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TYROSINE KINASES: NOVEL TARGETS FOR CANCER THERAPY

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ABSTRACT

Tyrosine kinases are the enzymes those play a major role in tumor invasion and metastasis. Preclinical and clinical data strongly support the involvement of specific tyrosine kinases in the formation and progression of solid and liquid tumors. Various inhibitors were designed and synthesized for the regulation of tyrosine kinase activity. The present review focuses on to explore distinct characteristics of tyrosine kinases and their involvement in the progression of cancer.

Keywords: Receptor Tyrosine Kinase, Cancer, Signal Transduction, Phosphorylation, Oncogenes

INTRODUCTION

Tumor invasion and metastasis are the major causes of treatment failure and death in cancer patients. About 30% of patients with newly diagnosed tumors already have detectable metastases. Of the remaining 70% who are clinically free of metastasis, about half of them develop metastatic spread during follow-up after a potentially radical treatment of primary tumor[1]. Cancer drug therapy is undergoing a major transition from the previous pregenomic cytotoxic era to the new post genomic era. New cancer drug targets are identified and validated in various ways. The determination of the normal human genome sequence, followed by that of multiple cancer genomes, is accelerating target discovery[2].

Many of the defining characteristics of cancer, including uncontrolled growth, survival, neovascularization, metastasis and invasion, result from perturbation of regulatory signaling pathways, which are normally under tight control. As advances are made in understanding the mechanisms underlying the development of cancer, it has become clear that particular pathways are more frequently deregulated. Deregulation whether as a result of deletion, mutation or amplification of component gene products, is manifested as aberrant activation of key regulators of these pathways, prime examples of which are kinases[3].

The kinase family is one of the largest target families in the human genome. Together, it is estimated that there are more than 500 members of the major classes of protein serine/threonine, tyrosine and dual specificity kinases within the human genome[3-4]. Protein phosphorylation is one of the most significant signal transduction mechanisms of kinases by which intercellular signals regulate crucial intracellular processes such as ion transport, cellular proliferation, and hormone responses.

The family's key function in signal transduction for all organisms make it a very striking target class for therapeutic interventions in many disease states such as cancer, diabetes, inflammation, and arthritis. Recent successful launches of drugs with kinase inhibition as the mode of action demonstrate the ability to deliver kinase inhibitors as drugs with the appropriate selectivity, potency, and pharmacokinetic properties[5-6]. The

largest group of kinases is Protein kinases, which act on and modify the activity of specific proteins. These are used extensively to transmit signals and control complex processes in cells.

A protein kinase is an enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation). This usually results in a functional change of the target protein (substrate) by changing enzyme activity, cellular location, or association with other proteins. Deregulated kinase activity is a frequent cause of disease, particularly cancer, where kinases regulate many aspects that control cell growth, movement and death. Drugs which inhibit specific kinases are being developed to treat several diseases.

Protein kinases involve various types of kinases[7], as:
1. Serine/ threonine specific protein kinases
2. Tyrosine specific protein kinases
3. Histidine specific protein kinases
4. Aspartic acid/ glutamic acid specific protein kinases
5. Mixed kinases
6. MAP kinases
7. Cyclin dependent kinases.

TYROSINE KINASES

The protein tyrosine kinases (PTKs) are a large and diverse multigene family found only in Metazoans. Their principal functions involve the regulation of multicellular aspects of the organism. Cell to cell signals concerning growth, differentiation, adhesion, motility, and death, are frequently transmitted through tyrosine kinases. In humans, tyrosine kinases have been demonstrated to play significant roles in the development of many diseased states, including diabetes and cancer. Historically, tyrosine kinases define the prototypical class of oncogenes, involved in most forms of human malignancies. Tyrosine kinase genes have also been linked to a wide variety of congenital syndromes[8].

Tyrosine kinases are enzymes that transfer γ- phosphate groups from ATP to the hydroxyl group of tyrosine residues on signal transduction molecules. Phosphorylation of signal transduction molecules is a major activating event that leads to dramatic changes in tumor growth.

