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Review

Tyrosine Kinases: Structural Insights and Mechanistic Roles in Cancer Progression and Therapeutics

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Abstract: Protein tyrosine kinases (PTKs) are key enzymes of cellular signaling, regulating key processes such as proliferation, differentiation, migration, metabolism, and apoptosis. TKs modulate protein functions in normal and disease states by phosphorylating tyrosine residues on target proteins. On this critical role, dysregulation of TKs is directly linked with disease progression, particularly in cancer, therefore making TKs an attractive target for therapeutic intervention. The PTK family is broadly classified into receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). Both classes play a critical role in signal transduction. High-resolution structural studies of PTKs have provided mechanistic insights into their regulation. This review aims at the organization, mechanistic activity, and therapeutic potential of PTKs, with a particular focus on epidermal growth factor receptor (EGFR) and Src kinase as representatives of RTK and NRTK, respectively. Additionally, this review also focuses on addressing emerging strategies to enhance TKI efficacy and overcome acquired resistance in cancer therapy.

Keywords: Protein tyrosine kinases; epidermal growth factor receptor (EGFR); receptor tyrosine kinases; non-receptor tyrosine kinases; cancer; Src kinase; exo-site

1. Introduction

Tyrosine kinases are crucial enzymes involved in signal transduction, which regulate key cellular processes such as proliferation, differentiation, migration, metabolism, and apoptosis[1–5]. By catalyzing the phosphorylation of tyrosine residues in target proteins, kinases mediate vital cellular communication and maintain homeostasis[6,7]. Hence, phosphorylation acts as a post-translational modification that plays a central role in normal cellular functions, but its dysregulation can lead to pathological conditions, including cancer [8–13]. Unusual activation of protein tyrosine kinases (PTKs) is usually associated with disease progression and therapy resistance, while making them critical targets for therapeutic interventions, particularly in cancer treatment[14–20].

1.1. Broad Classification of Tyrosine Kinases

The PTK family is diverse, with members varying in structure and function[21]. These kinases are classified into two major subgroups: receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs)[22,23]. RTKs are membrane-bound enzymes that transmit extracellular signals such as growth factors, cytokines, and hormones to the cytoplasm and nucleus, initiating a cascade of cellular responses[7,24,25]. The key function of RTKs is to rapidly and reversibly phosphorylate protein substrates, which leads to alterations in protein conformation and interaction, driving various

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cellular processes such as growth and survival[26]. On the other hand, NRTKs lack extracellular and transmembrane domains and are found in the cytoplasm or nucleus. These kinases are involved in mediating intracellular signals, often in response to receptor-dependent activation at the cell membrane[27–29]. While RTKs and NRTKs function similarly by regulating crucial cellular processes, including cell division, growth, and immune responses, their structures are strikingly distinct [30,31]. Due to their essential roles in cellular signaling, both RTKs and NRTKs are critical in the regulation of various physiological functions and are often implicated in the progression of cancers when their activation becomes dysregulated [32]. The discovery of the Src oncogene and the identification of the epidermal growth factor receptor (EGFR) as the first RTK laid the foundation for understanding the role of tyrosine kinases in cancer[33,34]. So far, over 90 tyrosine kinases have been identified, and these enzymes are now recognized as pivotal players in cellular signaling circuits that contribute to cancer development[35]. Hence, tyrosine kinases represent a significant portion of oncoproteins, and targeting these for therapeutic development is a promising strategy in the treatment of cancers associated with their dysregulation[14–16].

As described above PTKs are primarily classified as RTKs and NRTKs[22,23]. Based on the composition of the extracellular regions, the 58 identified RTKs in humans are further categorized into 20 distinct families (**Table 1**).

Table 1. RTKs classification is based on the sequence of the kinase domain. Table adapted and modified from[36].

Class	Family	Receptors
I	EGF/ErbB	EGFR, ErbB2/HER2, ErbB3/HER3, ErbB4/HER3
II	Ins	InsR, IGF1R, InsRR
III	PDGF	PDGFRα, PDGFRβ, CSF1R, KIT, FLT3
IV	VEGF	VEGFR1/Flt1, VEGFR2/KDR, VEGFR3/Flt4
V	FGF	FGFR1, FGFR2, FGFR3, FGER4
VI	PKT7	PKT7/CCK4
VII	TRK	TRKA, TRKB, TRKC
VIII	ROR	ROR1, ROR2
IX	MuSK	MuSK
X	HGF	MET, MST1R(RON)
XI	TAM	AXL, MER, TYRO3
XII	TIIE	TIE1, TEK(TIE2)
XIII	Eph	EphA1-8, EphA10, EphB1-4, EphB6
XIV	RET	RET
XV	RYK	RYK
XVI	DDR	DDR1, DDR2
XVII	ROS	ROS
XVIII	LMR	LMR1, LMR2, LMR3
XIX	ALK	LTK, ALK
XX	STYK1	STYK1

