Protein kinases: Role of their dysregulation in carcinogenesis, identification and inhibition

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ABSTRACT

Protein kinases belong to the phosphor-transferases superfamily of enzymes, which "activate" enzymes via phosphorylation. The kinome of an organism is the total set of genes in the genome, which encode for all the protein kinases. Certain mutations in the kinome have been linked to dysregulation of protein kinases, which in turn can lead to several diseases and disorders including cancer. In this review, we have briefly discussed the role of protein kinases in various biochemical processes by categorizing cancer associated phenotypes and giving their protein kinase examples. Various techniques have also been discussed, which are being used to analyze the structure of protein kinases, and associate their roles in the oncogenesis. We have also discussed protein kinase inhibitors and United States Federal Drug Administration (USFDA) approved drugs, which target protein kinases and can serve as a counter to protein kinase dysregulation and mitigate the effects of oncogenesis. Overall, this review briefs about the importance of protein kinases, their roles in oncogenesis on dysregulation and how their inhibition via various drugs can be used to mitigate their effects.

Introduction

Protein kinases are an important part of almost every aspect of cell functioning [1]. Ever since protein phosphorylation was discovered by Ed Krebs and Ed Fisher around 1950s, protein kinases got recognition as prominent regulators of cellular signalling [2]. Initially, the focus of researchers was metabolic pathways, mainly emphasizing on glycogen metabolism and insulin signalling. An extensive work in the field of biochemistry led to the origin of protein kinase, which includes working on insulin action, eventually leading to the mitogen-activated protein kinase identification [3, 4]. A complete set of protein kinases, which encode for them in the genome of an organism is called the kinome, especially in the fields of molecular biology, biochemistry and cell signalling. Another breakthrough for understanding protein kinome came from establishing their variation with earlier identified oncogenes, for example; Rous-sarcoma virus proto-oncogene (Src) encoded viral gene of cellular protein kinases [5, 6].

These protein kinases are involved in almost all cellular functions such as transcription, translation, cell division, other metabolism and even in apoptosis. Reversibility is maintained between phos-

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phorylation and dephosphorylation via balanced action of protein kinases and phosphoprotein phosphatases during the protein phosphorylation [1, 7]. The protein kinases mediate the phosphorylation process i. e. catalyse phosphate group transfer from a high energy source such as adenosine triphosphate (ATP) or guanosine triphosphate (GTP) to proteins or even lipids as depicted in ▶ **fig. 1**. Generally, a serine, threonine or tyrosine residue containing a hydroxyl group acts as the phosphate group receiver in proteins [8].

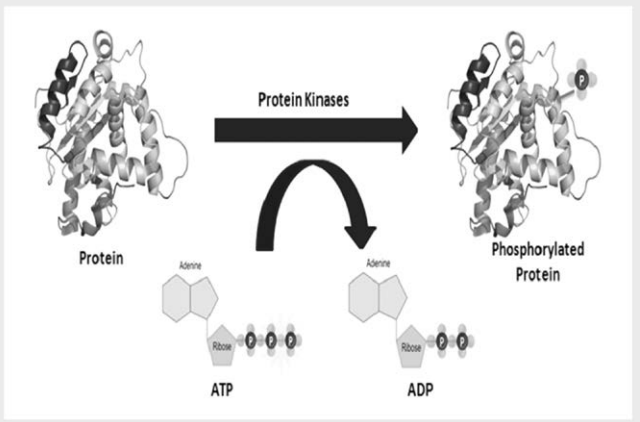
In disease pathophysiology, the importance of protein kinases was understood because mutations and alterations in kinases can cause altered cellular functioning, which can lead to various diseases [9]. According to Manning et al., the human kinome contains 518 protein kinases and about 106 pseudo protein kinases [1], among which, 218 protein kinases are such whose alterations can cause human diseases. Since the Manning study, the human genome's count has advanced, and as of now, 483 human proteins have been identified by Uniprot as possessing 496 typical kinase domains (https://www.uniprot.org/docs/pkinfam) [10]. For this reason, protein kinases are undoubtedly promising therapeutic targets for various diseases including some viral infections [1].

In this work, we have discussed the importance of kinome in context of cancer pathogenesis and how mutations of the kinome contribute to the same. An overview has been presented about the protein kinases as therapeutics, including novel kinase targets in oncology, their identification, causes of kinase gene mutations during cancer, various techniques for detection of protein kinase activities, different types of protein kinase inhibitors and various FDA approved drugs.

Active and Pseudo kinases

Protein kinase domains comprise of two main lobes. First is a small N-terminal lobe made up of an α-helix, also known as C-helix and five β-sheet strands that are prominent for coordinating ATP binding. Second is a large C-terminal lobe, which is composed of 6 α-helices for protein-substrate binding and phosphorylation catalysis [11, 12]. The activation and catalytic loops that combine to generate the ATP and substrate binding sites are found in the active site, which are situated between these two lobes [10, 13]. Protein kinase activity regulation takes place through multiple post-translational modifications (i. e. phosphorylation), autoinhibition, regulatory partner binding, including both activator and inhibitor proteins along with expression changes [14, 15]. Most of the protein kinases utilize the above-mentioned mechanism for stabilizing and promoting the active conformation and enhancing protein kinase domain capabilities such as supporting ATP and substrate binding [11].

