDKs initiate ifferent stages from G1 to M phase. [5] The various functions and role of different CDKs are summarized in the table 1.

Figure 1: Functions of Cyclin-Dependent Kinases: [3]

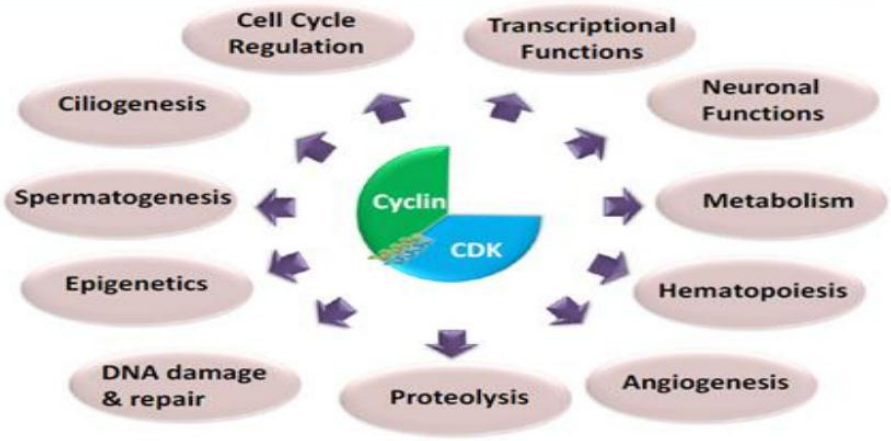

Table 1: Functions of CDK complexes in cell cycle.[6]

CDK	In complex with	Cell cycle function
CDK 1	Cyclin A Cyclin B	G2 – M progression # Nuclear envelope breakdown # Mitotic condensation # Spindle assembly
CDK 2	Cyclin E Cyclin A	G1 – S progression (DNA replication) # Hyper-phosphorylation of RB # Centrosome duplication # Induction of histone synthesis # Phosphorylation of replication factors
CDK 3	Cyclin C	G1 phase : DNA damage repair
CDK 4 and 6	Cyclin D	G1-S progression: # Phosphorylation of RB stimulates E2F
CDK 5	P39, P 35	Neuronal Viability (G1 – S control) # Phosphorylation of RB
CDK 7	Cyclin H	Basal transcriptional processes: # Transcription initiation # Transcriptional elongation # RNA processing
CDK 8	Cyclin C	
CDK 9	Cyclin T	
CDK 11	Cyclin L	

***Bona Fide* Cell cycle CDK/Cyclins in the cell cycle**

CDK1, CDK2, CDK4 and CDK6 and their associated Cyclins A, B, D, E can be considered as "*bona fide* cell cycle regulators" as they are regulating the cell growth, division and involved in the tight and timely control of cell cycle progression.[3] CDK 1 regulates the transition from Gap-2 (G2) to Mitosis (M) phase; CDK-2, CDK-4 and CDK-6 regulate the

transition from Gap-1 to DNA synthesis – S phase. In cell cycle, G1 and G2 phases act as check points for progression by ensuring all the necessary steps that have been completed before entering to the next phase. During G1 phase, the cell has two options at the restriction or R point that either to continue the cycling or entering the G-0, a non-diving phase.[7]

When quiescent cells (G0 phase) are stimulated to get into the cycle by mitogenic growth factors, mostly through Ras-signaling pathway, expression of D-type cyclins promote progression through G1 phase by activating CDK4 and CDK6 and thereby promoting phosphorylation of Retinoblastoma pocket protein. Phosphorylation of retinoblastoma inactivates its function as transcriptional repressor that leads to de-repression of E2F transcription factor as well as consequent expression of genes which are required for G1/S transition, including Cyclin E in the late phase of G1 (Figure 2).

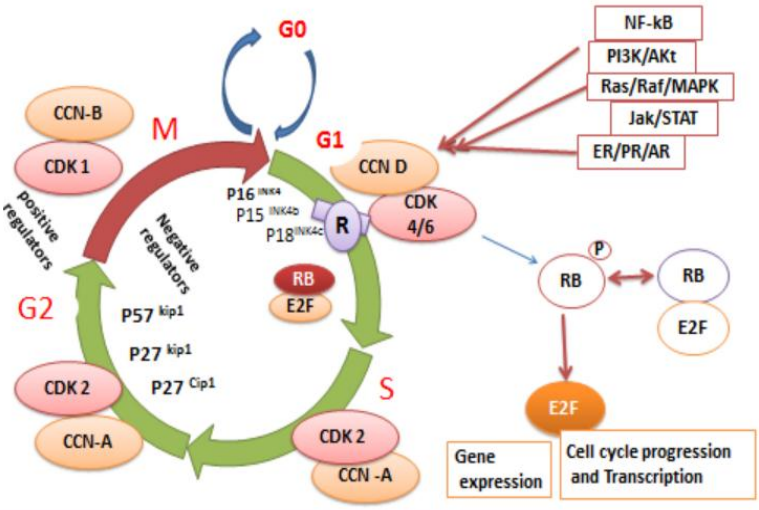

Figure 2: The cell cycle and regulatory process:[7] **AR**: androgen receptor; **CDK:** cyclin-dependent kinase; **ER,** estrogen receptor; **NF-κB**, nuclear factor κB; **PR,** progesterone receptor; **RB**, retinoblastoma; **R**, restriction point; **P,** phosphate; **p16INK4, p15INK4b, p18INK4:** CDK-interacting protein/kinase inhibitory protein (Cip/Kip) family.