Targeting receptor protein tyrosine kinases (RPTKs) as cancer chemotherapy has continued to become a compelling approach with time. Preclinical and clinical data strongly support the involvement of specific RPTKs in the formation and progression of a subset of solid and liquid tumors. The advances in our understanding of the oncogenic activation of these receptors have been matched by the identification of new structural classes of kinase inhibitors with improved potency, specificity and efficacy[9].

CLASSIFICATION

Tyrosine kinases are primarily classified as receptor tyrosine kinase (RTK) e.g. EGFR, PDGFR, FGFR and the IR and non-receptor tyrosine kinase (NRTK) e.g. SRC, ABL, FAK and Janus kinase. The human genome, as currently sequenced, contains 90 tyrosine kinase genes and five presumed tyrosine kinase pseudo genes. Of the 90 tyrosine kinase genes, 58 are of the receptor type as defined by encoding a protein with a predicted transmembrane domain. These 58 receptor tyrosine kinases can be grouped into 20 subfamilies based on kinase domain sequence. The 32 non-receptor tyrosine kinases fall into 10 subfamilies based on kinase domain sequence. The remaining five sequences are classified as pseudo genes by the lack of introns in the sequence, the truncation of the coding regions compared to other members of the family, the presence of in-frame termination codons, and the absence of evidence for expression[8].

STRUCTURE OF TYROSINE KINASES

The receptor tyrosine kinases are not only cell surface transmembrane receptors, but are also enzymes having kinase activity. The structural organization of the receptor tyrosine kinase exhibits a multidomain extracellular ligand for conveying ligand specificity, a single pass transmembrane hydrophobic helix and a cytoplasmic portion containing a tyrosine kinase domain. The kinase domain has regulatory sequence both on the N and C terminal end[10-11].

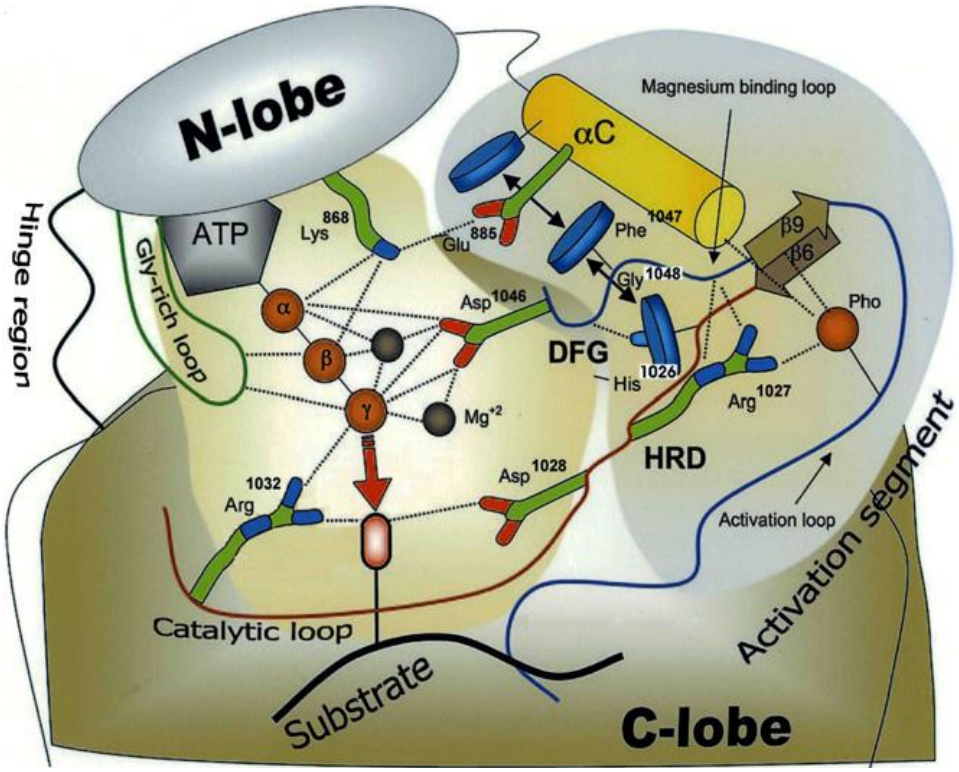

Fig. 1: Diagram of the inferred interactions between the human VEGF receptor 2 Protein– tyrosine kinase catalytic core residues, ATP, and a protein substrate.

