Journal of PharmaSciTech
2012; 1(2):36-45
Review Article

Tyrosine Kinase Receptor Inhibitors: A New Target for Anticancer Drug Development

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Abstract

Tyrosine kinases are important role in cell proliferation & differentiation of cell. Tyrosine kinases involved in diverse biological processes like growth, differentiation, metabolism and apoptosis in response to external and internal stimuli, mediator of signaling cascade. Tyrosine kinases are enzymes that selectively phosphorylates tyrosine residue in different substrates. Over expression of these tyrosine kinases receptor cause development of human cancer. So lot of work is done to understand role of tyrosine kinases in the pathophysiology of cancer. Defective regulation of growth processes plays a role in the genesis and progression of most type of cancer. Oncogenic activation of tyrosine kinase due to mutation, autocrine-paracrine stimulation in cancer cells can be blocked by selective tyrosine kinase inhibitors and thus considered as a promising approach for treatment of cancer. This review describes the role of TK receptors in development of cancer & the role of TK inhibitors on treating cancer. Glivec, Iressa and Terceva most successful tyrosine kinase inhibitor approved by FDA. The revolution in antibody technology allows producing humanized, human chimeric or bispecific monoclonal antibody for targeted cancer therapy. For e. g. Herceptin approved by FDA was first genome based targeted anticancer agent.

Keywords: Tyrosine kinase, cancer, oncogenic activation, TK inhibitor

Introduction

Membrane-spanning receptor tyrosine kinases control cell growth and differentiation. Tyrosine kinase is a subclass of protein kinase. Tyrosine kinase is enzymes that transfer a phosphate group to a tyrosine residue in a protein this have important faction in signal transduction. The binding of growth factors such as insulin, epidermal growth factor, and platelet-derived growth factor to the extracellular domain of this transmembrane receptor switches on the kinase activity of catalytic domain. This signaling cascade is altered in cancer cell, which cause overexpression of TK receptors.

The modes of oncogenic activation and the different approaches for tyrosine kinase inhibition, like small molecule inhibitors, monoclonal antibodies, heat shock proteins, monoclonal antibodies, antisense and peptide drugs are reviewed in light of the important molecules.[1-3] In cancer, angeogenesis is an important step in which new capillaries develop and develop for supplying a vasculature to provide nutrient and removing waste material. So tyrosine kinase inhibitor as an anti-angiogenic agent is new cancer therapy.[4] Natural drugs and Prodrugs also developed as TK inhibitor.[5] Low molecular weight substances, which inhibit tyrosine kinase phosphorylation block signaling pathway, initiated in the extracellular part of receptor. TK inhibitors can block tyrosine kinase reversibly or irreversibly.[6]

Classification

1. Receptor tyrosine kinase (RTK) e.g. EGFR, PDGFR, FGFR and the IR

2. Non-receptor tyrosine kinase (NRTK) e.g. SRC, ABL, FAK and Janus Kinase.

The tyrosine kinase receptors have multidomain extracellular Ligands for specific Ligand, a signal pass transmembrane hydrophobic helix and tyrosine kinase domain. The receptor tyrosine kinases are not only cell surfaces transmembrane receptors, but are also enzymes having kinase activity. Cytoplasm portion contains a tyrosine kinase domain. The kinase domain has regulatory sequence both on the N and C terminal end.[7-8]

The first non- receptor tyrosine kinases identified was the SRC. Non-receptor tyrosine kinase receptor has additional signaling or protein-protein interacting domains such as SH2, SH3, and the pH domain. SH2 domain bind phosphorylated peptides. In fact, they direct src and the protein containing them to activate receptor tyrosine kinase.[2]

Mechanism of action of tyrosine kinase

Until now four epidermal growth factor receptor have been identified HER1-HER4.These have three type of domain as

extracellular (ligand binding domain), a transmembrane (single transmembrane helix) and an intracellular (tyrosine kinase catalytic site) Tyrosine kinases are enzymes, which phosphorylates tyrosine residue in different substrates. Growth factor binding results in dimerisation of its cell surface receptors. This receptor dimerisation alters the three-dimensional molecular structure of monomeric receptor. When the Ligand bound and receptor dimerisation has occurred, cross phosphorylation of tyrosine kinase of other receptor can now take place in the intracellular domain of receptors. This autophosphorylation cause them to bind with SH2 (SRO homology 2)-containing cellular proteins. SH-2 binding to phosph-tyr activates the SH-2 containing protein.

