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Protein Kinase Structure, Function, and its Binding Sites for Understanding the Role in Anticancer Therapy

Adarsh Sahu

Department of Pharmaceutical Sciences, Dr Harisingh Gour University, Sagar, Madhya Pradesh - 470003, India

*adarshniper@gmail.com (Corresponding Author)

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ABSTRACT

Background: Protein kinases are involved in regulating different aspects of metabolism including cell growth, differentiation and regulation of cell cycle. Deregulated protein kinases are thought to affect various forms of tumor growth. The advent of protein kinase inhibitors has caused a great shift in the way we treat cancer.

Purpose: There were several inhibitors of protein kinases that had been approved by the FDA in the last few decades. Therefore the need of the hour is to know the structure of the protein kinase to design the effective therapy for cancer treatment. Interaction of the drug with the protein is successful if the drug binds to the cavity of the receptor or protein and brings conformational changes, therefore the binding site in the protein should be well understood.

Conclusion: Thus in this mini review we have systematically discussed the structure of protein kinase and their binding sites so the future designing of the potential molecules should have better efficacy.

1. Introduction

The protein kinases are associated with symptoms of more than 400 diseases, which make them promising drug targets. The protein kinases are involved in the phosphorylation of proteins, that can be inhibited by targeting them with small molecular weight compounds (Melnikova et al., 2004; Vlahovic et al., 2002). The dysregulation of the kinases cause a variety of diseases like cardiovascular, diabetes, cancer, autoimmune, nervous disorders and inflammatory dysregulations. There are multiple kinases which play a complex role in the initiation of tumour, proliferation of cells along with the transformation of the cells. The kinases can be randomly subdivided based on their particular roles on cancer. Kinase inhibitors are presently considered as one of the primary categories of chemotherapy medicine (Motiwala et al., 2006). Over 30 kinase inhibitors have been approved in the US for the treatment of cancer with more such drugs under development. The kinase inhibitors mainly affect the tyrosine kinases which are a class of enzymes that initiate the gamma signal transduction mechanism. There are certain other inhibitors which affect the threonine or serine residues which are building blocks of amino acids. The modern genetics based kinase inhibitors are developed based on the studies of cell cycle, DNA structures and the routes for molecular signaling (Patterson et al., 2014; Abramson et al., 2016). Thus the specific cancer cells can be targeted whereby the healthy cells remain unaffected and no destruction is caused to these healthy cells.

2. Structure of Protein Kinase

Protein kinases can be classified as enzymes which play significant role in cell biology by taking part in cell cycle progression, apoptosis, differentiation, immune responses, transcription and cytoskeletal rearrangement (Melnikova et al., 2004). They are notable drug targets as their disregulation are a cause of a variety of diseases like cardiovascular, diabetes, cancer, autoimmune, nervous disorders and inflammatory dysregulations. The kinases have been studied as drug targets since the last two decades. Presently around 38 kinase inhibitors have been approved as drug targets (Vlahoic et al., 2003). The human genome encodes 518 kinases which function to phosphorylate around one third of the proteome (Motiwala et al., 2006; Patterson et al., 2014). The process of transfer of signal transduction takes place through a cascade of phosphotransfer. Thus therapeutic intervention can be applied in the different nodes of the kinases in case of the irregular biological processes (Abramson et al., 2016). The aberration of the kinase function leads to different symptoms like cardiovascular, inflammatory, immunological, metabolic and degenerative diseases (Tomasini et al., 2019). The kinases have proved

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to be promising drug targets based on their clinical safety and druggability. It is worth mentioning that the arena of kinase inhibitor has a lot to be explored (Tourneau et al., 2007). The phosphorylation of the protein is catalysed by the protein kinases. The gamma phosphoryl group of ATP is transferred to threonine, serine and tyrosine in proteins. Again, the protein phosphatises are counteracting enzymes which acts as catalyst for the dephosphorylation.