The human genome contains many non-functional, non-enzymatic members of kinase genes. According to the data of sequence analysis, it was found that about 10 % of human kinase domains are catalytically inactive but have common core kinase scaffold as that of catalytically active protein kinases and lacks the presence of key motifs necessary for effective catalytic activity [10]. For instance, the absence of one of the amino acids like Lys30, Asp125, and Asp143 in the catalytic domain of the protein kinases. These inactive pseudokinases are divided into 3 groups [16]. In the first group, the Pseudokinomes are potential modulators of their catalytically active kinomes [17]. Second are those kinases, which have high similarity to the eukaryotic protein kinase (ePK) domain's canonical structure [18]. In the third group, members are dissimilar to their active domains. The three-dimensional structures of pseudokinases and protein kinases are very much similar. The pseudo-active site (ATP-binding site) is hidden in between the two lobes i. e., the N-terminal lobe, which is dominated by β-sheets and connected to the C-terminal lobe by the hinge region [19]. Pseudokinomes show almost zero levels of enzymatic activity but in few cases, these bind in normal body concentration of nucleotides and start working as a molecular signalling switch, which occurs in cells through druggable ligand-induced transitions. Sometimes, they indirectly take part in enzymatic action by allosteric modification, competition in substrate binding or change in the signalling pathway. Two well-known examples are Human epidermal growth factor receptor 3 (*HER*3) and Erb-B2 Receptor Tyrosine Kinase 3 (*ERBB*3), which are prominent *HER*2 signalling partners in cancerous cells. These activate and maintain the activity of Phosphoinositide 3-kinases (*PI3K*) and also take part in Kinase Suppressor of Ras, KSR1 and KSR2 in the Epidermal growth factor receptor-Ras-Mitogen-activated protein (EGFR-Ras-MAP) kinase pathway via allosteric interaction, with respect to its active kinase, which is RAF (Rapidly Accelerated Fibrosarcoma) relative.



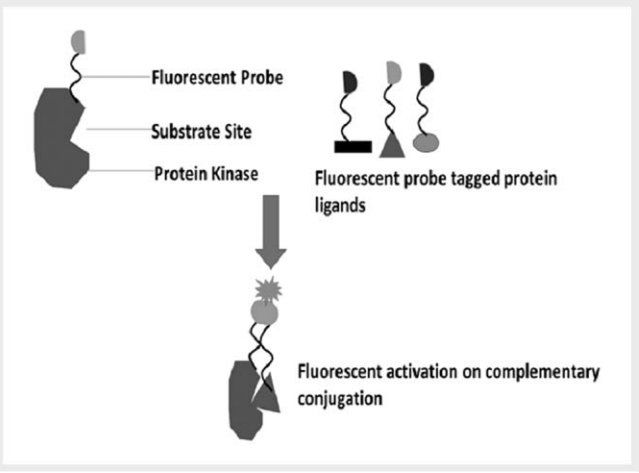
▶ **Fig. 1** Protein phosphorylation facilitated by protein kinases.

Identification of novel kinase targets in oncology

The human genome contains more than 500 different kinases [20] which comprise about 2 % of the entire genome. Most protein kinases promote cell survival, proliferation and migration, therefore when constitutively overexpressed, they can be associated with oncogenesis [21]. Deregulated kinases are often found in oncogenic cells and can be central in their survival and proliferation [22]. Hundreds of kinases play intricate and overlapping roles in cell transformation, tumour initiation, tumour survival and proliferation [21]. Therefore, to identify novel protein kinase targets in oncology, it becomes necessary to catalogue kinases in the human body so that any abnormalities leading to cancer and other disorders can be identified and treated.

▶ **Table 1** Tumorigenic phenotypes.

Cancer associated phenotype	Role in oncogenesis	Protein kinase examples
Immuneoevasion	Tumours escaping immunosurveillance and immunosuppression.	MAP4K1 (Mitogen Activated Protein Kinase)
Cell cycle progression	Proceed through cell cycles despite the damage.	B-RAF (B-Rapidly Accelerated Fibrosarcoma)
DNA damage response	Not arresting cell cycle or recruitment of DNA repair mechanisms despite significant unrepairable damage.	ATM (Ataxia-Telangiectasia Mutated), ATR (Ataxia-Telangiectasia & Rad3)
Angiogenesis	Increase blood supply and therefore nutrients in the tumor microenvironment.	VEGF (Vascular Endothelial Growth Factor)
Metabolism	Increased nutrient metabolism.	mTor (Target of Rapamycin), PI3K (Phosphoinositide-3-Kinase)/ATK,
Anti-apoptosis	Bypassing apoptosis leading to uncontrolled growth.	RIPK3 (Receptor Interacting Serine/Tyrosine Kinase 3)
Metastasis & invasion	Metastasis and cell invasion	RTKs (Receptor Tyrosine Kinases)



▶ **Fig. 2** Kinase-Substrate identification using Bimolecular fluorescence complementation (BiFC).

