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KINASE INHIBITOR CHEMISTRY – A REVIEW

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ABSTRACT

About 538 known kinases are encoded in the human genome, and these kinases maintain cellular function by turning protein function on, while corresponding phosphatases reverse this action. These counter mechanisms greatly improve the plasticity of epigenome by regulating protein activity in virtually every imaginable way. Biochemically, protein kinases catalyze the following reaction. Recent advances in our understanding of the fundamental molecular mechanisms underlying cancer cell signaling have elucidated a crucial role for kinases in the carcinogenesis and metastases of various types of cancer, Human cells have many different kinases, and they help control important functions, such as cell signaling, metabolism, division, and survival. Certain kinases are more active in some types of cancer cells and blocking them may help keep the cancer cells from growing. Kinase inhibitors may also block the growth of new blood vessels that tumors need to grow. Some kinase inhibitors are used to treat cancer.

KEYWORDS: human genome, and these kinases.

INTRODUCTION

Kinase inhibitor discovery is a very active area as developers are exploring more deeply into designing immune-modulatory agents as single or combination therapies, tackling chronic disease indications, such as inflammation and CNS disorders, as well as effectively harnessing allosteric modulators, and covalently binding compounds. This year will also be discussing the role of artificial intelligence, new and non-oncology drug targets, phosphatases, and protein degraders in kinase development. Cambridge Healthtech Institute's 11th Annual Kinase Inhibitor Chemistry conference will once again bring together academic and industry leaders to network, collaborate, and discuss advances in kinase inhibitor discovery and development.

The human genome encodes 538 protein kinases that transfer a γ-phosphate group from ATP to serine, threonine, or tyrosine residues. Many of these kinases are associated with human cancer initiation and progression. The recent development of small-molecule kinase inhibitors for the treatment of diverse types of cancer has proven successful in clinical therapy. Significantly, protein kinases are the second most targeted group of drug targets, after the G-protein-coupled receptors. Since the development of the first protein kinase inhibitor, in the early 1980s, 37 kinase inhibitors have received FDA approval for treatment of malignancies such as breast and lung cancer. Furthermore, about 150 kinase-targeted drugs are in clinical phase trials, and many kinase-specific inhibitors are in the preclinical stage of drug development. Nevertheless, many factors confound the clinical efficacy of these molecules. Specific tumor genetics, tumor microenvironment, drug resistance, and pharmacogenomics determine how useful a compound will be in the treatment of a given cancer. This review provides an overview of kinase-targeted drug discovery and development in relation to oncology and highlights the challenges and future potential for kinase-targeted cancer therapies.[1]

The enzyme- kinases

Kinases are enzymes that transfer a phosphate group to a protein while phosphatases remove a phosphate group from protein. Together, these two enzymatic processes modulate numerous activities of proteins in a cell, often in response to an external stimulus.[2] Approximately 538 known kinases are encoded in the human genome, and these kinases maintain cellular function by turning protein function on, while corresponding phosphatases reverse this action.These counter mechanisms greatly improve the plasticity of epigenome by regulating protein activity in virtually every imaginable way. Biochemically, protein kinases catalyze the following reaction.[3]

$$MgATP^{1-} + protein - O{:}H \rightarrow protein - O{:}PO_3^{2-} + MgADP + H^+$$

Recent advances in our understanding of the fundamental molecular mechanisms underlying cancer cell signaling

have elucidated a crucial role for kinases in the carcinogenesis and metastases of various types of cancer. Since most protein kinases promote cell proliferation, survival and migration, when constitutively overexpressed, or active, they are also associated with oncogenesis.[4] Genome-wide studies of kinase mutations have revealed genetically inherited variants of specific kinases are causally associated with cancer initiation, promotion, progression as well as recurrence. Over the last three decades, multiple human malignancies have been identified to be associated with modulation and dysfunction of protein and lipid kinases and deactivated phosphatases on account of chromosomal reshuffling and genetic mutations .[5]

Role of kinases in cancer

Targeting the kinases harboring oncogenic transformational capacity and metastasis has led to a notable change in the clinical management of cancer. Hundreds of kinases play overlapping and intricate roles in cell transformation, tumor initiation, survival and proliferation. Diving kinases while justifying their coinciding functionalities is difficult. However, in order to understand and discuss their oncogenic undertakings, they can be vaguely categorized based on their hallmark roles in cancer. The first group is the kinases that play a fundamental role in the primary oncogenic transformation and thus present themselves as prospective drug targets. Cytoplasmic tyrosine kinases are critical conveyers of extracellular signals, and mutations in these kinases have been reported to occur in various oncogenic conditions. This category includes the PI3K family of dual specific protein/lipid kinases, which are the most frequently mutated kinases implicated in 30–50% of human cancers.[6]