1.2. Cancer-Associated Receptor Tyrosine Kinases

The epidermal growth factor family (EGF) includes EGFR (HER1), HER2, HER3, and HER4[37,38]. These receptors are often overexpressed in epithelial tumors, such as colorectal, head and neck, non-small cell lung, breast, pancreatic, and renal cell cancers[39,40]. The insulin growth factor (IGF) and insulin receptor (InsR) family consist of the IGF1R and InsR receptors. Both IGF1 and IGF2 are capable of binding to and activating the IGF1R transmembrane receptor kinase. However, when IGF2 binds, it does not activate any downstream signaling pathways because the IGF2R lacks the kinase structural domain necessary for this activation[41]. Platelet-derived growth factor receptor (PDGFR), colony-stimulating factor 1 receptor (CSF1R), KIT proto-oncogene receptor (KIT), and FMS-like tyrosine kinase 3 (FLT3) receptors are critical for various cellular processes[42].

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PDGF is essential for tissue growth, division, and blood vessel formation. CSF1R, secreted by cancer cells to evade immune detection, promotes the growth and recruitment of tumor-associated myeloid cells, contributing to poorer survival in many cancers[43]. The vascular endothelial growth factor (VEGF) receptor family—VEGFR-1, VEGFR-2, and VEGFR-3—regulates processes like cell migration, angiogenesis, and metabolic homeostasis[44–46]. Likewise, the fibroblast growth factor (FGF) receptor family, including FGFR1-4, plays a role in tissue repair, regeneration, and the growth and differentiation of cells during development and organ formation[47,48]. Protein tyrosine kinase-like 7 (PTK7) and colon carcinoma kinase 4 (CCK4) receptors are involved in epithelial cell polarization and brain structure formation[49]. These receptors are catalytically active protein kinases and play roles in the Wnt and VEGF signaling pathways[50]. The neurotrophin receptor (NTRK) family includes TRKA, TRKB, and TRKC receptors, which are vital for the proliferation and migration of the nervous system[51,52]. TRKA responds to nerve growth factor (NGF), TRKB to brain-derived neurotrophic factor (BDNF), and TRKC to neurotrophin-3[53]. The RTK-like orphan receptor (ROR) family includes ROR1 and ROR2 receptors. ROR1 acts as a substitute receptor and co-receptor for Wnt signaling, regulating cell division, polarity, and tissue maintenance[54]. In contrast, ROR2's role in tumor development varies depending on the tumor type or stage; it can either repress or activate tumor growth through atypical Wnt signaling[55]. The muscle-specific kinase (MuSK) receptor is essential for the formation and organization of neuromuscular junctions in skeletal muscle[56,57]. The hepatocyte growth factor (HGF) receptor family includes MET (c-Met) and RON receptors. When HGF binds to MET, it activates the proliferation, migration, and morphogenesis of epithelial cells[58,59]. The TAM receptors (TYRO3, AXL, MER) are activated by the vitamin K-dependent proteins Gas6 and protein S, regulating cell proliferation, survival, adhesion, and migration[60,61]. They also have anti-inflammatory properties and are implicated in carcinogenesis in various malignancies[62–64]. The TIE receptor family, consisting of TIE1 and TIE2, regulates angiogenesis and lymph angiogenesis[65–67]. The Eph receptor family (EphA1–A10, EphB1–B6) controls angiogenesis, cell migration, patterning, and neuronal formation[68–70]. The RET receptor, activated by glial cell-derived neurotrophic factor ligands, is crucial for cell proliferation, neuronal navigation, migration, and differentiation[71,72]. The receptor tyrosine kinase (RYK) is characterized by extracellular Wnt-binding domains and is closely associated with Wnt signaling[73,74]. The discoidin domain receptor (DDR) family, which includes DDR1 and DDR2, regulates cell adhesion, proliferation, and metalloproteinase expression[75–77]. DDR1 also promotes tumor cell invasion and enhances the survival of tumor stem cells in collagen-rich environments[78,79]. The reactive oxygen species (ROS) receptor family is present in various malignant tumors, making it a promising target for anticancer drugs[79–81]. Lemur receptor kinases (LMR/LMTK) are linked to cancer and influence multiple signaling pathways involved in cell proliferation, migration, and invasiveness[82–84]. The anaplastic lymphoma kinase (ALK) receptor family includes ALK and leukocyte tyrosine kinase (LTK)[85,86]. ALK gene fusion is linked to the formation of various tumors[87,88]. Additionally, the serine/threonine/tyrosine kinase (STYK) receptor plays a role in cellular processes such as proliferation, differentiation, and survival[30,89,90].