Identifying and cataloguing protein kinases

Protein kinases enzymatically phosphorylate proteins to "activate" them. Up to 30 % of all human proteins may be modified by kinase activity. Since kinases play a role in several processes such as gene expression, proliferation, differentiation, metabolism, membrane transport, and apoptosis, it is not surprising that dysregulation of their activity may lead to different diseases and disorders including cancer [23]. Cancer-associated phenotypes as classified by Gross et al. [24], their role in oncogenesis and protein kinase examples are given below in ▶ **table 1**.

Therefore, it becomes clear that the first step in developing novel protein kinase targets is the identification of a particular protein kinase, which is associated with a specific cancer phenotype. This can be done by identifying the enzyme or receptor, which is abnormally expressed and then associating it with a kinase, which activates it.

One recent emerging method of associating protein kinases with their corresponding substrates is using an established process called Biomolecular Fluorescence Complementation (BiFC), which has been elucidated in ▶ **fig. 2**, where a fluorescent protein is divided into two parts; one attached with the protein kinase under study and another with the cancer phenotype proteins under study. In performing BiFC assay, the BiFC labelled protein kinase and cancer phenotype proteins can be reacted together and on conjugation, the associated fluorescent marker can be identified. This method has been used recently to study the interaction of regulatory 14-3-3 proteins with Cyclin B1 in tumour promotion sensitive mouse epithelial JB6P + cells [25].

Bioinformatics can also be used to statistically study common mutations across cancer cells compared to normal cells. Paired tumour normal exome sequencing has revealed millions of somatic mutations across many thousands of patients. However, only a fraction of them have any biological significance and a majority of those are inclined towards cancer development [26]. One possibility might also be to subject different variations of the same protein kinase to mutagenic screens to model and predict abnormal kinases, and their associated genotype [21]. With increasing computing power, large scale complex data analysis is becoming easier and hence is now widely used in research. One of the method of employing computational analysis is using reputed cancer genomic repositories such as The Cancer Genomic Atlas (TCGA) and putting it through a clinical kinase index (CKI) or kinases, whose mRNA expression seems to be associated with the prognosis of cancer [27].

Protein kinase structure prediction

Kinases are a class of enzymes and being so, their sensitivity and functionality largely depend on their structure. Therefore, to develop novel selective agents against protein kinases involved in cancer, it becomes necessary to identify and study their structure in detail. Advancements in X-Ray crystallography, NMR spectroscopy, cryo-electron microscopy and computational protein modelling have made it possible to study protein structures in greater detail.

All protein kinases contain an ATP binding site, where the ATP binds and "powers" the transfer of the phosphate group onto the target protein to activate it. Until recently, it was thought that these ATP binding residues were conserved among kinases and therefore it was not possible to selectively target them [21]. However detailed crystallographic studies have revealed some inter-class differences, which can be exploited to develop more specific inhibitors [28]. Blocking substrate binding sites such as exposed hydrophobic groove in Cyclin-Dependent Kinases (CDKs) reduce recruitment of cell cycle substrates [29]. Other non-active site regions such as blocking the polo box domains of Polo Like Kinase (PLK1) have been shown to

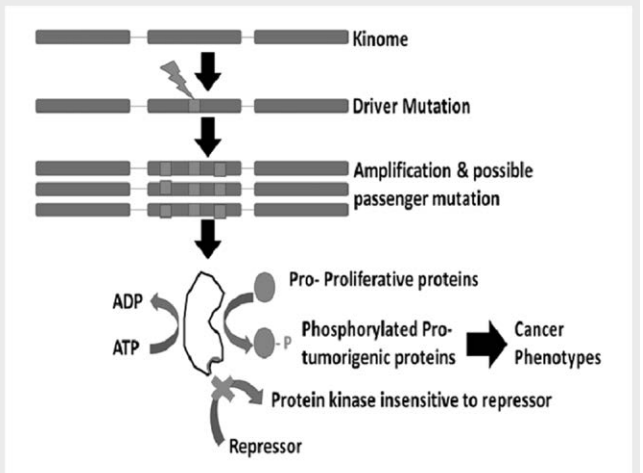
decrease its kinase activity [30]. Normal cells rely on a full complement of cell cycle checkpoint kinases, whereas most cancer cells have faulty G1 DNA damage checkpoint and thus depend on G2 or S phase checkpoints [29]. Using alteration of kinase-mediated cytological pathways like this, it may be possible to downregulate overexpressed kinases, while upregulating those, which are functional and can partially restore normal cellular function.