PI3KCA, perhaps the most notable member of PI3K family is associated with the pathology of colorectal cancer, breast cancer, ovarian cancer, endometrial carcinoma, and hepatocellular carcinoma. The PI3KCA kinase catalyzes the production of PIP3, a phospholipid which activates downstream signaling components such as protein kinase AKT and promotes tumor cell growth and survival. Similarly, active form of the protein kinase Akt/PKB contributes to oncogenic transformation of cells. Likewise, V599E and V600E mutations in BRAF kinase are associated with various carcinomas while BRAF somatic missense mutations occur in 66% of malignant melanomas. The oncogenic mutations in JAK2 kinase such as single point mutation (Val617Phe) and JAK2 exon 12 mutations are implicated in both myeloproliferative disorders and myelodysplastic syndromes. Similarly, genetic alterations in other kinases such as ALK, IGF-1R, c-Kit, FGFR1–4, c-Met, c-Ret, c-SRC, regulate fundamental molecular mechanisms for tumor cell growth and development.[7] Apart from tumor initiation, kinases are also vital for tumor cell survival and proliferation and may be present as downstream members of oncogenic kinase pathways. This category of kinases includes EGFR, a receptor tyrosine kinase, which has been shown to prevent autophagic cell death by maintaining intracellular glucose levels through interaction and stabilization of the sodium/glucose cotransporter 1 (SGLT1).[8]

Kinase inhibitors

A substance that blocks a type of enzyme called a kinase. Human cells have many different kinases, and they help control important functions, such as cell signaling, metabolism, division, and survival. Certain kinases are more active in some types of cancer cells and blocking them may help keep the cancer cells from growing. Kinase inhibitors may also block the growth of new blood vessels that tumors need to grow. Some kinase inhibitors are used to treat cancer. A protein kinase inhibitor is a type of enzyme inhibitor that can block the action of protein kinases. Protein kinases add a phosphate group to a protein in a process called phosphorylation, which can turn a protein on or off and therefore affect its level of activity and function. Protein kinase inhibitors can be subdivided according to the amino acid on a protein that they add the phosphate to (e.g serine, threonine or tyrosine) in order to inhibit phosphorylation of that amino acid. Kinases mostly act on both serine and threonine, but tyrosine kinase acts on tyrosine only and some dual-specificity kinases act on all three of these amino acid residues. Some protein kinases also phosphorylate other amino acids, such as histidine kinases that act on histidine residues.[9]

Type i kinase inhibitors

Type I kinase inhibitors represent ATP-competitors that mimic the purine ring of the adenine moiety of ATP. Functionally, they interact with the conformational phosphorylated active catalytic site of the kinases. These kinase inhibitors bind to the active conformational site and alter the structural conformation otherwise favorable to phosphotransfer.[10] Type I inhibitors usually contain a heterocyclic ring system that occupies the purine binding site, where it serves as a scaffold for side chains that occupy adjacent hydrophobic regions. These hydrophilic regions of the enzyme occupied by the ribose moiety of ATP may be used to exploit the solubility of the drugs or other active compounds. To date, many Type I kinase inhibitors for the treatment of cancer have been approved by the FDA viz. bosutinib, crizotinib, dasatinib, erlotinib, gefitinib, lapatinib, pazopanib, ruxolitinib, sunitinib, and vemurafenib. Apart from the large-scale clinical success, Type I kinase inhibitors also come with adverse side-effects. Type I inhibitors display an inclination for low kinase selectivity as the targeted ATP pocket is conserved through the kinome; therefore, increasing the potential for off-target side effects. This little selectivity for targeted kinases may result in cardiotoxicity and possible deterioration in cardiac function.

Type ii kinase inhibitors

Type II kinase inhibitors act by targeting the inactive conformation of kinases and interact with the catalytic site of the unphosphorylated inactive conformation of

kinases. Type II kinase inhibitors exploit new interactions inside the lipophilic pocket derived from the change of confirmation of the phenylalanine residue of the "Asp-Phe-Gly (DFG)" N-terminal loop conformation of kinases.[11] These inhibitors interact reversibly with the target kinase which leads to the formation of single or multiple hydrogen bonds with the protein in the 'hinge region' and also causes extra interactions in the open DFG-out conformation . These lipophilic interactions have a high degree of selectivity towards unwanted kinases affecting an increase in the safety profile of Type II kinase inhibitors.