Each tyrosine kinase receptor attached with an adenosine triphosphate (ATP) and the energy reach phosphate group is transferred to the amino acid tyrosine by this activation of tyrosine kinase and phosphorylation of tyrosine residue lead to activation. Schematic representation of mode of action of tyrosine kinase of intracellular signaling pathway is given below (Fig. 1).[3,7,8]

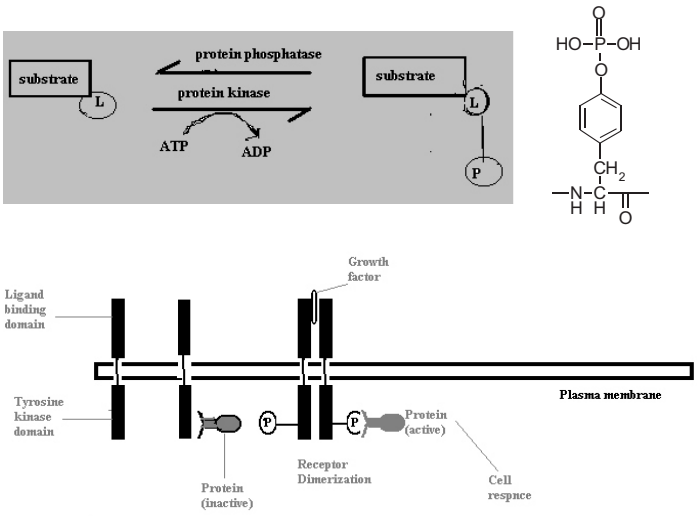

Fig.1: Mechanism of action of tyrosine kinase [9]

Oncogenic activation of tyrosine kinase

In general the level of cellular tyrosine kinase phosphorylation is tightly controlled by the antagonizing effect of tyrosine kinase and tyrosine phosphates. Many cancer-producing genes (oncogenes) encode altered signal transduction protein. Studies of mutant viruses revealed that many oncogenes encode receptor tyrosine kinases. The src gene is required for transformation (the education of cancer). The src gene acquired its name from its capacity to direct the synthesis of sarcoma producing protein. Normal cell contains a src gene that is very similar to viral one. The cellular gene called as c-src to distinguish from v-src. The viral version of src is constitutively active because its lacks inhibitory C-terminal region that is present in the cellular version.[2] Defective signal regulation by tyrosine kinase receptor plays an important role in the genesis of much type of cancers.

Mutation:

EGFRvIII is an example not containing extracellular ligand binding domain & become active without presence of ligand. Mutations within the extracellular domain e.g. EGFRv III mutant lacks amino acid 6-273, which give rise to receptor tyrosine kinase constitutive activity that lead to cell proliferation in the absence of ligand.[8, 10] EGFRv III is believed to cause many types of aggressive tumors most notably glioblastoma, breast and prostate cancer.[9]

Increased production of growth factors

1. BCR-ABL and Chronic Myelogenous leukemia

Chronic myelogenous leukemia is a clonal myeloproliferative disorder resulting from the oncogenic transformation of a pluripotent haemopoietic progenitor cell chronic myelogenous leukemia is a malignant, clonal haematopoietic stem cell disorder.[10] It accounts for 20% of all cases of leukemia with an incidence of 1-1.5 cases per 100 000. It affects predominantly older individuals even though all age groups are affected. Clinically, the course of CML is divided into three phases: a chronic phase, lasting an average of 3±4 years, an accelerated phase lasting 6-18 months and a blast phase lasting 3±6 months. The chronic phase of CML is characterized by myeloid hyperplasia with marked leucocytosis and circulating immature cells of the granulocytic series. Thrombocytosis is common and here is invariably some degree of basophilia. During the chronic phase of the disease, leukemia cells retain the capacity to differentiate generally. Familiar presenting symptoms consist of fatigue, night sweats and splenomegaly with abdominal discomfort and early satiety. Ultimately, the disease terminates in an acute leukemia or blast phase. The accelerated phase is an intermediate stage where patients show signs of disease progression, including a rising percentage of blasts (15±30%) and basophiles (>20%) in the peripheral blood or bone marrow, exclusive of meeting the criteria of an acute leukaemia.[11]

Molecular base of CML

The majority of cases of CML are associated with the presence of a specific chromosomal translocation (9,22) (q34; q11).[4] This reciprocal translocation between the long arms of chromosomes 9 and 22 results in a shortened chromosome 22, normally well-known as the Philadelphia chromosome (Ph).[12] The molecular consequences of this translocation occurrence is the fusion of the c-ABL oncogenes from chromosome 9 to sequences from chromosome 22, the breakpoint cluster region (BCR), give rise to achimeric BCR-ABL gene.[13] This gene encodes a fusion protein, of varying size, depending on the site of the breakpoint in BCR. The two most fusion proteins produced are given as p185 (185 kDa) and p210 (210 kDa). The p210 protein is seen in roughly 95% of patients with CML and up to 20% of adult patients with de-novo acute lymphocytic leukaemia (ALL), while the p185 form is seen in approximately 10% of adult patients with ALL and in the majority of

pediatric patients with Ph positive ALL (5% of pediatric ALL cases). These fusion proteins have constitutive tyrosine kinase activity, which is vital for their transforming ability.[14]