Kinase gene mutations in cancer

Analysing the role of protein kinases and thus kinome behind phosphorylation of a large section of proteins, it's quite easy to understand that dysregulation of the same would lead to many diseases and disorders including cancer. However, about 24 % of human kinases including pseudo kinases are still under study and only 8 % have been the target of any therapeutics, so a lot more needs to be done with regards to identifying the role of different kinases in cancer and the mutations which may result because of the same [27]. Mutations are random changes in the genome in both normal and cancerous cells. However, genomic mutations can be divided into three broad categories based on the extent of the mutation; base substitution, frameshift, and chromosomal. In base substitutions, one nucleotide is erroneously replaced with another, which may or may not change the associated protein functionality. One such mutated kinase gene is *BRAF* (encoding for B-Raf protein), which is regularly mutated at Val-600 and leads to colorectal, melanoma, thyroid or non-small cell lung cancers [22]. RAS point mutations are also responsible for about 10–20 % of thyroid cancers [23].

In case of frameshift defects, it is possible for genes to carry tens of thousands of aberrant insertions or deletions in tandem repeats, while most occur in intronic regions, few can happen in the exon regions also, leading to frameshift mutations, altering several codons. It is likely that some of these mutations may have a role in oncogenesis [31]. In gene or chromosomal level mutations, entire genes or portions of genes become translocated on one another, causing extensive damage. One of the most well-known examples of this is the Philadelphia Chromosome, which involves the translocation and fusion of Breakpoint Cluster Region (*BCR*) and Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) genes to form a fusion *BCR-ABL*1 gene, which is present in 95 % of people with chronic myelogenous leukemia and 25 % with acute lymphoblastic leukemia [18]. The *BCR-ABL*1 fusion gene produces a hybrid tyrosine kinase signalling protein, which is always activated leading to uncontrolled cell division by undermining many normal cell signalling pathways [32]. The same fusion gene is also responsible for imatinib resistance, which is a tyrosine kinase inhibiting anti-cancer drug, leading to poor prognosis [30]. Rearrangement and fusion of RET gene has also been associated with several RET dependent cancers such as 10–20 % of papillary thyroid carcinomas [23, 33]. Several kinase genes have been categorized as proto oncogenes and oncogenes.

Genomic study of protein kinases involves accurate identification of genes responsible for protein kinase, their sequencing and finally a comparison of the healthy kinomes with corresponding ones from cancer cells. Thereafter several statistical analytical tools can be applied to the mutations to reveal any reoccurring pattern. Two more terms, which must be discussed for the analysis of kinome mutations are driver and passenger mutations. Driver mutations are those mutations, which are significant for cancer progression because they offer an advantage in the clonal expansion [34]. For the individual cell, it is beneficial but for the organism, it leads to tumour formation and progression. Passenger mutations are mutations, which can be a result of driver mutations, or accumulated over the cell's life and are less significant than driver mutations because they do not directly contribute to oncogenesis [35]. However, their study may shed light on patterns of mutations leading to oncogenesis. This phenomenon is shown in ▶ **fig. 3**, where a random mutation on a kinome leads to the formation of a driver mutation, which gives a clonal advantage and may further lead to passenger mutations. The result is a protein kinome enzyme, which is insensitive to its repressor, and therefore catalyses excess of pro-proliferative proteins. One prominent example of kinase drivers is receptor tyrosine kinases because of the activating point mutations, which leads to oncogenesis [36]. In a report, resequencing 518 kinases across seven lung cancer cell lines and 26 primary lung neoplasms across 141 kinomes, 188 somatic mutations were found, three-fourths of which were probably passenger mutations [36]. In a more comprehensive study, 518 protein kinase genes amounting to 274 MB of genetic data across a total of 210 different cancers including breast, lung, colorectal, gastric, testis, renal, skin, brain, leukaemia, etc. amount to 1000 somatic mutations were analysed, where 158 were classified as driver mutations and the rest as passenger mutations [37]. Perhaps the most comprehensive tumour sequencing study preformed till date was by Wood and colleagues, where out of a total of 18,191 exon genes in eleven breast and colorectal tumours, 280 were found to be of candidate cancer genes [38]. These were then studied in a cohort of 96 patients, where it was found that most of these genes were mutated in less than 5 % of the tumours. An extreme exception to this would be in case of *BRAF* kinase mutations in melanomas, where about 60 % of the mutations were present in the catalytic core [36]. Specific protein kinase cancer drivers are rare; however, few hotspots do exist and are vital in our understanding of proto-oncogenes [36]. It may be an attractive approach to identify cancer driver mutations but recent studies on cancer genomics have found less domination of high frequency driver mutations than study of individual cancers seem to imply [39].



▶ **Fig. 3** Kinome mutations leading to cancer phenotypes.

Techniques to detect activities of Protein Kinases

Direct or indirect quantification of the catalysed products i. e. phosphorylated substrates, ATP or GTP can be detected by using enzymatic-activity and kinase functional based assays [40]. According to a worldwide survey, the functional assays formats are considered better as compared to cellular and binding based assays [41]. Radiometric, luminescence based, mobility shift based, and fluorescence-based assays are included in functional assays and some of them have been elaborated in the following section.