The protein kinase has been broadly categorised as eukaryotic protein kinase (EPK) and atypical protein kinase (APK) (Dhuguru et al. 2020). It was found that most of the APK structure was similar to the folds of EPK although they did not possess resemblance in the sequence. Thus the APK which resembles the structural fold of EPK where named as protein kinase like (PKL) (FDA 2020). The protein kinases (EPK and APK) are flexible and dynamic proteins (Manning et al., 2002) which behave as molecular switches as they transform between on and off states (USFDA 2017). There are two lobes in the centre of the protein kinase: the C-terminal lobe and the N-terminal lobe. The nucleotide is oriented and anchored by the N-lobe. The hinge region is a short loop which deliberates both the lobes. The transfer of the phosphoryl takes place at the deep cleft which is present between the two lobes and is coined as the active site. The combination of the ATP with the 2 Mg is instigated by the C-lobe and N-lobe. The substrate attaches to the C-lobe and brings it to the vicinity of the ATP which aids in the phosphorylation of the substrate.

The core of the protein kinase consists of three major elements: There are two hydrophobic spines which are nonlinear, which attaches the C-lobe with the N-lobe as observed from the local spatial pattern alignment of the EPK structures (Ficarro et al., 2002). The spines are coined as regulatory spine (R-spine) and catalytic spine (C-spine). Both the spines are attached to the αF-helix which is hydrophobic and fixes the position of the active catalytic sites (amino acid residues, ATP and substrates) (Cohen et al., 2000). An active kinase comprises of the regulatory spine (R-spine) which gets assembled dynamically during regulation (Manning et al., 2002). The binding to the adenine ring of ATP creates the catalytic spine (C-spine). There is no conventional sequence of amino acids for the spines as it comprises of amino acids that are included from various parts of the EPK sequence. However, the C-spine has a distinctive feature where the adenine ring present in ATP constitutes a part of this spine. It also binds the hydrophobic residues existing in the C-lobes and N-lobes. The shape of the R-spine remains unaltered during the process of phosphoryl transfer.

The EPK do not possess static inactive and active conformation (Muller et al., 2015). The position of the pheylalanine group in the activation loop of the DGF motif categorises the inactive state into two groups (Botta et al., 2014). The slight movement of the phenylalanine from the active conformation is termed as the DFG in one inactive conformation. The transposition of the DFG-phenylalanine to a far away position from the active site is termed as DFG-out conformation in another inactive conformation. The active state keeps on altering where the changes between closed and open conformation. The conformation (DFG-out) is the target position of the drugs. The movement of the αC-helix is responsible for the inactive DFG-in conformation. The structure of the protein kinase is shown in Figure 1.

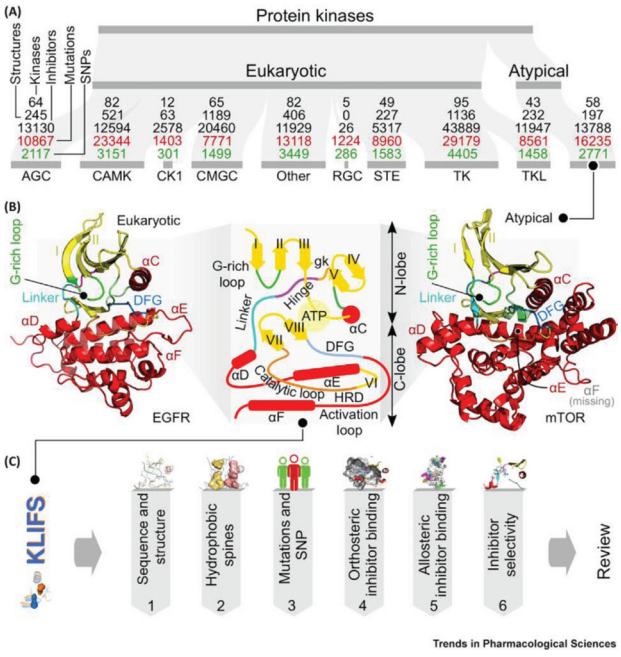

Figure 1: The structure of the APK and EPK.