The constitutive tyrosine kinase activity of BCR ABL causes activation of a variety of intracellular signalling pathways leading to alterations in the proliferative, adhesive and survival properties of CML cells.[15] However, all of these events are reliant on the tyrosine kinase activity of the BCR-ABL protein.Oncoprotein p210 BCR-ABL, a tyrosine kinase with hyper-phosphorylation capacity when compared to the c-Abl encoded protein has been found to participate a role in the pathogenesis of CML . Recently it was reported that the 39 kD SH2/SH3 adaptor protein CrkL, a predominant substrate for BCR-ABL oncoprotein, was constitutively tyrosine phosphorylated not only in neutrophils but also in platelets from CML patients.

Break point cluster region (BCR) sequences of chromosome-22 on translocation, which lead to the philadalphia (Ph) chromosome. Philadalphia is a foreshortened chromosome 22 resulting from an exchange connecting the long arm chromosome 9 and 22. The translocation result in the jextaposition of 3/ DNA sequence derived from the Abelson(ALB) proto oncogen on chromosome 9 with 5/ sequence of the BCR gene on chromosome 22, forming a fusion of BCRALB. Mutation produces a 210KDa mutant protein which first Exxon of c-ABL has been replaced by BCR sequences encoding either 927 or 902 amino acid, the BCR-ABL.[16-21]

It is clear therefore that inhibition of the tyrosine kinase activity of BCR-ABL should be an effective treatment of CML as BCR-ABL is present in the greater part of patients with CML, is the causative abnormality of the disease and its kinase activity is vital for transformation standard treatment options for patients in chronic phase are allogeneic stem cell transplantation, hydroxyurea, busulfan or Interferon-a based regimens.[22]

2. TEL-ABL and Chronic Myelogenous leukemia

TEL-ABL tyrosine kinase similar to ABL-BCR is constitutively phosphorylates due to reciprocal translocation in case of acute lymphocyte leukemia and with a complex karyotype [9,18,23] in patients with CML. TEL ,a putative transcription factor is complex in-frame with exon-2 of the ABL proto-oncogene, producing a fusion protein product with elevated tyrosine kinase activity.

3. FLT3 receptor and leukemogenesis 24

FMS-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase that appears to play a major role in leukemogenesis. Activating mutations of FLT3 are there in roughly one-third of acute myeloid leukaemia patients. FLT3, also called as fetal liver kinase-2 (FLK-2) or stem cell kinase 1 (STK-1), is prearranged by a 24-exon gene located on chromosome 13q12. It shares the identical structural features as other members of the type III receptor tyrosine kinase family for instance FMS, KIT and platelet-derived growth factor receptor a (PDGFR-a and b), namely an extracellular ligand-binding region with five immunoglobulin-like domains, a cell membrane-spanning domain, and a cytoplasmic portion comprising a juxtamembrane region and split tyrosine kinase motifs. Upon stimulation with FL, FLT3 dimerises and undergoes autophosphorylation, up regulating its tyrosine kinase activity and triggering signalling through an array of downstream pathways that help cell proliferation and inhibit apoptosis and also phosphatidylinositol (PI)-3 kinase/ AKT and RAS/mitogen-activated protein kinase. The FLT3 receptor is expressed at high levels in 70–100% of classes of AML and virtually all cases of B-lineage acute lymphoblastic leukaemia. FLT3 emerged as a potential therapeutic target mostly following the discovery of activating mutations of FLT3 that take place in approximately one-third of AML patients.

In AML, though, FLT3 is single one among several genetic 'hits' that merge to cause the disease; more analogous to BCR/ ABL in CML blast crisis/Philadelphia positive ALL, or HER2, a member of the ErbB receptor tyrosine kinase family that is overexpressed in 20–30% of breast cancer patients. In these situations imatinib and transtuzimab, a monoclonal antibody directed against HER2, produce favourable clinical responses, but not outright cures.