Fluorescence resonance energy transfer (FRET) based assay

FRET is fluorescence based microscopic technique. It involves the radiation less transfer of energy from a donor, which can be a dye or a chromophore molecule (eg. coumarin) to an acceptor chromophore (eg. fluorescein) [42]. FRET is frequently used in many biomedical researches and in drug delivery. The advantages of this technique include its simple applications and homogenous format, which means, the donor and acceptor must be in closer proximity. The energy transfer in FRET is distance dependent. This assay gives a quantitative analysis of kinase reaction by calculating the ratio between the emission of donor and acceptor. In this method, the user must avoid the materials, which have autofluorescence property to avoid any variation in the signal intensity. One of the important challenges of this assay is designing of specific substrate for binding with a particular kinase. This assay consists of limited number of substrates.

Time-resolved fluorescence (TRF) based assay

TRF is based on the use of fluorophores, which have long decay time. In this, the fluorescence output is observed as a function of time after excitation of the complex [43]. The lanthanide ions like samarium, europium and terbium are frequently used in this assay, due to their longer emission lifetimes. The use of lanthanide ions reduces the background noise which in turn enhances the sensitivity of detection.

The commercially available TRF based assay is PerkinElmer's DELFIA1 kit [44]. This includes the fluorophore-conjugated substrate binding, separation and detection. In this assay, the biotinylated substrate has been used for the reaction, then the mixture is transferred onto a streptavidin coated plate. After multiple washings, the europium labelled antibody is used to detect the phosphorylated complex. Now for the dissociation of lanthanide ions, an enhancement solution is added and the dissociated lanthanide ions forms a fluorescent chelating complex with the enhancement solution [42]. One of the biggest disadvantages of this assay is that, it is time consuming due to its multistep process like transferring, washing, developing and detecting. In addition to this, the selection and development of antibody is another challenging task.

Time-resolved fluorescence resonance energy transfer (TR-FRET) based assay

TR-FRET is a combined assay of FRET and TRF but it only detects the phosphorylated substrate. In this, the signal is produced through FRET between donor and acceptor compound in close proximity. This assay contains an acceptor fluorophore tagged with peptide substrate for the detection and an anti-phospho-peptide antibody tagged with a donor fluorophore is used [45]. The commercially available TR-FRET assay kits are PerkinElmer's Lance1 and Life Technology's LanthaScreen™ [46]. The PerkinElmer's Lance1 assay uses lanthanoids (eg. europium) as a chelating agent, which binds with the anti-phosphorylated antibodies and uses streptavidin–allophycocyanin as an acceptor. In the LanthaScreen™ assay, the peptide substrate is tagged with fluorescein (acceptor), which reacts with a donor (terbium chelating agent). The product of this reaction is terbium-labelled anti-phosphopeptide antibody which is used for the detection. Due to this, there is no requirement of additional acceptor fluorophore. This makes it more cost-effective, highly sensitive, robust and can be performed in 384 and 1536 well plates. The major challenge of the assay is to develop a specific detection antibody.

Fluorescence lifetime technology (FLT) based assay

FLT is widely used as an alternative of traditional fluorescence-based methods. It is an automated assay, free from antibodies and free from the interference due to fluorescent molecules. Developments in recent years have pointed the target class suitability, assay authorization and assay reagent suitability problems using FLT. The market available assay is AlmacFLEXYTE™ protein kinase assay. In this assay, a fluorescent tagged peptide substrate is phosphorylated by kinase reaction [47]. Then a small molecule chelating agent (SMC) or Lifetime Modulator (LiM) is used to bind with the phosphate group, which results in decrease in the fluorescence lifetime. This method is only limited to some tyrosine kinases and serine/threonine kinases due to the availability of a limited number of generic peptide substance.

Luminescence-based assay

These assays are used to calculate the depletion of ATP employing luciferase, which in the presence of ATP converts luciferin to oxyluciferin and give rise to the emission of light. Luminescence had been extensively employed in numerous kinase assays developed by various companies like PerkinElmer and Promega [48]. The commercially available luminescent kinase assay Promega's Kinase-Glo™ records the depletion of ATP or the formation of ADP. The other available luminescence assay is PerkinElmer's ATPLite™, which is based on the similar approach of Promega's Kinase-Glo™ [49]. These assays usually show low sensitivity towards the low ATP concentration.

Enzyme-linked immunosorbent (ELISA) based assay

ELISA was extensively used for the detection of phosphorylated substrates prior to the use of latest fluorescence-based technologies. In this assay, the substrate is picked by the matrix and washed several times before the detection so that the compound fluorescence could not interfere with the measurement. After washing, a specific anti-phosphorylated antibody is used for its detection. In this, highly fluorescent dye labelled antibodies are used for more sensitive measurements. But somehow, the High-throughput screening (HTS) applicability of ELISA is restricted due to separation and continuous washing steps [49]. In addition to this, the major challenge in the application is to design specific antibodies that can bind to serine / threonine kinase. Kinase assay kits based on the phenomena of ELISA, dedicated for few specific kinases are available in the market for use. These are provided via various companies like Abcam, Life Technology, Ray Biotech, and Cell Biolabs, etc.