3. Binding Sites

There is a strong competition between the ATP and the kinase inhibitors to combine to the adenine pocket existing near the hinge region. The significant binding sites are HRD motif, hinge region, αC-helix, G-rich loop, activation loops and the catalytic loops (Health et al., 2003). These fragments offer as binding sites to the kinase inhibitors based on their flexibility, which aid them to be available in different conformations (Scheef et al., 2005). The DFG pattern comprises of the Asp-Phe-Gly residues which can either activate or inactivate the unit of protein kinase through flipping. Additionally, the DFG initiates the random orientation of the catalytic hydrophobic spine (C-spine) and regulatory hydrophobic spine (R-spine) (Huse et al., 2002). The protein kinase in its active form is made stable by both the spines which thereby undergo phosphorylation.

There are multiple structural similarities of the binding sites of EPK and APK as shown in Figure 2.

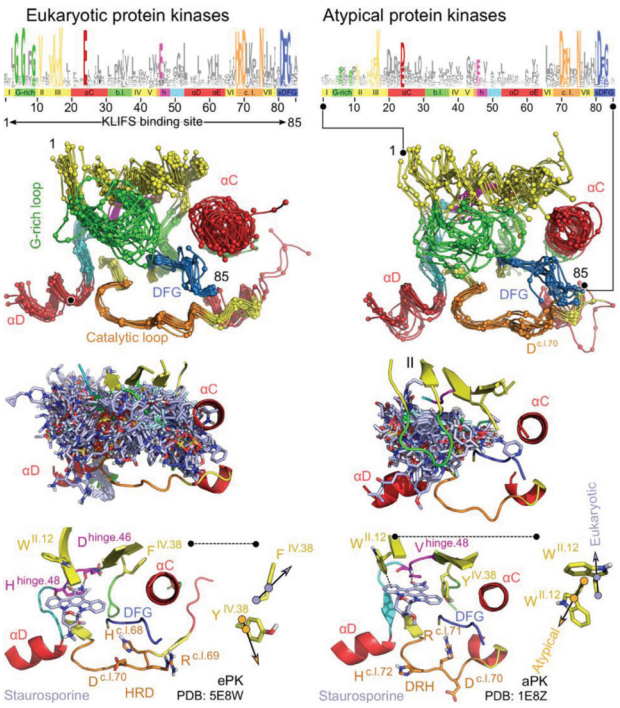

Figure 2: The binding site of the APK and EPK (Shaw et al., 2014).

However, the shape of the binding site has the probability of alteration between the EPK and APK. The binding sites of APK are deeper and open from the hinge to the HRD motif. The amino acid placed at 12th position of the side chain present in the residues of the binding site in the APK faces towards the pocket. This allows special interaction of the APK with the inhibitors forming the specificity pocket (Taylor et al., 2011). Thus the residues encapsulating the pocket can be turned around so the capping residue can protrude out and substitute positions occupied by other various residues. Thus the side chains in APK are easily rotated through 90° along the backbone. The amino acid existing at 38 position in the APK present in the affinity pocket is interacted through orthosteric binding under the condition when the side chain is not rotated (Kornev et al., 2006).

Conversely, the binding sites of EPK are wider from the αC-helix to αD-helix. The side chains of EPK remain static. The gatekeeper prevents the interaction of the amino acid at 38 position in EPK. Two chambers are formed for aromatic amino acids and hydrophobic witin the catalytic region by the hydrophobic spines. Thus the R-spine activation is regulated along with the C-spine (Kornev et al., 2008). The conserved interacted motifs in EPK and APK are the DFG-in, although the amino acids present at 11 and 15 position of EPK are not conserved (Taylor et al., 2004). The EPK after binding with the ATP completes the C-spine and interacts with these two particular amino acids through the adenine molecule (Levinson et al., 2006). The highly conserved moiety in the EPK is the histidine moiety present in the HRD motif. Thus there exists connection between the phenyalanine of the DFG motif in 93% of the DFG-in structures (Hemmer et al., 1997).

