Targeting cyclin dependent kinases in management of human cancer

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Abstract

Uncontrolled proliferation is the hall mark of cancer and abnormal cell cycle regulation in cancer. CDK plays very important role in the control of the cell cycle and its proliferation. CDK2 is the "superstar" among the CDK family.CDK2 has cyclin A and cyclin E in its complex which the cyclin A complex require to progress through S phase regulated by phosphorylation and cyclin E require to transition from the G1 to S phase in cell cycle. Also, the mechanism of binding of CDK2 with its inhibitors as well as the changes of binding mechanisms following conformational variation of CDK2 are compared. Considering this fact, inhibition or disruption of the CDK2/cyclin complexes should be possible to suppress the hyper activation of CDK2 and hold back the infinite cell proliferation. There are main four binding site of CDK inhibitors. Competitive binding sites (site 1), Noncompetitive binding sites (site 2 and 3), Allosteric binding site (site 4). CDK inhibitors are mainly used in cancers including leukemia, melanoma, solid tumors and other types are being targeted.

Keywords: Cancer, CDK, CDK2, CDK2 Inhibitors

Introduction

Cyclin dependent kinases are the specific kinases that play most important role in cell cycle regulation allowing transition between its different phases of cell cycle. Many of the genes involved in cell cycle progression events causes over activity of the cell cycle in human cancer, and their inhibition two process occur of the cell cycle arrest and apoptosis. The different CDKs having different activity in cell cycle process that the specific targeting cancer to specific CDK inhibition process.

Method

1. **Cyclin Dependent Kinases: From cell cycle control to Physiological Regulation:** Cyclin-dependent kinases [CDKs] was the first protein identified and involved in regulation of the cell cycle division. These serine/threonine/proline-directed kinases, which are inactive in their monomeric form, associate with a family of regulatory subunits, cyclins, named after their periodic profiles of expression and degradation, to form functional heterodimeric complexes. The first CDK/Cyclin complexes to be characterize regulators of cell growth and division, involved in the tight and timely control of cell cycle progression, through phosphorylation of substrates involved in DNA replication, chromatin condensation, assembly of the mitotic spindle and disassembly of the nuclear envelope. This is the reason they are considered as "masters regulators" of cell cycle progression, molecular engines that drive cell cycle transitions.

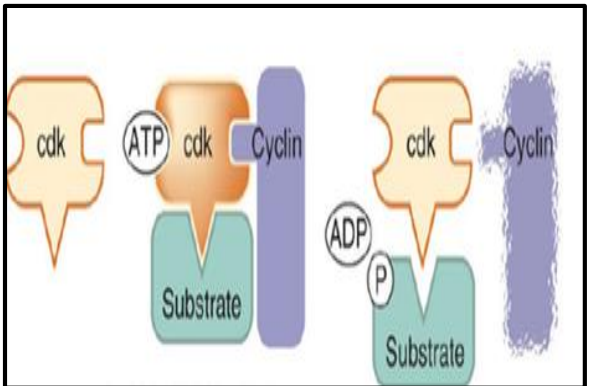

Fig. 1: Cyclin dependent kinase

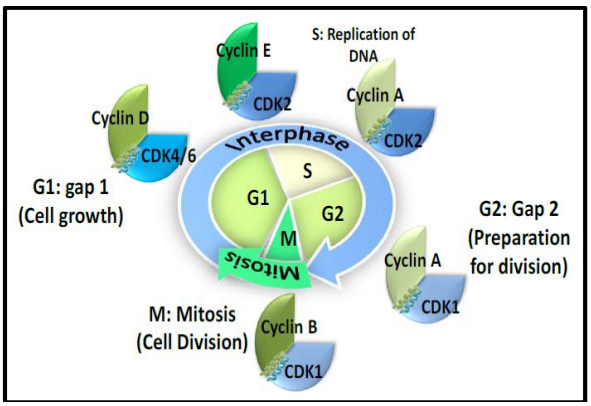

Fig. 2: Regulation of CDK/Cyclin

2. **Cyclin Dependent Kinase involved in Cancer:** In physiological conditions, activation of CDK/Cyclin kinases is tightly control. However, CDK/Cyclins are deregulated in several human cancers, which wreaks havoc in the coordinated cycle of cell growth and proliferation and contributes to the uncontrolled proliferation characteristic of cancer

cells. In fact, together with mutations in proto-oncogenes, mutations leading to hyper activation of CDK activity have been reportedly found in human cancer genomes, and confer selective growth advantage to cells, whilst mutations that inactivate checkpoint regulators, tumors suppressor genes or CKIs result in loss of cell cycle inhibition CDK/Cyclin hyper activation may result from one of several causes, including gene amplification and protein over expression of either the CDK or cyclin subunit, alternative splicing and expression of truncated cyclin variants, untimely expression and mislocalization or constitutive activation of CDK/Cyclins by preventing their inactivation through binding to INK or KIP/CIP inhibitors. A representative panel of mutations which occur in CDKs and Cyclins may be found in the catalogue of Cosmic Mutations in Cancer, which integrates all mutations identified through sequencing of human cancer tissue samples.