Radiometric filtration binding based assay

These are one of the most common and effective kinase profiling assays. This method directly detects the original products without doing any modification in substrates or enzymes coupling. This assay working involves incubation of test compounds along with kinase, reactants, appropriate co-factors and radio-isotopically labelled ATP like ^{33}P-g-ATP or ^{32}P-g-ATP. The radioisotopically labelled catalytic product obtained from the incubated reaction mixture is spotted on p-81 phosphocellulose filter papers. After that, continuous washing is done to remove an excess of radioactive ATP. The inclusion of radio-isotopically labelled phosphate along with the kinase substrate is evaluated in order to detect the transfer activity of kinase phosphorylation, which directly depends on the extent of substrate phosphorylated [50]. The biggest advantage of this assay is that it is applicable to all the protein kinases. This assay does not require any modification in the substrate and its detection is also free from background interference (fluorescence), but its major disadvantage is that it can't be applied on a large-scale HTS. The commercially available radioisotope filtration binding assays are Eurofins's Kinase Profiler™ and Reaction Biology Corporation (RBC)'s HotSpot℠, which are used for assessing a large amount of kinase. The RBC assay provides largest kinase panel among the commercially available assays. It has total of 578 kinases, which have 366 wild type, 175 mutant, 20 atypical and 17 lipid kinases [51].

Most of the detection techniques for protein kinases with their advantages and disadvantages have been summarized in ▶ **table 2**. It was observed that no single technique is fully acceptable for drug discovery against kinases. The radioisotope filter binding assay remains the much accepted amongst the others, due to its various advantages and applicability to all the protein kinases in an HTS format.

Protein Kinase Inhibitors

Enzymatic protein kinase inhibitors are special class of inhibitors that hinder the phosphorylation of a protein. These inhibitors are employed to block the action of protein kinases generally on serine, tyrosine, and threonine amino acids of the protein, as these are the most readily phosphorylating targets in protein kinases. Generally, most of the kinases function on serine and threonine, whereas for tyrosine, tyrosine kinase is employed. Some kinases can act with dual specificity and can phosphorylate any protein based on the three above-mentioned amino acids of the protein. Since these protein kinases can mutate or alter the cellular functioning and can cause various diseases including cancer, hence these protein kinase inhibitors can be utilized as a promising candidate for treating diseases including cancer, which might have been caused because of the abnormal protein kinases. Due to this, quite substantial work has been carried out to develop a therapeutic tool for inhibiting protein kinases and further examining cellular functioning [9]. Nowadays, numerous examples of protein kinase inhibitors are available having high selectivity and apt pharmaceutical applicability properties, which can bring about a significant change in the concerned field [52]. Imatinib is an excellent example of such inhibitors, which is employed for inhibition of *BCR-ABL*1 in case of acute lymphoblastic leukemia and chronic myelogenous leukemia (CML) with the Philadelphia chromosomes [53]. Some other examples are vemurafenib for mutant tumor, which is caused because of the proto-oncogene *BRAF* [54]; crizotinib and alternative anaplastic lymphoma kinase (ALK) inhibitors for cancer caused via ALK fusions [55]; erlotinib and gefitinib for mutated tumor caused by EGFR and lapatinib employed for HER2/ ERBB2 amplified tumor [56]. Focusing on the target inhibiting specificity of the protein kinase inhibitors, the multiple targeted kinase inhibitors are the most prominent inhibitors of all, which are discussed in the following section.

Multiple Targeted Kinase Inhibitors

As mentioned earlier, there are about 518 protein kinases, which have pivotal roles in regulating various cellular signalling pathways, having different cellular metabolisms such as cell apoptosis, cell division, angiogenesis, and cell separation, etc. Several kinases like BCR-ABL, mutant EGFR, BRAF and tyrosine protein kinases are pro-