Autocratic and paracrine growth factors in cancer[18]

Autocratic, paracrine stimulation plays vital role in constitutive activation TK receptor (Fig. 2). It has in been observed that as compared to normal cell, many tumour cells are abnormally expressed or overexpressed to growth factor. Fact 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 overexpressed in presence of its associated ligand.[25]

EGFR in autocrine-paracrine loops[26]

The epidermal growth factor family is comprised of EGFR (ErbB1), HER-2/neu (ErbB2), HER-3 (ErbB3), and HER-4 (ErbB4). All are found in reproductive tissues. Growth factors and their receptors are identified to play critical roles in cell growth and differentiation.[27] Growth factors such as EGF are strong mitogens for several human epithelial cell types including the endometrium[28-29] and have been implicated in cancer development.[30] EGFR has been revealed to be highly expressed in normal endometrium and overexpressed in endometrial cancer specimens[31] where it has been associated with a poor prognosis.[32-34] Increased expression of EGF related protein and EGFR may contribute to a drug resistant phenotype.[35] Inhibition of EGFR with monoclonal antibodies leads to growth arrest, and a similar and potentially synergistic effect is anticipated with inhibition of EGFR tyrosine kinase activity.

TGF-a and IGF receptor have been found in human breastcancer.[18-19]

EGFR is over expressed in most of solid human cancers including non small-cell lung, breast, head and neck, bladder and ovarian carcinomas. One important process is overproduction of TGF-a(Transforming growth factor-a) involved in autocratic paracrine growth loops in 20-40% of lung cancer[36] 40-80% Increased expression of EGFR is detected in non small cell lung cancer and is also overexpressed in 50% of primary lung cancer.[19]

PDGFR (platelet derived growth factor receptor) in Autocrine paracrine Loop [37,21]

Vascular endothelial growth factor-A (VEGFA) is the origin member of the VEGF family consisting of dimeric glycoprotein that belongs to the platelet-derived growth factor (PDGF) superfamily.[38] A cytokine that increases microvascular permeability to plasma proteins, VEGF-A has been further characterized for its many effects relevant to generating and preserving tissue vasculature. These effects comprise the induction of endothelial cell division and migration, promotion of endothelial cell survival through protection from apoptosis.[39] VEGF exerts its biological effect through interaction with transmembrane tyrosine kinase receptors present on the cell surface (VEGFR, neuropilin receptor). Upon binding of VEGF to the extracellular domain of the receptor, dimerization and autophosphorylation of the intracellular receptor tyrosine kinases occurs and a cascade of downstream proteins are activated.40 VEGFR-2 appears to be the major receptor responsible for mediating the proangiogenic effects of VEGF. It has been found recently by several researchers that VEGF overexpression in conventional RCC samples compared to normal renal tissue.[41] For that reason, inhibition of VEGF signalling has been pursued as a therapeutic target in advanced RCC.

Abnormal PGDF-induce cell proliferation has been found to lead to disorder such as atherosclerosis, restinosis following percutaneous coronary intervention, glomerulosclerois, liver cirrhosis, and certain cancer. In the sub families of PDGF mutated c-Kit has been found in mastocytosis, acute mylogenous leukemia. Constitutive activation of PDGFR and its cognate ligand PDGF-A and PDGF-B has been observed in most astrocytic brain tumors and gliomas.[43]

Insulin like growth factor receptors in Autocrine growth loop

IGF-1 and IGF-2 are though to have Autocrine, endocrine, and paracrine roles in regular mammary development and in the etiology of breast cancer.[44-46] There is now large evidences showing that IGF activation involve in malignant processes. Co-expression of IGFR and its ligand IGF I and IGF II is reported in the pathogenesis of breast cancer, prostrate cancer and small cell lung cancer.[47] In breast cancer it is seen that there is elevated IGF-I R autophosphorylation and kinase activity.[42] Elevated prostrate cancer risk is also correlated association of Autocrine paracrine loops has been implicated in the pathogenesis of several cancers.[48]

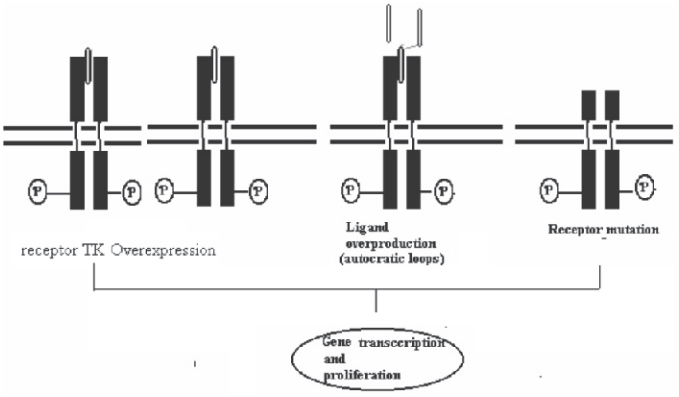

Fig. 2: Schematic view of different mechanisms leading to the constitutive activation of tyrosine kinase

