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Review Article

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**CYCLIN DEPENDENT KINASES (CDK) – A KEY REGULATORY PROTEIN**

**Sumit Dadhwal<sup>1</sup>, Pushpa Sharma\*<sup>1</sup>, Gurkirpal Singh<sup>1</sup>, Pankaj Garg<sup>1</sup>, Sakshi Sharma<sup>2</sup>, DK Sharma<sup>3</sup>**

<sup>1</sup>M.M. College of Pharmacy, Maharishi Markandeshwar University, Mullana, (Ambala), Haryana, India.

<sup>2</sup>Ranbaxy Laboratories (Gurgaon), Haryana, India.

<sup>3</sup>Surya School of Pharmacy, Bapror, (Patiala), Punjab, India.

*Email: pushpamullana@rediffmail.com*

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**Abstract**

Cell is the basic unit of the life and cancer is the major disease of the uncontrolled cell division and cell proliferation. Cell cycle is regulated by the CDK's and is the main site of action for many anticancer therapeutic agents. Flavopiridol is a one of the promising anticancer agent acting against cancerous cells by inhibiting the CDK's activity. It is the first CDK's inhibitor going in the clinical trials phase-II. Different analogues are reported that are investigated for their anticancer activity. The review deals with cell cycle, CDK regulations of cell cycle, role of Flavopiridol in cell cycle regulation of cancerous cells.

**Key Words:** Flavopiridol, Cyclin Dependent Kinase, Cyclin kinase inhibitor, Cell Cycle

**Introduction**

Many diseases have afflicted humankind since antiquity and can persist with serious medical consequences throughout a patient's life time. Cancer is one of them, related with cell proliferation and cell growth and is increasing day by day. It is one of the major diseases affecting the major population without having any effective measures of treatment. Cancer is primarily an environmental disease with 90-95% of cases due to lifestyle and environmental factors and 5-10% due to genetics [1]. Every year, at least 200,000 people die worldwide from cancer related to their workplace. Millions of workers run the risk of developing cancers such as lung cancer and mesothelioma from inhaling asbestos fibers and tobacco smoke, or leukaemia from exposure to benzene at their workplaces. Currently, most cancer deaths caused

by occupational risk factors occur in the developed world. It is estimated that approximately 20,000 cancer deaths and 40,000 new cases of cancer each year in the U.S. are attributable to occupation. Cancer therapy has been dominated by chemotherapeutic agents from last many decades. The different cancer treating agents have different mode of action related mostly with either inhibition of cell proliferation or the others as killing the cancerous cells. The Cyclin-dependent kinases represent a class of enzymes that play a central role in cell-cycle progression and cellular proliferation [2]. Flavopiridol is the first CDK inhibitor to undergo clinical trials against a variety of cancers. Flavopiridol was shown to inhibit the proliferation of mammalian cell lines at nanomolar concentrations. Flavopiridol is non-selective, showing in vitro activity against CDK1, CDK2, CDK4 and protein-tyrosine kinase, with some activity for the EGF-receptor tyrosine kinase [3]. Kinetic studies have shown that Flavopiridol binds at the ATP binding site of the CDKs. The total synthesis of Flavopiridol and some SAR around Flavopiridol has been reported.

### **CDK: Cyclin-dependent kinases**

Cyclin-dependent kinases (CDK), a family of serine/threonine protein kinases are activated at definite points of the cell cycle. Transition from one cell cycle phase to another phase regulated by different cellular proteins. Key regulatory proteins are the Cyclin-dependent kinases (CDK).

### **Types of CDK**

Nine CDK have been identified until now, out of these, five are active during the cell cycle, i.e. during G1 (CDK4, CDK6 and CDK2), S (CDK2), G2 and M (CDK1) Table 1. When CDK is in active state, downstream processes are induced by after phosphorylating selected proteins [5]. CDK7 and Cyclin H act in combination as CDK activating kinase (CAK) [6]. The remaining CDK have not yet been shown to have an essential role in normal cell cycle progression [7-8]. During the cell cycle, CDK protein levels remain stable, in contrast to their activating proteins, the Cyclins. The level of cyclin protein rise and fall during the cell cycle and in this way they episodically activate CDK [9-10].