NRTK are cytoplasmic proteins, exhibiting considerable structural variability. The NRTK have a kinase domain and often possess several additional signaling or protein-protein interacting domains such as SH2, SH3 and the PH domain[12]. The tyrosine kinase domain spans approximately 300 residues and consists of an N terminal lobe comprising of a 5 stranded β sheet and one α helix, while the C terminal domain is a large cytoplasmic domain that is mainly α helical. ATP binds in the cleft in between the two lobes and the tyrosine containing sequence of the protein substrate interacts with the residues of the C terminal lobe. RTK are activated by ligand binding to the extracellular domain followed by dimerization of receptors, facilitating trans-phosphorylation in the cytoplasmic domain whereas the activation mechanism of NRTK is more complex, involving heterologous protein-protein interaction to enable transphosphorylation[13].

SIGNAL TRANSDUCTION PATHWAY

Protein tyrosine kinases (PTKs) modulate a wide variety of cellular events, including differentiation, growth, metabolism and apoptosis[14-16]. Phosphorylation of tyrosine residues in target proteins is essential for maintaining cellular homeostasis, yet this post-translational modification also provides the means by which a number of cellular oncogenes deregulate various signaling pathways and induce transformation. PTKs are therefore important targets for both basic research and drug development efforts[17].

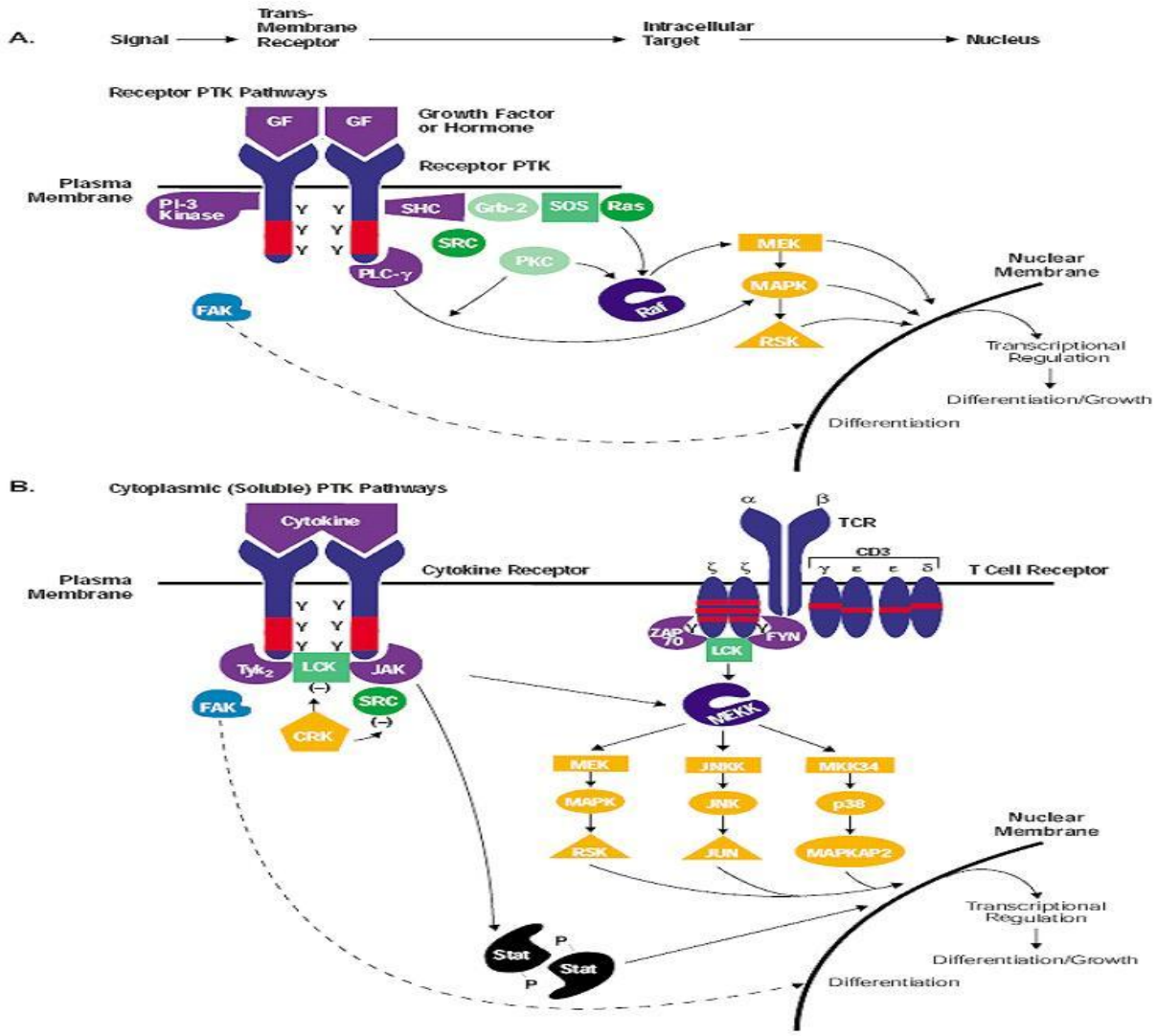

Fig. 2: Signal Transduction Pathway

PTKs represent a diverse and rapidly expanding super family of proteins, including both transmembrane receptor tyrosine kinases (RTK) and soluble cytoplasmic enzymes also known as nonreceptor tyrosine kinases (NRTK). Activation of the PTK domain of either class of PTK enzymes results in interaction of the protein with other signal transducing molecules and propagation of the signal along a specific signal transduction pathway.