This in turn allows activation of CDK2/cyclin E, which further phosphorylates retinoblastoma, thereby promoting complete release of E2F factors. So released E2F initiates subsequent progression through cell cycle by inducing its maximal action as transcriptional activator.

Transcriptional CDK/Cyclins

There are three CDKs (CDK7, CDK8, and CDK9) which regulate transcription regulation. They facilitate efficient transcriptional initiation and elongation through phosphorylation by targeting the C-terminal domain (CTD) of RNA polymerase II (Pol II).[8] Inhibition of these

CDKs alter the accumulation of transcripts with short half-lives which include those encoding anti-apoptosis family members, cell cycle regulators, as well as p53 and NF-kB-responsive gene targets.

CDK ABERRATIONS ASSOCIATED WITH CANCERS.

Any amount of aberrations in the CDKs can lead to unchecked cell division and can result in mutation. Occurrence of various cancers have been to traced back and found to be originated from the aberrations in CDKs. Table 2 summarizes the above mentioned facts.[3,6,8]

Target	Deregulation	Cancer
CDK1	Overexpression	B lymphoma, advanced melanoma
CDK 2	Overexpression	Laryngeal squamous cell cancer
CDK 3	Overexpression	Glioblastoma
CDK 4	Amplification	Osteosarcoma, glioblastoma
	overexpression	Melanoma, lung cancer
	Amplification/ overexpression	Uterine cervix cancer, sporadic breast cancer
CDK 5	Amplification/overexpression	Pancreatic cancer and breast cancer
CDK 6	Amplification	Squamous cell carcinoma
CDK 7	24 simple coding mutation, 1 nonsense substitution, 19 missense mutations, 3 synonymous mutations.	Solid cancers
CDK 8	Overexpression, amplification, 65 simple coding mutation, 9 nonsense substitution, 42 missense mutations, 12 synonymous mutations, 2 in-frame deletions.	Colon, colorectal and gastric cancer
CDK 9	Over expression, mutation	Lymphoma and neuroblastoma
CDK 10	Down regulation	Biliary tract cancer

RATIONALE FOR TARGETING OF CELL CYCLE CDKs.

The regulation of CDKs activity mainly depends on:

- binding by activating cyclins
- binding by inhibitory cyclin-dependent kinase inhibitors (CKIs)
- inhibitory phosphorylation of the CDK, and
- activating phosphorylation of the CDK

Hyper activation of CDKs results from one of several causes including gene amplification, protein overexpression of either CDKs or Cyclin subunits, alternative splicing, expression of truncated cyclin variants.[3] In many human cancers CDK/Cyclins are dysregulated, which

affect the coordinated cycle of cell growth and proliferation leading contribution to the uncontrolled proliferation characteristic of cancer cells. Therefore the pharmacological inhibition of CDKs can cause cell cycle arrest and induces apoptosis selectively in transformed cells.[9]

- Inhibition of cdk4/6 leads to potent Rb-dependent G1 arrest
- Inhibition of cdk4/6, cdk2, and cdk1 results in arrest at the G1-S and G2-M boundaries
- More selective inhibition of cdk2 and cdk1 leads to less potent G1 arrest, S and G2 effects, and E2F dependent apoptosis
- Inhibition of cdk9 preferentially depletes mRNAs with short half-lives (eg, Mcl-1, cyclin D1, c-myc, p53-induced p21Cip1, and hypoxia-induced VEGF)

STRATEGIES FOR TARGETING CELL CYCLE CDKs

In the past 20 years, different strategies for targeting the cell cycle have been described. The traditional strategies have focused at targeting cancer cells by interfering with DNA integrity/ replication and mitosis (targeting tubulin and Mitotic kinases – polo-like kinase 1 and aurora kinases) through alkylating agents, anti-metabolites, topoisomerase inhibitor drug administration. At present, the attention has been shifted towards targeting chemical inhibition of Cyclin dependent kinase catalytic activity.[10]

These strategies can be divided into

a) Direct acting compounds that target catalytic subunit of CDK by interacting with ATP-binding site of CDKs; these drugs provide the opportunity for rationale design of drugs,

b) Indirect compounds that target the regulatory pathway that regulate CDK activity, i.e. by altering the synthesis and expression of CDK/Cyclin subunits or inhibition of phosphorylation of CDKs.

DRUGS TARGETING CYCLIN DEPENDENT KINASE

ATP- competitive inhibitors

The great majority of known CDK inhibitors are ATP-competitive drugs. They interact with CDKs within their catalytic ATP site. To date, this strategy has been the most successful one in order to develop powerful inhibition of CDKs implicated in cell cycle.[1,11]

First generation.