Tyrosine kinases as targets for anticancer agents

Protein kinase catalyzed protein phosphorylation, is the most general and frequent mechanism by which almost all cellular function are reversibly regulated. Due to better understanding of pathophysiology of cancer shown that tyrosine kinases found upstream or downstream of epidemiological relevant oncogen or tumor suppressor, in particular the receptor tyrosine kinases. More attention is been given to tyrosine kinase that catalyzes the phosphorylation of specific tyrosine residue.[49]

Target Site of action

Inhibiting the activity of tyrosine kinases by low molecular weight compounds capable of interfering with either ligand binding (in the case of receptor tyrosine kinases)[50] or with protein substrate (in case of non receptor tyrosine kinase) has proved to be difficult.[51] Although the bisubstrate inhibitor approach offered promise, but with very little practical progress. Approaches to generate non-competitive or allosteric inhibitors have also failed. The ATP competitive inhibitors appear to be the target of choice.[52]

ATP binding site

The ATP binding sites have the following features (Fig. 3): (a) Linker region: Is the conserved linker region of protein kinase catalytic core formed by interaction 6-amino (N-6) and N-1 nitrogen of purine base. N-7 nitrogen and its interaction with the enzyme is a region for potential modification of ATP competitive inhibitor.44; (b) Hydrophilic region: a sugar region except a few e.g. EGFR. This region has been utilized to design ATP based specific inhibitor.Hydrophobic pocket: though not used by ATP but plays an important role in inhibitor selectivity; (c) Hydrophobic channel: not used by ATP and may be exploited for inhibitor specificity; (d) Phosphate binding region: the key residues that hold the lig? and and ? phosphate in position are the phosphate anchor, the metal site, and Lys? 168. It can be used for improving inhibitor selectivity.[53-56]

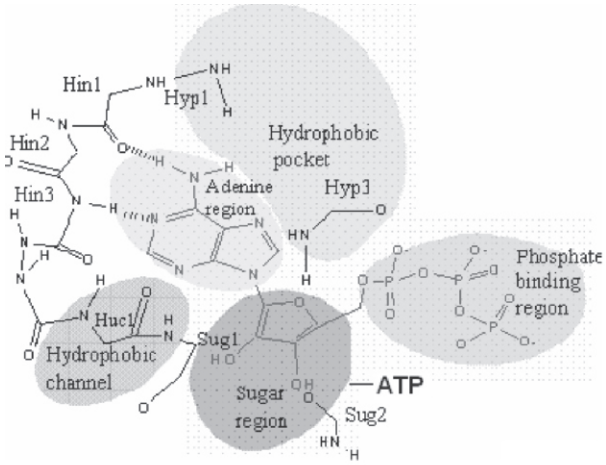

Fig. 3: Model of the ATP-binding site of protein kinases. Sug1, Hyp1 and Hyc1 are residues lining the sugar region, hydrophobic pocket (Hyp), hydrophobic channel (Huc) and hinge region (Hin) respectively (redrawn)[56]

Tyrosine kinase inhibitors

Numerous small molecules, synthetic tyrosine kinase inhibitors are in clinical development for the treatment of human cancers. These all falls in three broad category: (1) inhibitor of EGFR tyrosine kinase, e.g.Iressa and Tarceva; (2) inhibitor of split kinase receptor tyrosine kinase e.g. PTK787; (3) inhibitor of tyrosine kinase from multiple subgroup e.g. Glivec [8, 9, 57]

The first synthetic tyrosine kinase inhibitors, the tyrphostins, were described in 1988.[58] Working in parallel, scientists at Ciba-Geigy (now Novartis, Basel, Switzerland), identified a 2-phenylaminopyrimidine as a lead tyrosine kinase inhibitor by screening a large library of compounds. Using structure-activity relationships, compounds of increasing potency and specificity were synthesized.[59] STI571 was initially developed as a specific platelet-derived growth factor receptor (PDGF-R) inhibitor, but was also found to be a potent ABL tyrosine kinase inhibitor. Of all the tyrosine kinase inhibitors the most successful are Glivec, Iressa and Tarceva. The novel anticancer drug Glivec/ Glivec/ Imatinib mesylate (Novartis STI571) is a success for CML and c-kit positive metastatic GIST. Glivec selectively and effectively inhibits the kinase activity of BCR-ABL fusion protein, which is responsible for the constitutive proliferate signaling. TEL-ABL and TEL-PDGFR are fusion proteins. STI571 remains bound to the ATP binding cleft of the unphospholrylated (activation loop) ABL, thus establishing extensive contacts [1, 15, 57, 60] with residues lining the cleft and with peptide segments just outside the cleft. A large change in conformation of the nucleotide binding domain is accompanied with the binding of the drug. The binding of STI571 prevents ATP to access the ATP binding cleft and thus inhibits subsequent tyrosine phosphorylation of the substrate56-61 Iressa is a selective inhibitor of EGF receptor tyrosine kinase in non-small cell lung cancer and squamous cell carcinoma (Fig. 4).[62]

In May 2003, the FDA approved gefitinib for third line treatment of advanced or metastasis. Iressa blocks several of these tyrosine kinases, including one associated with Epidermal Growth Factor Receptor (EGFR). EGFR is expressed on the cell surface of many normal cells and cancer cells.