▶ **Table 2** Some common kinase profiling technologies

S.No	Techniques	Available assays	Advantage	Disadvantage
1	Fluorescence resonance energy transfer (FRET)	▪ AlphaScreen™ ▪ AlphaLISA1	▪ Highly sensitive in detection ▪ Applied to all fluorescent derived molecule ▪ Effectively used for Biological macromolecules	▪ Modified molecular system can change the fluorescence properties. ▪ Tagging of fluorescent probes can change the character of substrate.
2	Time-resolved fluorescence assay (TRF)	▪ DELFIA1	▪ No background disturbance from sample	▪ Use of limited lanthanide dye. ▪ More expensive
3	Time-resolved fluorescence resonance energy transfer (TR-FRET)	▪ LanthaScreen™	▪ Does not depend on radioactive material for detection ▪ High sensitivity ▪ HTS Friendly	▪ Modification of substrate for fluorophore labeling. ▪ Development of specific antibody
4	Fluorescence lifetime technology (FLT)	▪ FLEXYTE™ ▪ ATPLite™	▪ Homogenous ▪ Antibody free assay ▪ HTS compatible	▪ Limited to few kinases ▪ Requires specifically designed peptide substrate
5	Luminescence-based assay	Kinase-Glo™	▪ Homogenous set up ▪ HTS compatible ▪ Less interference	▪ Low sensitivity in case of low ATP ▪ Requires anti screening against coupling enzyme
6	Radiometric filtration binding assay	KinaseProfiler™ HotSpot℠	▪ No background interference ▪ Applicable to all protein kinases ▪ No modification in substrate is required	▪ Additional washing step required ▪ Radio isotopic materials safety issues.

claimed to be oncogenic and their hindrance/ inhibition is beneficial for anticancer treatment [57]. Continuous efforts are being made to convert the most efficient kinase inhibitors to work on multiple kinases by the collegial action [58]. This multi-targeted inhibition using a single reagent, also known as polypharmacology, offers considerable therapeutic benefits like satisfactory patient consent, interruption of drug-drug cooperation, simple and minimal combined off-target effects of protein kinases [59–62]. Sorafenib was the first multiple targeted inhibitor drug, which was approved in 2005. Sorafenib can be utilized for the treatment of non-resectable hepatocellular and final stages of renal cell carcinomas [63–65]. Other than sorafenib, many other FDA approved multi-kinase targeting inhibiting drugs are nilotinib, imatinib, pralsetinib, and dasatinib, etc., which are mentioned in ▶ **table 3** [66, 67]. These are validated to be inhibitors of various kinases such as p38, ABL, B-RAF, and c-KIT kinases, etc. [68–74].

FDA Approved Drugs as Targets

Kinases play a vital role in cell survival, proliferation, transformation and tumour initiation. A key challenge is to understand their role in oncology process and then to develop drugs against them [75]. On the other hand, it is hard to understand the functionalities of all the kinases, due to which these are categorized in different groups on the basis of their roles in oncology. Many intensive efforts have been made for the detection of novel targets in the human genome. Many clinical trials have been done successfully for drugs discovery to mark the infection. The advancement in kinase inhibitors had started in the mid-1970s. From the past few years, the protein kinase attracts a lot of attention in targeted drug discovery in cancer [76]. Currently, Structure-based medication discovery is made easier by more than 5,000 protein kinase structures that are publicly available. Additionally, the pharmaceutical industry uses a greater number of proprietary structures in the drug discovery process. Around 180 kinases showed the potential as primary targets of oral kinase inhibitors, which are under clinical trial. One can find a comprehensive list of these agents, which is updated periodically, at www.icoa. fr/pkidb/[77–79]. Since there are vast number of drugs in clinical Phase I-III, related to protein kinase inhibition, therefore, it is too difficult to cover all of them in this review. However, this article tends to review some of recent FDA approved drugs that bear high clinical importance for kinase inhibition, and these have been summarized in ▶ **fig. 4** along with their targets [21, 80].

The first successful protein kinase Rho-associated Protein Kinase (ROCK) inhibitor Fasudil was approved for treatment in 1995 in China and Japan. After 4 years (in 1999), Rapamycin, which is an inhibitor of mammalian target of rapamycin complex 1 (mMTORC1) complex was approved by the FDA [81]. Rapamycin was the first kinase inhibitor, which got FDA approval [82].