Type II inhibitors also display a high conservation of distinctive H-bond pattern between the inhibitor and the glutamic and aspartic acids of the kinase.[12] Due to the exclusivity of inactive protein kinase conformations, it was theorized than type II kinase inhibitors would be more selective. However, there is considerable overlap of selectivity between type I and type II inhibitors. The discovery of Type II kinase inhibitors such as imatinib and sorafenib was serendipitous, and it wasn't until much later that their mode of action was discovered. The role of imatinib in the consequent development of small molecule protein kinase inhibitors cannot be overstated. All Type II inhibitors share a similar pharmacophore and hydrogen bonds that interact with DFG-out kinase conformational structure as revealed by the discovery of the Type II kinase inhibitor co-crystal structure.[13]

Type iii or allosteric inhibitors

The third class of kinase inhibitors bind outside the catalytic domain/ATP-binding site and modulates kinase activity in an allosteric manner. Some authors have divided the allosteric inhibitors into two subtypes where type A inhibitors bind to an allosteric site next to the adenine-binding pocket whereas the type B inhibitors bind elsewhere. Overall, Allosteric or Type III inhibitors exhibit the highest degree of target kinase selectivity as they exploit binding sites and physiological mechanisms that are exclusive to a particular kinase.[14] With respect to ATP, these drugs are steady-state noncompetitive or uncompetitive inhibitors because ATP cannot prevent their interaction with the target kinase. One of the earliest allosteric inhibitors was CI-1040, an orally active, highly specific, small-molecule inhibitor of the MEK1/MEK2 pathway.[15]

A recent chemical proteomics study confirms the allosteric activity of type III inhibitors as they showed a higher selectivity, but also stated that these are special cases as most of them are designated MEK1/2 inhibitors that bind to a particular cavity adjacent to the ATP-binding site. Another allosteric kinase inhibitor GnF2 binds to the myristate binding site of BCR–ABL1. GnF2 also displays sound IL-3 reversible anti-proliferative and apoptotic effect on two mutants identified as E255V and Y253H. Likewise, TAK-733 binds to the MEK1-ATP complex in the gate area and the back cleft adjacent to the ATP-binding pocket; however, it cannot bind to the adenine pocket owing to its occupation by ATP. Other examples include RO0281675 and analogs thereof. Overall, targeting kinases using allosteric inhibitors is thought to be a crucial approach for overcoming hurdles in kinase inhibitor research, such as limited selectivity, off-target side effects, and drug resistance. In future, more active and target specific allosteric inhibitors will be discovered as larger stress is placed on cell-based assays in whnative cellular context.[16]

Substrate-directed inhibitors

These are also called Type IV kinase inhibitors and undergo a reversible interaction outside the ATP pocket, located in the kinase substrate-binding site. These inhibitors don't compete with ATP and offer a higher degree of selectivity against targeted kinases. Substrate-directed inhibitors include ATP-noncompetitive inhibitors such as ON012380 which are targeted against Philadelphia chromosome-positive leukemias. More importantly, ON012380 was found to override imatinib resistance at physiologically relevant concentrations of < 10 nM.[17]

Type v or covalent inhibitors

The covalent kinase inhibitors form an irreversible covalent bond with the kinase active site and target a catalytic nucleophile cysteine within the active site of the enzyme. The chemical rationale for developing Type V inhibitors is based on exposed cysteine side chain in the ATP site which can be targeted for covalent reaction with a drug candidate with an electrophilic Michael acceptor in the right position.[18] This type of kinase inhibition takes place via trapping of a solvent-exposed cysteine residue either by S_N2 displacement of a leaving group or by reacting with a Michael acceptor incorporated within the kinase inhibitor. Covalent inhibitors target respective kinase by formation of a rapidly reversible collision complex followed by an irreversible enzyme-inhibitor complex.[19] Afatinib (targets EGFR (ErbB1), ErbB2, and ErbB4) and ibrutinib are currently FDA-approved drugs that form a covalent bond with their target kinase. Afatinib, unlike the first-generation EGFR-TKIs such as gefitinib and erlotinib, is a mutant-selective EGFR inhibitor with low toxicity profile despite its irreversible mechanism. Similar to Afatinib, ibrutinib also targets mutant-EGFR kinase with a distinct binding conformation.[20]

Jak inhibitors

Janus Kinases are one form of tyrosine kinase. When discovered they were given the name JAK for "just another kinase", but these were later renamed for the Roman god Janus. Several drugs have been developed to block JAK; only one, Ruxolitinib, is used for cancer patients.

Alk inhibitors

ALK stands for anaplastic lymphoma kinase. The first ALK inhibitor, *crizotinib*, is now considered the first-generation of drugs in this class. Crizotinib works on

ALK and also on the pathways ROS1 and MET. In early tests scientists found this medicine inhibited the ALK pathway and that this pathway might be a good target for anti-cancer drugs. Some (not all) patients with non-small cell lung cancer have a mutation in the ALK gene system.