Imatinib acts by inhibiting the kinase activity of the BCR-ABL oncoprotein and has exhibited impressive results in the treatment of Ph+ CML. The amazing success of Imatinib, a blocker of the BCR-ABL kinase in chronic myeloid leukemia has shown that the drugs based on these strategies can improve cure rates in cancer. Iressa blocks growth signals in cancer cells.

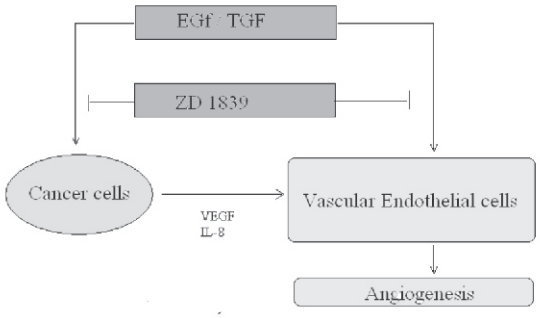

Fig. 4: A model for antiangiogenic effects by ZD1839. EGF/TGF up-regulates expression of angiogenic factors (VEGF and IL-8) in cancer cells

The Na^+/H^+ antiproton amiloride, the Ca^+ antagonist chlorpromazine, imipramine and dibucain, quercetein and other flavonoids, genisteine & series of 4-hydroxycinnamides & a-cynocinnamide have been reported to show TryK inhibitory activity. Genisteine inhibited in vitro the tyrosine specific protein kinase activity of epidermal growth factor (EGF) receptor, pp60 and pp110. The inhibition was competitive with respect to ATP and noncompetitive with a phosphate acceptor, histone H2B and the enzyme activity of serine/threonine specific protein kinase. Genisteine inhibited the GF stimulated increase in phosphorylation level in A431 cells. The structures of 4-hydroxycinamides are given in Fig. 5.

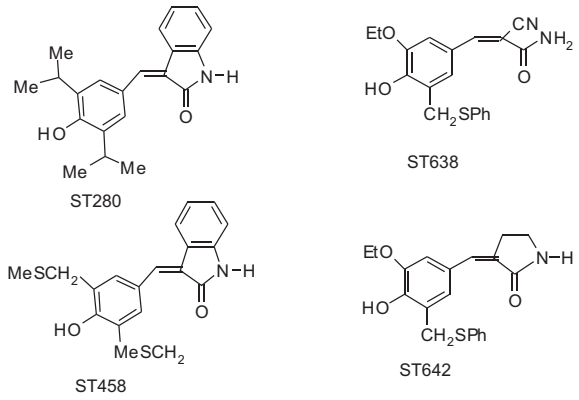

Fig.5: Structures of ST-280, ST-458, ST-638, and ST-642 that inhibits in vitro tyrosine specific protein kinase activity in the EGF receptor.[1]

Tarceva inhibit phosphorylation of HER-1 tyrosine kinase Tarceva inhibit binding of adenosine triphosphate (ATP) to the HER1tyrosine kinase domain competitively and reversibly so the effectiveness depend on intracellular ATP concentration.[3, 8, 63]

Ranjita Banerjee et al. synthesis prodrug designed to release multiple inhibitors of epidermal factor Receyor tyrosine kinase and an alkylating agent which is the novel approach for treatment of cancer. Acetoxymethyletriazine designed to be a prodrug of multiple inhibitors of EGFR and a methylediazonium species. Acetoxymethyletriazine degrade to the corresponding hydroxymethyl triazene, which in tern converted to monoalkyletriazine. Further hydrolysis produces aromatic amine (6-amino-4-quinazoline) inhibitor of EGFR & an alkylating agent. [64]

Lapatinib ditosylate (GSK572016) is an epidermal growth factor receptor (EGFR) and ErbB-2 (Her2/neu) dual tyrosine kinase inhibitor under development by GlaxoSmithKline as a treatment for solid tumors such as breast and lung cancer. If preliminary findings are supported by data from larger phase III trials then Lapatinib could become an important new treatment option for breast cancer patients and potentially those with other difficult-to-treat solid tumors.