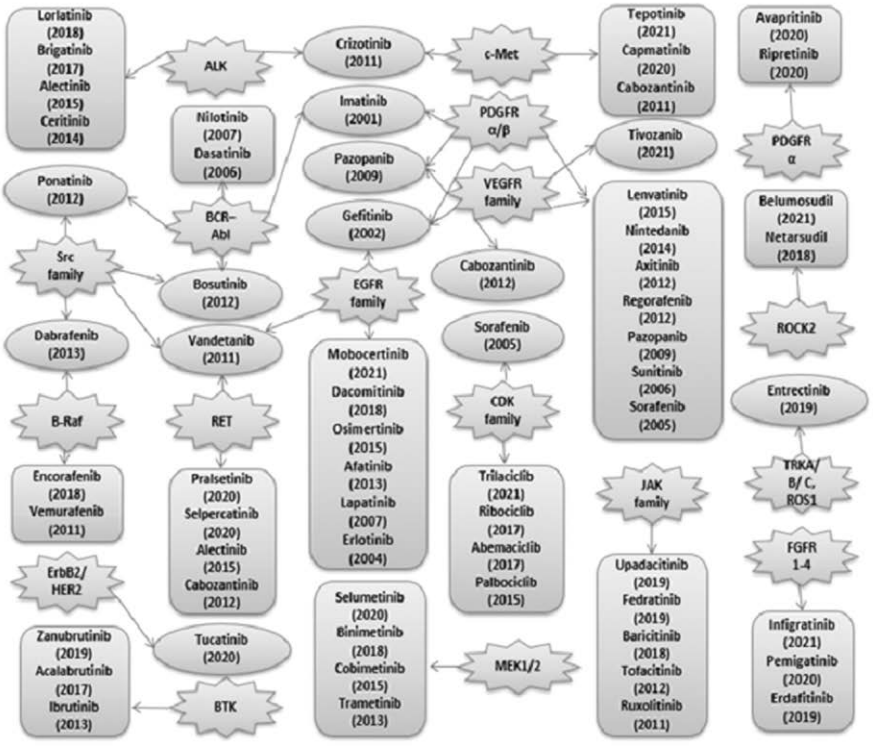
One of the biggest breakthroughs was the discovery of CGP-57148Bin 2001, which was later named as Imatinib [83–85]. It showed potential binding to the inactive conformation of ABL1 kinase for the treatment of chronic myeloid leukaemia (CML). Imatinib targets the *BCR-Abl* gene, the BCR part of the gene is found on chromosome 22 and the *Abl* gene is located at chromosome 9 [86], which showed the clinical improvement in the leukaemia patients [87]. This discovery leads to the development of other drugs for kinase inhibition like nilotinib, which was used against the imatinib resistant CML [88]. After this, sunitinib, which has a broad-spectrum activity against the FMS-like tyrosine kinase 3 (*FLT3),* a receptor tyrosine kinase *(KIT),* fibroblast growth factor receptors *(FGFR),* Platelet-derived growth factor receptors *(PDGFR)* and vascular endothelial growth factor receptors *(VEGFR)*, got approved by FDA for the treatment of renal cell carcinoma (RCC) and second line therapy of GIST (imatinib-resistant gastrointestinal stromal tumour) [89]. Similarly, a drug sorafenib which effectively binds with the inactive conformation of VEGFR kinase, was approved for the cure of RCC and hepatocellular carcinoma [90]. In 2009, a 2-amino pyrimidine derivative pazopanib was approved for the advanced stage of RCC. After 2009, an increase was observed in the approval of kinase inhibitor drugs. From 2011 to 2013, total of 14 kinase inhibitor drugs were approved by the FDA for various types of cancers. Since 1999, around 70 drugs got approval from FDA till Dec 2021 and still many more drugs are under clinical trial for many new kinase inhibitors [25, 91, 92].

Outlook and Future Prospects

Protein kinases, encoded by genes collectively called the kinome of the organism, mainly function to phosphorylate proteins and thus activate them. Since this form of protein activation is so com-

▶ **Table 3** FDA Approved Multi-Kinase Targeting Inhibitors.

Drug	Target Protein Kinase	Targeted Cancer	Developing Company
Imatinib	ABL1–2, KIT, PDGFR	MML, GIST. CML, CEL, Ph+ B-ALL	Novartis
Dasatinib	SRC, ABL1–2, KIT, PDGFR	CML	Bristol Myers
Sorafenib	KIT, BRAF, VEGFR2, FLT3, PDGFR	RCC	Onyx and Bayer Pharmaceuticals
Lapatinib	ERBB2, EGFR	Breast Cancer	Glaxo SmithKline
Gefitinib	EGFR	NSCLC	AstraZeneca
Temsirolimus	mTOR	RCC	Wyeth
Erlotinib	EGFFR	Pancreatic Cancer, NSCLC	Roche, OSI, Genentech Inc
Nilotinib	KIT, ABL1–2, PDGFR	CML	Novartis
Everolimus	mTOR	RCC	Novartis
Pralsetinib	RET	NSCLC, Thyroid Cancer	Genentech and Blueprint Medicines
Selpercatinib	RET	NSCLC, Thyroid Cancer	Eli Lilly and Company
Ripretinib	PDGFRA, KIT	GIST	Deciphera Pharmaceuticals



▶ **Fig.4** FDA approved kinome therapeutics with their approval year and major kinase targets.

mon, this highlights the omnipresence and importance of protein kinases and by extension of kinomes in the physiological and biochemical functioning of the body. Mutations in the kinome may lead to alterations in the protein kinase, resulting in their deregulation, which leads to several diseases including cancers. To throw some light on this significant issue, we have briefly discussed the mechanism of kinase action, various methods for their detection, and their roles in direct phosphorylation and indirect cell signalling. Use of bioinformatics for comparison of mutated kinomes in cancer patients against healthy kinomes to identify patterns, mutation hotspots and detailed structural study of protein kinases to develop more specific targeted therapies is very essential. We have also discussed the potential, importance, and types of kinome silencing using protein kinase inhibitors to check deregulated protein kinases and a selection of FDA approved drugs, to address this dysregulation. Detailed genomic studies to identify common mutation hotspots aided by computational methods to predict possible mutations resultant in dysregulated protein kinases would help in diagnostics.

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Conflict of interest

Authors declare no conflict of interest regarding the present work.

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