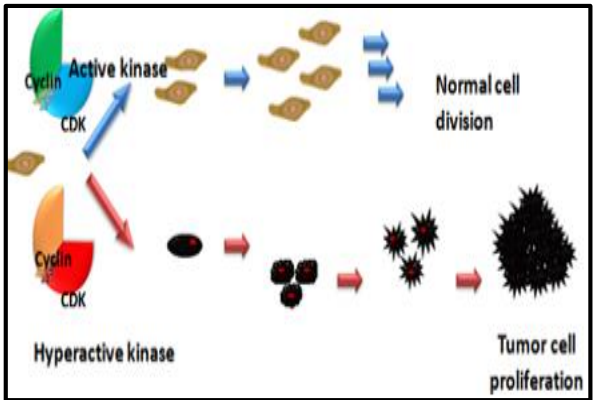

Fig. 3: Cyclin dependent kinases in cancer

3. **Cyclin Dependent Kinase 2 Inhibitor:** Cyclin-dependent kinase 2 (CDK2) is the most important regulator of the eukaryotic cell cycle. However, it is well established that monomeric CDK2 lacks regulatory activity, which needs to be aroused by its positive regulators, cyclins E and A, or be phosphorylated on the catalytic segment. These activation steps bring some dynamic changes on the 3D-structure of the kinase, especially the activation segment. Until now, in the monomeric CDK2 structure, three binding sites have been reported, including the adenosine triphosphate (ATP) binding site (Site I) and two non-competitive binding sites (Site II and III). In addition, when the kinase is subjected to the cyclin binding process, the resulting structural changes give rise to a variation of the ATP binding site, thus generating an allosteric binding site (Site IV). All the four sites are demonstrated as being targeted by corresponding inhibitors, as is illustrated by the allosteric binding one which is targeted by inhibitor ANS (fluorophore 8-anilino-1-naphthalene sulfonate). In the present work, the binding mechanisms and their fluctuations during the activation process attract our attention. Therefore, we carry out corresponding studies on the structural characterization of CDK2, which are expected to facilitate the understanding of the molecular mechanisms of kinase proteins. Besides, the binding mechanisms of CDK2 with its relevant inhibitors, as well as the changes of binding mechanisms following conformational variations of CDK2, are summarized and compared. The summary of the conformational characteristics and ligand binding mechanisms of CDK2 in the present work will develop our understanding of the molecular mechanisms regulating the bioactivities of CDK2.

a. **Characterization of Monomeric CDK2:** As a typical protein kinase, the monomeric CDK2 consists of 298 amino acids and folds into a typical bilobal structure, with a smaller N-terminal lobe that contains an antiparallel five-strand β-sheet and a major C-helix, together with a larger C-terminal lobe predominantly composed of α-helix. Actually, the two terminal domains are connected through a single peptide strand which acts as a hinge linker to ensure that the two lobes can rotate with respect to each other without disruption of the secondary structure of this kinase. There are two important segments in the large C-terminal domain, including the catalytic residues that are in charge of phosphorylation promotion, as well as the activation segment. Among them, this activation segment spans residues between the conserved DFG (residues 145–147) and APE motifs (residues 170–172), and also includes the phosphorylation site, i.e., residue Thr160. In the inert CDK2 monomer (PDB code: 1HCK),the Thr160 among the activation segment conformation is buried away from solvent facing toward the conserved glycine-rich loop. The unique sequence of the CDK family is the PSTAIRE motif (residues 45–51) embodied in the N-terminal C-helix, which has a main role in the interface. There are four binding sites in the CDK2 structure and one binding cavity in cyclin A have been discovered. Among the four sites on CDK2, one shows up only after particular structural transformation, whereas other three can be found on the original CDK2.

b. **Binding Sites of the Monomeric Cyclin-Dependent Kinase 2 (CDK2):** CDK2 promotes the G1/S boundary checkpoint and allows the cell cycle through the S phase by the bindings of cyclins E and A respectively. The CDK2 overexpression leads to loss of cell control. However, if there is no corresponding cyclin, CDK2 will not be rapidly activated to take effects. The incorporation of the cyclin subunit on one side

of the catalytic cleft connecting both the N- and C-terminal lobes of CDK2, and the phosphorylation on Thr160 forms a large, continuous protein−protein interface. Fascinatingly, neither cyclin binding nor phosphorylation alone is sufficient to achieve full activation of CDK2. Considering this fact, inhibition or disruption of the CDK2/cyclin complexes should be sufficient to suppress the hyper activation of CDK2 and hold back the infinite cell proliferation.