Monoclonal antibody

Numerous of monoclonal antibodies that block EGFR at the cell surface have been developed. The EGFR is the family of four members: the EGFR, HER-2, HER-3 and HER-4/. EGFR and HER-2/neu are amplified in tumor samples of breast, lung and colorectal tissues. Their overexpression leads to elevated MAP kinase and PI3 kinase recruitment and subsequent suppression of apoptosis, deregulation of cell cycle and proliferation. The best known monoclonal antibody is trastuzumab (proprietary name: Herceptin) developed by Gentech.[63] It is a humanized monoclonal antibody which blocks overexpression of EGFR and HER2 in breast cancer. Other important anti-EGFR monoclonal antibodies are Cetuximab (IMC-C225) by Imclone and 2C4 by Gentech and Osidem by Mederex. C225 or Cetuximab is chimeric monoclonal antibody targeted against EGFR/ Erb B1.2C4 is another humanized monoclonal antibody targeted against HER-2/ Erb B2 member of the EGFR family. In vitro experiment revealed that Cetuximab effective against a wide range of human cancer including those of the pancreas, kidney, breast, colon, prostate, and head and neck.[65] Antigens like are overexpressed in cancers Antibody directed towards P12 antigens might serve as important contributors to cancer therapeutics as exemplified by the results of preliminary trials. Example of this approach is MAb P12 reacts with the carbohydrate sequence present on high molecular weight glycoproteins. Researchers are interested in newer MAbs as promising agents for the treatment of cancer.

Heat shock protein 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 largely involved in the proper folding of other proteins and therefore called to as molecular chaperons.[66-67] 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.[68] Chaperon based inhibitors other than interacting with protein kinases prevents the associated chaperon(s) from maintaining the activation competent conformation of the kinase. The result is the proteosomal degradation of misfolded kinases thus, diminishing the level of many kinases. The Hsp-s has a unique ATP binding site, including a Bergerat fold characteristic of bacterial gyrase, topoisomerases and histidine kinases. Thus the ATP binding site serves as a robust antitumor target for kinase related chaperone machinery.

Inhibitors of the molecular chaperone heat shock protein 90 (Hsp-90) were the first class of compounds described to have inhibitory effects on FLT3. Herbimycin A, a naturally occurring product of Streptomyces sp., was found to both inhibit the in vitro growth of FLT3-ITD transformed cells and reduce tumour size in a FLT3-ITD-driven mouse tumour model. FLT3-ITD appears to require Hsp-90 for proper folding and stabilization: the anti-FLT3 effect of Herbimycin A occurs indirectly through dissociation of the complex formed between FLT3-ITD and Hsp-90. Another Hsp-90 inhibitor, the geldanamycin analogue 17-N-allylamino-17-demethoxyl geldanamycin (17-AAG) has shown promising in vitro activity against FLT3-ITD AML. The other small molecules that are currently in development as FLT3 inhibitors belong to a several of chemical classes, some are all heterocyclic compounds that directly inhibit FLT3 by mimicking the purine ring structure of adenosine and thus compete with ATP for binding to the ATP binding pocket of the kinase domain of the FLT3 receptor. These agents may bind to the active, inactive or transitional state of FLT3, with their selectivity being greatly influenced by changes in the tertiary structure of the binding pocket that result from single amino acid changes, such as those caused by FLT3-TKD mutations.24 Important examples are Geldanamycin, Cisplatin, Novobiocin, Radicol and other purine based inhibitors. Geldanamycin affects ErbB2, EGF, v-Src, Raf-1.[69-70]

Antisense oligonucleotide

Antisense oligonucleotide are nucleic acid molecule that block the signaling pathway of TK at there site of action in the nucleus .Oligonucleotide that are designed to interact with the mRNA and block the transcription and thus translation that is correct reading of genetic information of target proteins. Antisense oligodeoxynucleotides (ODN) targeting IGF-1R induces apoptosis in malignant melanoma and is also effective in breast cancer.[71]

Inhibitors of angiogenesis

Angiogenesis is a multistep process. Angiogenesis, the formation of new blood vessels to the tumor site is mainly induced and

regulated by VEGF. Blocking angeogenesis is now considered to be a promising approach for anticancer therapy Tyrosine kinase plays an important role in this process. In cancer angeogenesis is crucial for supplying vasculature to provide nutrient and remove waste material. It is found as a crucial step in tumor transition from benign to malignant form; capable of spreading throughout the body.[72-73] Vascular endothelial growth factor (VEGF) is release by many tumors. Angiogenic activity is mediated by two high affinity receptors VEGRF-1 and VEGRF-3. The VEGRF receptor are the members of receptor tyrosine kinase having an extracellular ligand binding domain, a transmembrane domain, and an intracellular split kinase domain [74,75] This VEGF-1 receptor tyrosine kinase mediated angeogenesis of human endothelial cells and growth of multiple tumor types is inhibited by SU5416.76 PD173074, ZD1839 is a potent inhibitor of FGFR1 and also VEGFR2.[77] PD98059 is an important inhibitor of MAPK cascade that lies downstream of Ras pathway and thus is effective in many tumors. Antiangiogenic drugs stops new vessels from forming around a tumor and break up the existing network of abnormal capillaries that feeds the cancerous mass, thus shrinks the tumor by limiting blood supply.4