Activation of transmembrane PTKs is typically initiated by binding of a ligand (e.g., hormone or growth factor) to a specific site within the extracellular domain of the receptor. Upon ligand binding, these receptors commonly undergo dimerization, resulting in autophosphorylation of tyrosine residues within the cytoplasmic domain[18-19]. This autophosphorylation event can occur in *trans* (between receptor molecules within the dimer) or in *cis* (within a single receptor molecule in the dimer). These phosphorylation events activate the kinase, thereby increasing its intrinsic PTK activity, and produce new binding sites for intracellular adapter molecules that bring signal transduction molecules into close proximity[14, 16-17].

ONCOGENIC ACTIVATION OF TYROSINE KINASES

Normally the level of cellular tyrosine kinase phosphorylation is tightly controlled by the antagonizing effect of tyrosine kinase and tyrosine phosphatases. There are several mechanisms by which tyrosine kinase might acquire

transforming functions, but the ultimate result is the constitutive activation of normally controlled pathways leading to the activation of other signaling proteins and secondary messengers which serves to hamper the regulatory functions in cellular responses like cell division, growth and cell death[20]. Constitutive activation of tyrosine kinase may occur by several mechanisms.

Activation by mutation

An important mechanism leading to tyrosine kinase deregulation is mutation. Mutations within the extracellular domain e.g. EGFRv III mutant lacks amino acid 6-273 which gives rise to receptor tyrosine kinase constitutive activity, that leads to cell proliferation in the absence of ligand in glioblastomas, ovarian tumors and non small cell lung carcinoma[21].

Autocrine-Paracrine loops

Autocrine-paracrine stimulation serves as an important mechanism for the constitutive activation of tyrosine kinase specially receptor tyrosine kinases. This activation loop is stimulated when a receptor tyrosine kinase is abnormally expressed or over expressed in presence of its associated ligand or when there is an over expression of the ligand in presence of its cognate receptor. A role of autocrine paracrine stimulation has been immanent in a variety of human cancers[22].