1.3. Cancer-Associated Non-Receptor Tyrosine Kinases

Non-receptor tyrosine kinases (NRTKs) include Ack, Jak, Fes, Fak, Tec, Src, Csk, Abl, and Syk kinases[91,92]. These NRTKs typically consist of the N-terminal kinase domain, which is around 300 residues long, and the C-terminal region, which contains several functional domains[92]. NRTKs share significant sequence similarity within their kinase domains, and their catalytic domains are like those of Ser/Thr protein kinases[93,94]. In addition to their catalytic domains, NRTKs also feature non-catalytic domains that regulate their activity[95,96]. The classification of NRTKs into distinct families is based on molecular analysis of their domain structures, variations in amino acid sequences, and genomic organization of the kinase domains[36,97–99]. Below is a brief overview of the most common NRTK families.

The Ack is a large protein of 120 kDa whose kinase activity can be mediated by the phosphorylation of its tyrosine residues[100–102]. Ack1 is a non-receptor tyrosine kinase with a

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unique multidomain structure, including an SH3 and CRIB domain, which regulates cellular functions like migration and adhesion and plays a critical role in cancer progression[103–105]. Furthermore, Ack1 promotes tumor growth, resistance to chemotherapy, and recurrence through gene amplification, mutations, and epigenetic regulation[106,107]. The Jak/Janus family consists of four kinases (JAK1, JAK2, JAK3, and TYK2), each with two kinase domains, one functional and one pseudo-kinase[108,109]. These kinases are activated by cytokine receptor ligation, leading to transphosphorylation and downstream signaling[110–112]. JAKs play crucial roles in immune cell regulation and tumor development through the JAK-STAT pathway[113,114]. JAK3 is primarily found in hematopoietic cells, while other JAKs are involved in diverse cytokine signaling processes[115–117]. Feline sarcoma (Fes) and Fes-related (Fer) kinases are a subgroup of NRTKs with similarities to viral oncogenes from feline sarcoma virus and avian Fujinami poultry sarcoma virus[91,118,119]. Fes kinases have a unique FCH domain, coiled-coil motifs, an SH2 domain, and a C-terminal kinase domain[120,121]. Fes and Fer kinases are implicated in cancer progression, with Fes playing a role in cell signaling pathways that influence cell migration, proliferation, and survival, contributing to tumorigenesis[122–124]. The Fak family includes Fak, Pyk2, Cak-beta, Cadtk, Raftk, and Fak2, with varying expression in organs like the brain, liver, and hematopoietic cells[125,126]. Fak family kinases feature a FERM domain that mediates interactions with integrins and RTKs and a C-terminal FAT region involved in focal adhesion targeting[127–131]. Fak plays a crucial role in tumor cell signaling, including transcriptional regulation within the tumor microenvironment[132,133]. Overexpression of Fak is linked to aggressive cancers, including breast, colon, ovarian, and pancreatic and is commonly associated with metastasis and poor prognosis [134–139]. The Tec family consists of five NRTK members, including Tec, Itk, Btk, Txk, and Bmx, characterized by domains such as PH, TH, SH3, SH2, and a kinase domain[140,141].Tec kinases are involved in immune cell signaling, with specific expression in T, B, and NK cells[142,143]. The Src family is one of the largest NRTK family that includes eight members, such as Fyn, Yes, Fgr, and Lyn, divided into two subfamilies: Src-A (Fgr, Fyn, Src, Yes) and Src-B (Blk, Hck, Lck, Lyn)[144–146]. These kinases share a similar structure with SH4, SH3, SH2, and kinase domains, but differ in their C-terminal regulatory regions[147–149]. Src family kinases are involved in diverse cellular processes, with distinct expression patterns in hematopoietic and other tissues[146,150,151]. FRK (Fyn-related kinase) is a member of the breast tumor kinase (BRK) family, closely related to Src family kinases[152–154]. FRN kinases feature an SH3, SH2, and kinase domain, but lack the N-myristoylation site, which prevents membrane localization and allows nuclear localization[155,156]. Unique to FRK and IYK kinases is the presence of a nuclear localization signal (NLS) within the SH2 domain[157–160]. The NLS is a bipartite motif that enables nuclear targeting and functional regulation in the cell[161]. The Abl family includes Abl and Arg kinases, which are widely expressed, with high levels in the thymus, spleen, and brain[162,163]. Both kinases have structures similar to Src family members but feature a unique C-terminal actin-binding domain and nuclear localization signals[164–167]. Abl activation, through mutation or phosphorylation, is linked to leukemia and solid tumors like brain, lung, and prostate cancers[168].[169–171] The Syk family includes Syk and Zap70 kinases, which share a similar structure containing two SH2 domains followed by a catalytic kinase domain[172–174]. These kinases are cytosolic proteins lacking fatty acid modification sites, and upon cell stimulation, Syk and Zap70 translocate to immune receptor complexes at the membrane to trigger downstream signaling[175–177]. A tabular representation of kinases that play a significant role in various cancer types is presented in Table 2.