**Table1. Cyclin-CDK complexes are activated at specific points of the cell cycle. CAK, CDK activating kinase.**

CDK	Cyclin	Cell cycle phase activity
CDK4	Cyclin D1, D2, D3	G1 phase
CDK6	Cyclin D1, D2, D3	G1 phase
CDK2	Cyclin E	G1/S phase transition
CDK2	Cyclin A	S phase
CDK1 (cdc2)	Cyclin A	G2/M phase transition
CDK1 (cdc2)	Cyclin B	Mitosis
CDK7	Cyclin H	CAK, all cell cycle phase

### CELL CYCLE REGULATION BY CDK

At different phases of the cell cycle (Table 1) different Cyclins are required. Binding of three Cyclin D (Cyclin D1, Cyclin D2, Cyclin D3) to CDK4 and to CDK6 form complexes which is essential for entry in G1 [11]. Cyclin D is synthesized as long as growth factor stimulation persists [12], but it is not expressed periodically. Cyclin E is another G1 Cyclin which associates with CDK2 to regulate progression from G1 into S phase [13]. Binding of Cyclin A and CDK2 form complex, this complex is required during S phase [14-15]. During late G2 and early M, Cyclin A and CDK1 form complexes to promote entry into M. Further mitosis is regulated by Cyclin B and CDK1 complex [16-17]. Sixteen Cyclins have been identified but not all of them are cell cycle related [18-20]. Destruction box is contained by Cyclin A & B and PEST sequence (proline (P), glutamic acid (E), serine (S) and threonine (T) residues) is contained by

Cyclin D & E. These are sequence of proteins required for efficient ubiquitin-mediated Cyclin proteolysis at the end of a cell cycle phase [21-22].

In addition to Cyclin binding, phosphorylation on conserved threonine and tyrosine residues also regulates CDK activity. Phosphorylation of threonine 161 (threonine 172 in CDK4 and threonine 160 in CDK2), brought about by the CDK7-Cyclin H complex, also called CAK which is required for full activation of CDK. In CDK, conformational changes and enhancement in the binding of Cyclins is induced by phosphorylation [23-24]. The Myt1 and Wee1 kinesis phosphorylate CDK1 at threonine-14 and /or tyrosine-15, thereby inactivating the kinase .Dephosphorylation by the enzyme Cdc25 at these sites is required for activation of CDK1 and further development through the cell cycle [25].

### **CDK INHIBITORS**

The inhibitors which inhibit the activity of CDK are known as cell cycle inhibitory proteins, called CDK inhibitors which both bind to CDK alone or to the CDK-Cyclin complex and control CDK activity.

Two different families of CDK inhibitors have been reported

1. INK4 family
2. Cip / Kip family (Table 2) [26]

INK4 family includes p15 (INK4b), p16 (INK4a), p18 (INK4c), p19 (INK4d), which particularly inactivate G1 CDK (CDK4 and CDK6). Before Cyclin binding to CDK enzyme, CKI form strong complex with CDK enzyme which prevent association with Cyclin D [27]. The Cip / Kip family includes p21 (Waf1, Cip1), p27 (Cip2), p57 (Kip2). CDK-Cyclin complexes are inhibited by these inhibitors [28-30]. They inhibit the G1 CDK Cyclin complexes, and CDK1-Cyclin B complexes but they inhibit CDK1-Cyclin B complexes to a lesser extent [31]. DNA synthesis also inhibited by p27, it binds to and inhibits the proliferating cell nuclear antigen[32-33]. Internal and external signal regulate the CKI i.e. the expression of p21 is transcriptional controlled by the p53 tumour suppressor gene. p53-binding site is present in p21 gene promoter, that allows p53 to transcriptionally activate the p21 gene [34]. Respectively, the expression and activation of p15 and p27, increases in response to transforming growth factor- $\beta$  (TGF-  $\beta$ ), contributing

to growth arrest [35-36]. A different intracellular localized, cell cycle-regulating protein also contributes to a correct cell cycle progression. Nuclear exclusion signal is present in Cyclin B and is actively exported from the nucleus until the beginning of the prophase. The CDK inactivating kinases Wee1 and Myt1 in the Golgi complex and nucleus protect the cell from premature mitosis [37-38].