TYROSINE KINASES AS TARGETS FOR ANTICANCER AGENTS

The role of tyrosine kinases in cancer molecular pathogenesis is immense and recently kinases have come in vogue as potential anticancer drug targets, as a result a couple of anticancer drugs are in the market. The complexity and the number of tyrosine kinases have greatly increased with the sequencing effort of the Human Genome Project, thus providing more opportunities for drug discovery. Recent understanding of the molecular pathophysiology of cancer have highlighted that many tyrosine kinases are found upstream or downstream of epidemiologically relevant oncogenes or tumor suppressor, in particular the receptor tyrosine kinases[23].

The ATP binding site

ATP binds within a deep cleft formed between the two lobes of the tyrosine kinase domain. Though the ATP binding site is highly conserved the architecture in the regions proximal to the ATP binding site does afford some key diversity for designing new drug and has potential application in drug discovery [24].

Small molecule inhibitor

Tyrosine kinase forms a significant share of all oncoproteins thus they take centre stage as possible targets for cancer therapy. Hence low molecular weight tyrosine phosphorylation inhibitors (tyrphostins) have been proposed to be prospective anti-proliferating agents. By late 1980s it was proved that low molecular weight EGFR inhibitors could block EGF dependent cell proliferation[25].

Monoclonal antibody

The extracellular domain of the receptor tyrosine kinase provides an excellent target for monoclonal antibodies. With the advancement of genomics, design, selection and production of therapeutic monoclonal antibodies have become much easier. The revolution in antibody technology now allows us to produce humanized, human chimeric or bispecific antibody for targeted cancer therapy[26].

Hsp 90 and other novel strategies

Heat shock proteins (Hsp-s) are ubiquitous proteins known for the maintenance of cellular homeostasis and are inducible under variety of stresses. Hsp-s are mainly involved in the proper folding of other proteins and hence referred to as molecular chaperons[27-28]. The accumulation of Hsp-s is seen in pathological conditions and tumors. Most kinases require molecular chaperons to maintain their activation competent conformation. Hsp-s interacts with and stabilizes various kinases[29]. Chaperon based inhibitors other than interacting with protein kinases, prevent the associated chaperon(s) from maintaining the activation competent conformation of the kinase. The result being the proteosomal degradation of the misfolded kinases, thus diminishing the level of many kinases.

Antibody drug conjugate

The efficacy of the antibodies that targets specific molecules expressed by tumor cells can be increased by attaching toxins to them[30]. Existing immunotoxins are based on bacterial toxins like pseudomonas exotoxin, plant exotoxin like ricin or radio-nucleotides. The toxins are chemically conjugated to a specific ligand such as the variable domain of the heavy or light chain of the monoclonal antibody. Normal cells lacking the cancer specific antigens are not targeted by the targeted antibody[31].

Antisense strategies and peptide drugs

Antisense are small pieces of synthetic oligonucleotides that are designed to interact with the mRNA by Watson-Crick base pairing to block the transcription and thus translation of target proteins. Antisense oligodeoxynucleotides (ODN) targeting IGF-1R induces apoptosis in malignant melanoma and is also effective in breast cancer[32]. Protein–protein interaction is very important in cellular processes. Hence peptide and peptido mimetics that interfere with this interaction are important.

CONCLUSION

Tyrosine kinase plays an important role in angiogenesis and neovascularization. This process though occurs normally during embryonic development, female reproductive cycle or wound healing is found as a crucial step in tumor transition from benign to malignant form, capable of spreading throughout the body. Targeting receptor protein tyrosine kinases (RPTKs) as cancer chemotherapy has continued to become a compelling approach with time. The advances in our understanding of the oncogenic activation of these receptors have been matched by the identification of new structural classes of kinase inhibitors with improved potency, specificity and efficacy.

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