- **Competitive Binding Site (Site I)**: The major ATP binding site, located deep at the junction of the N and C domains, includes the pivotal catalytic residues with high sensitivity and linker region (residues 81–83), and consists of 136 consecutive amino acids (10–145) on the CDK2. This pocket is characterize by a lipophilic feature and tends to bind a highly polar/charged heterocyclic molecule. Many inhibitors bind to the ATP binding pocket and normally occupy the adenine ring (subset) of the ATP. In fact, it is recognized that the ATP binding site is conservative, and ATP recognition contains residues from both N and C lobes. Small agents inhibit CDK2 through competition with ATP for the catalytic site to hit the mark of catalytic subunit. Researchers arranged this site to six subsections: (1) the adenine region; (2) the ribose pocket; (3) the triphosphate binding region; (4) hydrophobic region I (opposite of the ribose pocket); (5) hydrophobic region II (cleft adjacent to the ribose pocket) and (6) the hinge region (connecting the N- and C-terminal domains), or normally the adenine, ribose and triphosphate subsists. The hydrogen bonds formed with this hinge region of the kinase are set as the primary criteria to assess a multitude of inhibitors as all known inhibitors form hydrogen bonds with the linker region of CDKs' ATP binding pocket. However, it is worth noting that, owing to high conservation of the PSTAIRE helix among CDKs, the ATP site is not the perfect binding pocket for highly specific inhibitors. The core portion of these inhibitors roughly overlaps with the adenine region, and these ligands' binding does not bring substantial change to the domain orientations when compared with the ATP–enzyme complex. In fact, the two ring systems have a rotation with respect to ATP, and this movement allows inhibitors to have contacts with the enzyme, which is not observed in the ATP bond. The three conservative H-bonds formed with the backbones of residues Glu81 and Leu83 are cardinals, at least two of which can be seen in this binding fashion. With H-bonds, lipophilic and van der Waals interactions are also important complements in this mechanism. Most of the scaffolds following this binding mode are similar to the purine skeleton. The main representative flavonoid molecule flavopiridol stands for the second binding mode.
- **Non-competitive Binding Sites (Site II and III)**: In the structure of CDK2, there are two non-competitive binding sites. Targeting the protein−protein interaction interface is a applicable but also challenging strategy in that the interface typically spans a wide contact area without any apparent dominant contacts to target. The top 10 residues involved in the formation of CDK2/cyclin interface in inactive states (when disassociated from its cyclin partner) rank as Tyr180, Glu208, Asp235, Lys178, Leu174, Arg126, Val154, Ile173, Gly176 and Arg150, while in active states (in complex with cyclin) rank as Tyr179, Trp227, Pro228, Pro155, Lys178, Tyr180, Val456, Pro271, Met233, Cys177. From this, Lys178 and Tyr180 come into view to be the favorite residues to target in Site III. And there is indication that peptides tend to bond to the active form of CDK2; but it is not easy to block the formation of CDK2/cyclin E complex. Knowing the theoretical structure analysis of CDK1, CDK4 and CDK6, Site III at the interface is only detected in CDK1. Therefore, this suggests that the binding pocket at the interface, i.e., Site III, may be less conserved and thus provides a more suitable and accessible target than the ATP binding site for designing relatively specific inhibitors of CDK2.

Cyclin binding at proper timing is one step for the activation of CDK2, and the other step is the phosphorylation by the CDK-activating kinases, which will be explained in detail in next part. Cyclins connect with one side of the catalytic cleft and interact with both the N and C lobes of the kinase. To accommodate cyclins well, CDK2 rearranges its structure, where the missing active site residues are restored to their correct positions. Additionally, most of the T-loop including both the structure and position is reconstructed, which contains rebuilding this flexible structure, and restoring missing active site residues to their correct positions. When cyclin binds to CDK2, the N-terminal domain particularly the αC-helix, shows some structural changes which lead to a slight broadening of the active site, and then releasing the catalytic cleft and exposing the phosphorylation site on the T-loop. After structural restoration upon links with cyclins, CDK2 is well-controlled mainly through hydrophobic interactions which are formed by residues of the cyclin linker between α5 and α1' at the CDK2/cyclin interface with cyclins. However, the cyclin subunit holds basically invariant conformation compared to the free phosphorylated form. The formation of dimer after elastic structural rebuilding generates a continuous protein−protein interface, and then facilitates further