Future development

The promising approach made by Glivec, Gefitinib and Tarceva has given new genomic-based treatment of cancer. Other agent such as Cetuximab, Erlotinib, Canertinib are in advance stage of clinical trials and may be available for general clinical use in the next few years.[38] Promising approach based on the use of protein derived vector peptide has been recently reported. These peptide are capable of translocating covalently tagged, membrane impermeable compound across the plasma membrane directly in to cytosol. Different inhibitors of protein tyrosine kinases synthesized have been internalized inside intact cell using this carrier. Cancer is a disease characterized by extensive genomic abnormalities and aberrations in gene expression. Tumor-specific mutations, DNA amplifications and translocations can all distort the normal programs of gene expression and function, resulting in misregulated activity of apoptosis, angeogenesis and cell proliferation. The selectivity becomes important consideration in development of tyrosine kinases inhibitor. Hence strategy's to move kinases drug discovery in a rapid and efficient way includes tyrosine kinase target validation, selectivity and durability are the future demand. Fluorescent polarization assays, monoclonal antibody based multiimmunoblotting, Nonradioactive HTS used recently.[78] A number of protein kinase inhibitors are in clinical trials (Table 1):

Table 1: Protein kinase inhibitors in clinical trials

Inhibitor	Target kinase(s)	Company	Cancer
Small molecule inhibitors			
STI-1571 , (Approved)	Abl, c-kit, PDGFR	Novartis	CML, GIST
ZD1839(Iressa,gefitinib) (Approved)	EGFR	AstraZeneca	NSCLC, SCC
Erlotinib(Terceva-OSI pharmaceutical) (Phase III)	EGFR	OSI/Genetech	SCC, BC, LC
Canertinib(CI-1033), (Phase-II)	EGFR,Her2, Her3	Pfizer	BC, LC, Overian cancer
EKB-569 , (Phase-II)	EGFR, Her2	Wyeth	
PTK-787, (Phase III)	VEGFR	Novartis	CR cancer
SU5416, (Phase III)	VEGFR	Sugen	solid tumor
Monoclonal antibody			
Trastuzumab(Herceptin) FDA (Approved)	Her-2/neu	Genetech	BC
ABX-EGF	EGFR	Abgenex	NSCLC, SCC
h-R3 (Phase-II)	EGFR	Cuben institute of Oncology	solid tumor NSCLC, SCC
MAb-806	EGFR, Her-2	Ludwing institute of cancer research	NSCLC, SCC
2C4, (Phase II)	Her-2/neu	Genetech	BC, PC, OC
Cetuximab(IMC-C225), (Phase III)	EGFR	Imclone	Pan.C, BC, RenC

Antisense			
ISIS 3521 , (Phase II)	PKC-α	ISIS	NSCLC, BC, Pan.C
GEM 231 (Phase I)	PKA	Hybridon	CR, Pan.C, LC
Immunotoxins			
Anti-Tac (Fv)-PE38 , (Phase I)	CD25	–	B, T cell leukemia
DAB389IL2 , (Phase III)	IL-2R	–	CTCL, HD, B-NHL
Hsp90 inhibitors			
Geldanamycin, (Phase I)	Hsp90	Conforma. Inc.	Thyroid cancer
Egf-p64 , (Phase-II)	EGFR	National institute of oncology	Solid tumor including breast, lung
17-AAG , (Phase I)	Hsp90	–	BC

Conclusion

Inhibition of signal transduction inhibition has become a viable and attractive avenue in biomedical cancer research based on the discovery of a large number of somatic mutations in many different types of cancer that lead to deregulated growth signal transduction and subsequent aberrant growth, invasion, tumor-derived angiogenesis and metastasis. In these three situations it has been possible to fashion potent and selective tyrosine kinase inhibitors that inhibit the catalytic sites of these receptors thereby inhibiting tumor growth. Glivec (STI571), for instance, is an inhibitor of the Bcr Abl kinase that is well-tolerated in animals and man leading to major hematological responses in almost 100% of patients with interferon-resistant CML. Gefitinib ("Iressa", ZD1839) is an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor, and the single agent is clinically effective in non–small cell lung cancer . PKI166, on the other hand, is a dual inhibitor of EGF receptor (HER 1) as well as ErbB (HER 2) that is highly active inhibiting growth of EGF-receptor over expressing tumors in animals and that has entered clinical trials as well.

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