### **SUBSTRATE OF CDK**

The target proteins become phosphorylated on CDK consensus sites, when CDK is active, resulting in changes that is physiologically important for cell cycle progression. CDK4 / 6-Cyclin D is the most frequently studied target i.e. the product of the retinoblastoma tumour suppressor gene (pRb). pRb becomes phosphorylate during early G1, and this leads to disruption of the complex with the histone deacetylase protein and release of E2F-1 and DP-1, the transcription factors which regulate the transcription of genes whose products are required for S phase progression, including Cyclin A, Cyclin E, Cdc25 [39-41]. For the remainder of the cell cycle, pRb remains hyperphosphorylated, CDK2-Cyclin E participates in maintaining this hyperphosphorylated state. CDK2-Cyclin E complex also phosphorylates its inhibitor p27, inducing its proteasome-dependent degradation [42-43]. CDK2-Cyclin E also phosphorylate NPAT (nuclear protein mapped to the ATM locus) which is nuclear protein mapped to the ATM locus. At the G1 / S boundary, the protein level of NPAT peaks, and is thought to play a role in S phase entry [44]. Phosphorylation of Histone H1 by CDK2-Cyclin E is important for chromosome condensation required during DNA replication. For CDK1-Cyclin B, Histone H1 is also a substrate [45]. The initiation of DNA replication is regulated by Cyclin A-dependent kinases by phosphorylation of DNA polymerase alpha primase [46]. There are other CDK substrates, which include CDK's own regulators Wee1 and Cdc25, and cytoskeletal proteins such as nuclear microtubules, lamins and vimentin, which are necessary for correct mitosis [47-50].

### **DK DURING CANCER**

In cancer, alterations in CDK molecules have been reported with low frequency. Role of CDK in nuclear replication of DNA viruses is investigated previously [53]. Over expression of CDK4 occurs as a result of

amplification that has been identified in cell lines, sarcoma, glioma and melanoma [54]. Due to mutation in CDK4 and CDK6 genes results in loss of CKI binding have also been identified [55]. Over expression of CDK1 and CDK2 have been reported in a subset of colon adenomas, and greater over expression was seen in focal carcinomas in adenomatous tissue [56-57].

### **CYCLINS DURING CANCER**

Cyclin D act as growth sensor, it provides a link between cell Cycle mitogenic stimuli and cell cycle. In early G1, Cyclin D1 binds to CDK4 and CDK6. Abnormal expression by Cyclin D1 has been reported in many human cancers. Earlier study implicated Cyclin D1 in human tumours; its gene was linked in parathyroid adenomas to the parathyroid hormone gene [58][60]. Translocation of Cyclin D1 gene is associated with B-cell malignancies, including mantle cell lymphoma. Amplification of Cyclin D1 gene, occurs in, oesophageal, bladder, breast, lung and squamous cell carcinomas [59]. Over expressed Cyclin D2 and Cyclin D3 have also been reported, in some tumours and Cyclin E has been found to be over expressed, amplified or both in some cases of colon and breast cancer and in acute lymphoblastic and acute myeloid leukaemia [60-64]. In lung carcinoma, both Cyclin A and Cyclin E are over expressed and prominent expression of Cyclin A but not Cyclin E correlated with shorter survival [65].

### **ACTIVATION OF CDK DURING CANCER**

CDK activation is regulated through Dephosphorylation by members of the Cdc25 phosphatase family. During G1 / S-phase transition, Cdc25A plays an important role, during S-phase Cdc25B activation occurs and CDK1-Cyclin B becomes activated during entry into mitosis by Cdc25C. Unscheduled activation of CDK-Cyclins occurs due to over expression or deregulation of Cdc25 which can be associated with tumour formation. The potential human oncogenes are Cdc25A and Cdc25B [66]. Cdc25B is over expressed in 32% of primary breast cancers. An oncogene c-myc found to be found to be frequently mutated in human cancers. Transcription of Cdc25A and Cdc25B genes is activated by c-myc [67].