conformational changes in the region of the C-helix of the activation segment as well as in the relative orientation of the N- and C-terminal lobes, which finally result in a correct conformation for ATP triphosphate recognition site. Integration with cyclins cause great changes in the kinase structure. Taking cyclin A as an example, after it binds to CDK2, the T-loop, which is the main obstacle of the substrate access in the monomeric Apo enzyme, is displaced outside of the catalytic cleft exposing to the solvent. The crystal structures of CDK2/cyclin B and CDK2/cyclin E complexes with small ligands are not available for the present. There is evidence that inspite of the almost equivalent enzyme conformations extruded by cyclin B and A, the recruitment sites on the two cyclins and their functional features to promote the conformation activation are different. The cyclinA recruitment site inclines to attract those substrates containing a RXL motif. Due to the sequence differences between cyclins A and B, binding interaction between cyclin B and RXL motif in this groove is relatively weak. And the canonical substrate recognition motif SPXK in cyclin A is found absent in cyclin B, which is replaced by the sequence SPXX in cyclin B. As a result, the CDK2/cyclin B phosphorylation is independent of the canonical sequence SPXK. As a blessing in disguise, both two sites on cyclin B could be phosphorylated while merely the Ser640 site can be phosphorylated on cyclin A.

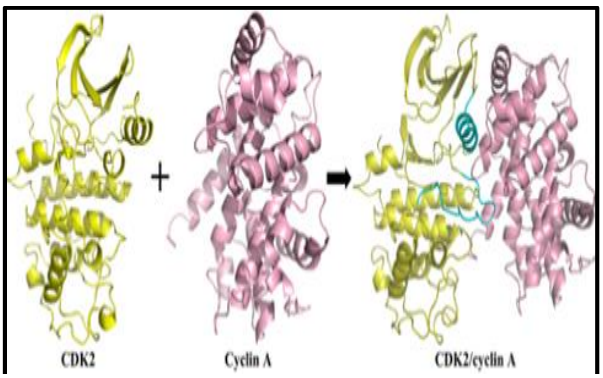

Fig. 4 : Formation of the CDK2/cyclin heterodimer. The cyan fragments are regions undergoing structural changes upon association with the cyclin subunit. Coordinates used for CDK2 and CDK2/cyclin A are 1HLC and 1OL1, respectively

- **Allosteric Binding Site (Site IV):** It is the conformational changes caused by the assembly of cyclin and CDK2 result in a binding site on CDK2, which is called as ANS (fluorophore 8-anilino-1-naphthalene sulfonate) binding pocket (i.e., allosteric binding site, Site IV). This site is located in a region which is adjacent to the C-helix, away from the ATP site, and in fact located approximately halfway of the ATP site and the C-helix.

Table 1: List of CDK Inhibitors

Sr. No	Drug name	Clinical Trials
1.	Flavopiridol	Phase 2
2.	P276-00	Phase 2
3.	Roscovitine	Phase 2
4.	PHA-848125 AC	Phase 2
5.	UCN-01	Phase 2
6.	R547	Phase 1/ 2
7.	AT-7519	Phase 1 /2
8.	Dinaciclib	Phase 1
9.	SNS-032	Phase 1
10.	RGB-286638	Phase 1
11.	BAY-1000394	Phase 1
12.	TG02	Phase 1

Conclusion

Cyclin dependent kinases are the protein which plays an important role in the regulation of cell cycle. From which Cyclin dependent kinase 2 (CDK2) is a crucial regulator of the eukaryotic cell cycle. Monomeric CDK2 lacks regulatory activity, which needs to be activated by its positive regulators, cyclin E and A. or be phosphorylated on the catalytic segment. Activation causes some dynamic changes on the 3D-structure of the kinase. CDK2 promotes the G1/S boundary checkpoint and drives the cell cycle through the S phase by the bindings of cyclin E and A, respectively. The overexpression of CDK2 may lead to loss of cell control. Here CDK inhibitors plays a crucial role in regulation of cell cycle and control of cancer. Some of CDK inhibitors are Flavopiridol, Dinaciclib. Major ongoing efforts are to develop. CDK inhibitors as monotherapy and rational combination with chemotherapy and other targeted drug.

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