The second generation of ALK inhibitors include ceritinib, alectinib, and brigatinib. Other drugs are in development and lorlatinib was given orphan drug status by the FDA, The drugs work on cells that have "chromosomal rearrangements" of ALK. These are a form of personalized therapy. The doctor can order a test of the biopsy tissue removed from the cancer. If it indicates the malignant cells have the ALK mutation (are said to be ALK-positive), these inhibitors are thought to be a good potential form of treatment.

Natural drugs as kinase inhibitors

Overexpression of kinases is observed in multiple carcinomas. In recent years, there has been a major paradigm shift in discovery and screening of natural compounds as potential kinase inhibitors. Emerging data has revealed numerous mechanisms by which natural compounds mitigate kinase mutations. Classically, many of the biological actions of small molecule compounds, especially polyphenols, have been credited with their antioxidant properties, either through their reducing capacities or their possible influence on intracellular redox states. These small molecule bioactives can directly bind receptor tyrosine kinases and alter their phosphorylation state to regulate multiple cell signaling pathways. Elevated levels of the EGFR and HER-2 have been identified as common components of multiple cancer types and appear to promote solid tumor growth.[21] EGFR inhibition is exhibited by multiple polyphenols including resveratrol, quercetin, curcumin, and green tea extracts. HER-2 overexpression in tumor cells is also attenuated by these bioactives. Fibroblast growth factors are involved in a variety of cellular processes, such as tumor cell proliferation, drug resistance, and angiogenesis. Oncogenic alterations of RTK kinases including FGFR1, FGFR3, and FGFR4 are inhibited by natural compounds. Similarly, curcumin and chrysin block expression of receptor d'origine nantais (RON) in tumor cells.[22]

Future developments

Kinase inhibitor drug discovery has progressed dramatically in the past decade. Clinical evaluation of kinase inhibitors has shown that therapeutic responses vary widely in individual patients and across patient populations, and seem to depend on many diverse factors. Many new candidate molecules have entered clinical trials, and much more are still at the preclinical stage. Most of the current kinase inhibitor discoveries have developed through rational drug design rather than through random screening and analysis of structure-activity relationships. An important strategy required for future development is to understand the basis of unexpected toxicities related to kinase inhibitors. Improvement in the documentation of toxicities of kinase inhibitor would provide a valuable database for understanding whether there are particular kinases of which inhibition should be avoided or specific substructures that result in problematic metabolites.

This strategy will help to develop kinases with better selectivity benefitting the vast patient population. Also, there is a critical need for better ways to monitor target kinase inhibition in humans using minimally invasive techniques. This may include monitoring of cancer biomarkers that may serve as benchmarks for the clinical development of kinase inhibitors. The development of such technologies will help to discover and eradicate tumors using targeted kinase inhibition with minimal toxicities. There is also an urgent need for developing more non-ATP-competitive kinase inhibitors as the current collection of kinase inhibitors is limited to ABL, IKK, AKT, CHK1, MEK, SRC, IGF1R inhibitor.[22] Furthermore, there is need to develop sophisticated modeling of chemotherapy resistance in response to kinase inhibitors. This will help to overcome kinase resistance and allow for the systematic application of combinations of kinase inhibitors.

Furthermore, novel pre-clinical models are required to identify the best cocktails of kinase inhibitors combined with natural bioactives. Advanced high-throughput cell-based screening using well-defined phosphorylation readouts should be established. However, it may prove challenging to screen and develop natural kinase inhibitors using the cellular readout only. It is also important to understand that kinase inhibitors are not only important for the treatment of cancer, but also help us better understand the physiological roles of kinases. In the field of oncology, kinase inhibitors are proving to be well tolerated compared with conventional cytotoxic chemotherapeutic treatments. 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.

CONCLUSION

As many human diseases result from mutations and overexpression of kinases, this enzyme class symbolizes an important targeted strategy for drug development. Kinases also play indispensable roles in signaling pathways that regulate tumor cell functions. Deregulation of kinases leads to a variety of pathophysiological changes triggering cancer cell proliferation and metastases. Hyperactivation of kinases also increases anti-apoptotic effects. Currently, about one-third of all protein targets under research in the pharmaceutical industry are kinase-based. Kinase inhibitors represent targeted therapy resultant of the understanding of molecular genetics and molecular signaling pathways. Most of the FDA-approved kinase inhibitors target ATP binding site of kinase enzymes and display therapeutic

indications against tumorigenesis. This class of therapeutics represents a transformation from conventional chemotherapy to targeted cancer treatment.

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