## **CKI AS GROWTH INHIBITORS DURING CANCER**

CKI shows inhibitory activity by growth suppression through activation of pRb. Alteration in the gene p16 occurs in a high percentage of human tumours and can be inactivated by a variety of mechanisms including point mutations, deletion and hyper-methylation. Through G1, Cells with altered p16 will be unrestrained to proceed. Specific inhibitor of CDK-Cyclin is p16 protein, which prevents the phosphorylation of the pRb protein and arresting cells in G1 phase (Table 2). CDK / Cyclin D and pRb, p16 are cell cycle regulators. They are functionally interconnected, perturbations in any of these cell cycle regulators are likely to have similar consequences. In nearly all human cancers, alterations of at least one of these regulators are found.[68-72]

## **ROLE OF pRb DURING CANCER**

The most important CDK substrate during G1 is pRb, which is frequently mutated in human lung cancer and retinoblastoma [73-74]. Truncated, non-functional pRb results due to deletion and mis-sense mutations, while binding of certain tumor virus proteins (e.g. human papilloma viruses (HPV) E7, adenovirus E1A[75-76]. Loss or Absence of pRb function is associated with uncontrolled cell cycle progression and is common in acute lymphoblastic leukaemia [77-78]. Abnormalities in some component of the pRb pathway occurs around 90% of human cancers [74].

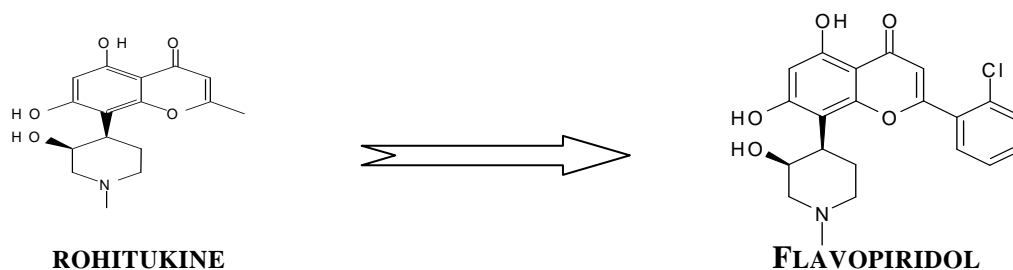
## **CHECK POINT DURING CANCER**

In all types of cancer, mutations of checkpoint proteins are frequent. Protein p53 is the tumour suppressor protein which is a sequence-specific DNA-binding protein that is able to induce apoptosis or either cell cycle arrest at the cell cycle checkpoints. But now this p53 gene becomes the most frequently mutated gene in human cancer [79-80]. Conformational changes and inactivation of the protein mainly occurs due to point and mis-sense mutations [81].

## **FLAVOPIRIDOL: A PROMISING ANTICANCER AGENT**

Flavopiridol is a derivative of Rohitukine which is obtained from indigenous plant *Dysoxylum binectariferum*. It is a potential anticancer agent, the first potent Cyclin-dependent kinase inhibitor to enter

clinical trials [83-85]. Flavopiridol inhibits proliferation [86-89] and induces apoptosis in a range of human cancer cells and cell lines [90-98].



### RECENT APPROACH OF FLAVOPIRIDOL TOWARDS CANCER

Flavopiridol is investigated for treatment of hormone-refractory prostate cancer [99,101]. It induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity in vivo against human leukaemia and lymphoma xenografts [100]. It also stimulates the ATPase activity of MRP1 (multi drug resistant proteins) in a dose-dependent way [101]. Also it has been shown to potently inhibit CDK1 and 2 (Cyclin-dependent kinases 1 and 2) and most recently it has been found that it also inhibits CDK9 [102]. Effect of P-glycoprotein (Pgp) on Flavopiridol sensitivity is observed by many researchers. It is found that Flavopiridol is less active in cells that over express Pgp [103]. Flavopiridol down regulates hypoxia mediated hypoxia-inducible factor-1 $\alpha$  expression in human glioma cells by a proteasome-independent pathway: Implications for in vivo therapy [104]. It induces mitochondrial injury and cell death in human leukaemia cells that over express bcl-2 [105]. The metabolism of Flavopiridol, a novel anticancer drug currently undergoing clinical development, was investigated in rat and human liver microsomes [106]. It induced apoptosis in hematopoietic cell lines is also researched out by researchers [107]. Study of acquired cellular resistance to Flavopiridol in a human colon carcinoma cell is also previously reported [108]. Phase II study of Flavopiridol in patients with advanced colorectal cancer is also reported [109]. It helps in suppressing tumour necrosis factor-induced activation of activator protein-1 [110]. It inhibits transcription, mechanism of chronic lymphocytic leukaemia cell death [111]. It induces G<sub>2</sub> arrest with inhibition of Cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells [112]. Flavopiridol