There is substantial difference in the binding sites of the spines of APK and EPK. There are some kinases which are devoid of αF-helix responsible for the rearrangement of the C-spine in the lower part. Instead, the position occupied by αF-helix is replaced by a group of hydrophobic residues which can interact with the desirable moieties in the C-spine (Wu et al., 2015).

A comparative study between the inhibitor binding sites of APK and EPK put forth that the APK is less targeted than the EPK (Kornev et al., 2008). The inhibitors of APK are type 11/2 which binds to both the back as well as the front cleft in the DFG-in conformation or type I inhibitors which target the binding site of the ATP (front cleft site). There are APK binding sites in DFG-out conformation till present date. Additionally, there are no atypical type II and type III inhibitors (Kanev et al., 2019). The type I inhibitors of kinase bind through hydrogen bond to the front pocket by anchoring to the hinge region. The presence of the adenine pocket demands selectivity of the type I inhibitors (Berndt et al., 2010). The popular binding sites of EPK are the gatekeeper region, back and front cleft and the different sites around the catalytic domain (Elmenier et al., 2019).

4. Future Perspectives of Kinase Inhibitors in Cancer Therapy

Currently small fraction of kinome is being targeted, drug discovery of newer kinase inhibtors have been increased in the past decade. Several new candidate entered clinical trials and some are still at preclinical stage. Structure-activity relationships and random screening resulted in discovering kinase inhibitors which are being used currently (Taylor et al., 2011). There is an crucial need for evolving more non-ATP competitive kinase inhibitors as the current collection of kinase inhibitors is limited to ABL, IKK, AKT, CHK1, MEK, SRC, IGF1R inhibitors (Bhullar et al., 2018). Thus the need of hour is to develop sophisticated modelling of chemotherapy resistance in response to kinase inhibitors. This will resolve the problem of kinase resistance and allow for the systematic application of combinations of kinase inhibitors. Also research is needed to identify the best cocktails of kinase inhibitors combined with natural bioactives. Progressive high-throughput cell-based broadcast using well-defined

phosphorylation details should be established. However, it may prove challenging to screen and develop natural kinase inhibitors using the cellular readout only. This is important that kinase inhibitors are not only important for the treatment of cancer, but it also help us to understand the physiological roles of kinases. Compared with the conventional cytotoxic chemotherapeutic for cancer kinase inhibitors are well tolerated so more research is needed to develop novel molecules. The future of kinase-targeted therapeutics in cancer appears promising, and implementation of these strategies will help to achieve therapeutic advances and overcome treatment hindrances (Bhullar et al., 2018).

Conclusion

The paradigm change in cancer therapies has been caused by protein kinase inhibitors. They, in general, are less harmful and kinase inhibitors are more potent in the right patient population than Classical chemotherapy. Nevertheless, like traditional growth in chemotherapy the drawbacks on kinase inhibitors are resistance and unintended side effects. In this mini review we have summarised the structures of protein kinase and their binding sites. The protein kinase has been broadly categorised as eukaryotic protein kinase (EPK) and atypical protein kinase (APK). The shape of the binding site has the probability of alteration between the EPK and APK. The binding sites of APK are deeper and open from the hinge to the HRD motif. A comparative study between the inhibitor binding sites of APK and EPK put forth that the APK is less targeted than the EPK (Bhullar et al., 2018).

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Adarsh Sahu: Conceptualization, methodology, investigation, resources, writing - Original Draft, Writing - Review & Editing

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Conflict of Interest

The author declare no conflict of interest.

Declaration

It is an original data and has neither been sent elsewhere nor published anywhere.

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Chitkara University, Saraswati Kendra, SCO 160-161, Sector 9-C, Chandigarh, 160009, India

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