Purine analogues: Roscovitine , Flavopiridol

Second generation

Purine analogue: R- roscovitine (seleciclib)

Others: Dinaciclib, palbociclib, bemiciclib, SNS032, EM-1421, RGB-286638

Flavopiridol

It is also called alvocidib and is a flavonoid derived alkaloid from an Indian plant "rohitukine". This ATP competitive drug was jointly developed by safoni-Aventis and the US NCI. It exhibits a broad-spectrum anticancer activity.[12] It is active against CDKs 1, 2, 4, 6 and 7 by interacting with the adenosine triphosphate (ATP) binding site. It also inhibits the CDK9/cyclin T complex, broadly repressing transcription and decreasing cyclin D1 mRNA expression. It induces cell-cycle arrest in G1 and is cytotoxic to cells undergoing DNA synthesis. It also inhibits kinases like PKC and PKA at higher concentrations, inducing apoptosis, and is active in many xenograft models.

R- Roscovitine[13]

- Is highly selective, oral, small molecule known as CYC202 or Roscovitine
- Is a 2,6,9-tri-substituted purine analogue of olomoucine
- Entered into clinical trials by Cyclacel pharmaceuticals
- Inhibits primarily CDK1,-2,-7 and -9
- Poor compound for inhibition of CDK 4, CDK6.
- Currently evaluated as a potential drug to treat cancers, neurodegenerative diseases, inflammation, viral infections, polycystic kidney disease and glomerulonephritis A

The phase I clinical trial with R- roscovitine had shown no tumour response, but disease stabilization was observed in 38% patients (8/21), when treated with doses of 100, 200 and 800 mg twice daily. The dose-limiting toxicities which developed at 800 mg were fatigue, skin rash, hyponatremia and hypokalaemia. Reversible abnormal liver function and emesis were also seen.[14] In another phase I clinical trial which was done with the 56 patients of advanced solid malignancies who were treated according to three schedules: schedule A consisted of 5 consecutive days every 3 weeks with1600 mg bid, schedule B of 10 consecutive days with 800mg bid followed by 2 weeks off and schedule C of 3 consecutive days every 2 weeks with 1800mg bid. One patient was responded partially while six other patients achieved tumour stabilization. Patient in schedule A, B,C had shown intolerable toxicities with asthenia, nausea, vomiting and hypokalaemia.[15]

Palbociclib: First Global Approval

- Palbociclib is an oral, reversible, selective, small molecule inhibitor of CDK4 and CDK6
- Developed by Pfizer for the treatment of cancer
- Received breakthrough therapy designation from the US FDA in April 2013 and the New Drug Application (NDA), based on final data from the phase II PALOMA-1 trial, was accepted for priority review in October 2014.
- It has got accelerated approval in the US in February 2015 based on clinical data available from a phase III Paloma II trial, for the first-line systemic treatment of postmenopausal women with estrogen receptor (ER)-positive, HER 2-negative locally advanced or metastatic breast cancer along with leterozole .
- PALOMA II study has shown the median progression-free survival of 9.2 months with palbociclib–fulvestrant and 3.8 months with placebo–fulvestrant.[16]
- Available as "IBRANCE®"
- Dosage: 125 mg once daily, orally for 21 days followed by 7 days off treatment in a 28-day cycle.

Dinaciclib[17]

- It is a novel, Potent CDK inhibitor and also known as MK-7965, SCH727965
- Developed by Merck for the treatment of cancer
- Selectively inhibits CDK1,CDK2, CDK5 and CDK9
- Inhibits cellular proliferation and induces apoptosis in cancer cells by suppressing Rb phosphorylation
- The most common adverse effects developed with dinaciclib are nausea, anemia, neutropenia, vomiting and fatigue.
- Used in advanced breast cancer treatment, as well as in other solid and haematological malignancies.

AT7519[18,19]

- Developed by Astex/Novartis pharmaceuticals
- Inhibits CDK1, CDK2,CDK4, CDK5, CDK9 and affects cell cycle regulation
- Also a potent inhibitor of RNA polymerase II dependent transcription
- In phase trial in the treatment of MYCN-dependent neuroblastoma.

Milciclib[20]

- Also called PHA-848125
- Developed by Nerviano pharmaceuticals
- Selective towards CDK1, CDK2, CDK5
- Currently in phase II clinical trials for treating thymic carcinoma

BAY-1000394

- Developed by Bayer pharmaceuticals
- Inhibits CDK1, CDK2,CDK3,CDK9
- IC value range is 5-25Nm
- Currently in phase-I trials.

CONCLUSION

CDKs play a critical role in cell biology, especially in proliferation control and transcription processes. To date, significant progress has been made in the development of specific CDKs inhibitors. However, the main drawback is to know which CDK or CDK/cyclin should be targeted for selective action so that other vital cellular function can be avoided. After Palbociclib, drugs like dinaciclib, PHA-848125 AC, BAY-1000394 are leading the way. Nevertheless, there are many information regarding the efficacy and safety are required to get to know about the implications of CDKs in cell cycle as well as in tumour growth.

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