Metabolism in Cancer Patients is Associated with the Occurrence of Diarrhea [113]. Potently it induces Small Cell Lung Cancer Apoptosis during S Phase [114]. Phase I study of Flavopiridol with oxaliplatin and fluorouracil / leucovorin in advanced solid tumours is also performed [115]. Flavopiridol induces cellular flice-inhibitory protein degradation by the proteasome and promotes trail-induced early signalling and apoptosis in breast tumour cells [116]. Phase I clinical and pharmacokinetic study of Flavopiridol in children with refractory solid tumours is also reported [117]. It Inactivates P-TEFb and Blocks Most RNA polymerase II Transcription in Vivo [118]. Flavopiridol having neuroprotective action in colchicines induced apoptosis [119]. Antiproliferative and CDK2-Cyclin A inhibitory activity of novel Flavopiridol analogues is also reported, [120]. It might be a useful targeted therapy strategy for hepatocellular carcinoma [121]. Flavopiridol is also having significant activity against drug-resistant strains and mutant strains of HIV-1 and HSV-1[122].

#### **MECHANISM OF ACTION OF FLAVOPIRIDOL**

Flavopiridol strongly inhibit kinases (CDK-1, 2 ,4, 6, 7) dependent on Cyclins but having low activity towards receptor tyrosine kinases and signal transducing kinases. Flavopiridol shows cytotoxic activity towards both Cycling and resting cells. Hence it can be assumed that CDKs inhibition and transcriptional control of Cyclin expression could contribute to anticancer activity of Flavopiridol. Also inhibition of receptor activation and kinases (pp60 Src, PKC.Erk-1) that are involved in signal transduction pathway, could participate in Flavopiridol antiproliferative activity. However many question regarding mechanism of action of Flavopiridol are still pending[123]. Antitumor activity of Flavopiridol shows

1. High rate of apoptosis in all type of cancer cell
2. Independent effect on pRb, p53 and Bcl-2 expression
3. It has synergistic anticancer activity with other antitumor agent
4. It has no interference with P-glycoprotein and Multi drug resistant protein
5. It is a strong antiangiogenic agent

Neuroprotective activity of Flavopiridol is independent of cell cycle but depend on inhibition of CDKs [124]. Flavopiridol inhibits growth and induces mitochondrial apoptosis in large cell lymphoma cells in vitro. Flavopiridol treatment has recently been associated with a decreased vascular endothelial growth factor mRNA half-life in monocytes and a reduction in metalloproteinase's in breast cancer cell lines. Flavopiridol treatment also inhibits the increase in pRb phosphorylation and the elevation of E2F1 protein. Activation of CDKs is required for the death of adult neurons evoked by stroke and Flavopiridol belongs to the class of drugs that inhibit CDKs and hence is effective in stroke treatment and prevention of cell death [125].

*Flavopiridol inhibit* the elongation phase of transcription through the inhibition of P-TEFb and was the most potent P-TEFb inhibitor. Flavopiridol inhibition of P-TEFb was not competitive with ATP [126].

The anticancer drug Flavopiridol may interfere with glucose homeostasis by inhibiting glycogen phosphorylase b and glycogen phosphorylase a. As a result there is an increase in glycogen accumulation in cancer cell [127], administration of Flavopiridol in tumour xenografts and head and neck squamous cell diminished cdc2 and CDK2 activity, as a result remarkable reduction of Cyclin D1 expression [128].

Flavopiridol having unique mechanism of action [129] its ability to kill no Cycling tumour cells[130] and its promising antitumor activity in xenograft models[131-133]. Flavopiridol has entered phase I [134] and phase II testing as a single agent as well as phase I trials in combination with cisplatin or paclitaxel [135].

## **Conclusion**

Flavopiridol is a first promising anticancer agent entered the Phase II clinical trials having inhibitory activity against CDK which is involved in cell cycle regulation and control of cell proliferation. Many analogues of Flavopiridol are synthesized by substituting different functional groups on different ring positions. Some of analogues have increased efficacy and selectivity for CDK inhibition. However much is yet to be revealed out of these analogues and their efficacy against other activities of CDK as cardiovascular, nervous system disorder, viral disease, renal and reproductive disorder etc. is yet to be researched out.

Further, ring modifications and functional group replacement with other groups can be the future area of interest for research.

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**Corresponding Author:**

**Pushpa Sharma\*<sup>1</sup>,**

**Email: [pushpamullana@rediffmail.com](mailto:pushpamullana@rediffmail.com)**