Table 2. Summary of cancer-associated tyrosine kinases.

Class of Tyrosine Kinase	Cancer type
EGFR (HER1, HER2, HER3, HER4)	Epithelial tumors-Lung, Breast and Colon[39,40].
VEGFR-1-3	Regulates angiogenesis and cell migration in tumors[44–46]
FGFR-1-4	Tissue cancer [47]

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TRKA, TRKB, TRKC (NTRK family)	Neuronal cancer[51,52]
RET	Implicated in multiple cancers[71,72]
RYK	Contributes to tumorigenesis[73,74]
DDR1, DDR2	Regulate adhesion, invasion, survival in collagen-rich tumors[75–77]
ROS	Present in many cancers types[79]
LMTK/LMR	Cancer-linked; influence proliferation, migration [84,85]
ALK, LTK	Fusion-driven cancers (e.g., ALK fusions in lymphoma, lung cancer[86,88,89]
STYK	Involved in proliferation, survival; emerging cancer target [90]
Ack1	Promotes tumor growth, chemoresistance, gene amplification[94,95]
JAK1, JAK2, JAK3, TYK2	Crucial for immune modulation in cancers [108,109]
Fes, Fer	Signal for migration, survival; linked to oncogenesis[122,123,125]
FAK family	Adhesion, motility; high expression in aggressive tumors[135,136]
Src family	Major signaling mediators; upregulated in various tumors[151]
Abl, Arg	Leukemia[169–171]
Syk, Zap70	Hematologic cancers[176]

3. Structural and Regulatory Mechanism of Tyrosine Kinases

PTKs play a critical role in cellular signaling pathways, and their catalytic activity is tightly regulated. Numerous atomic structures of PTKs reported in the literature have provided structural and mechanistic insights into the regulation of both receptor and nonreceptor PTKs[145,178–180]. As several PTKs are available in the PDB, the current review will focus on EGFR kinase as a representative of receptor PTK and Src for nonreceptor PTKs.

3.1. PTK Domain Architecture

RTKs are composed of three main regions: a large extracellular region, which binds to polypeptide ligands, a transmembrane helix, and a cytoplasmic region, which possesses tyrosine kinase activity. The extracellular region of RTKs is classically composed of a diverse array of distinct globular domains, including immunoglobulin (Ig)-like domains (domain-1), fibronectin type-III-like domains (domain-2), cysteine-rich domains (domain-3), and EGF like domains (domain-4). In the case of EGFR kinase, the extracellular region includes amino acids 1-165 (domain-1), 166-310 (domain-2), 311-480 (domain-3), 481-621 (domain-4). However, the cytosolic region of RTKs domain organization is simple, consisting of the juxtamembrane region (amino acids 643-685), immediately followed by the transmembrane helix, a tyrosine kinase domain (amino acids 686-952), and a carboxy region (amino acids 953-1186) (Fig. 1A, B, C). Unlike RTKs, the extracellular and transmembrane regions in NRTKs are absent, and most of the NRTKs are present in the cytosol. The NRTKs comprise intrinsically disordered regions (IDR) and folded domains. At the N-terminus IDR region, unique myristoylated Src homology 4 (SH4) fragments, a smaller SH3 domain (~60 residues), a short Src homology 2 (SH2 ~100 residues), SH2 kinase linker, catalytic tyrosine-protein kinase domain (SH1), and a short intrinsically disordered C-terminal tail. While the kinase domain (KD) has a catalytic function, the SH2 and SH3 domains are commonly involved in non-catalytic regulatory properties. However, all these three domains are essential in signal transduction[21,181–185] (Fig. 1D, E).

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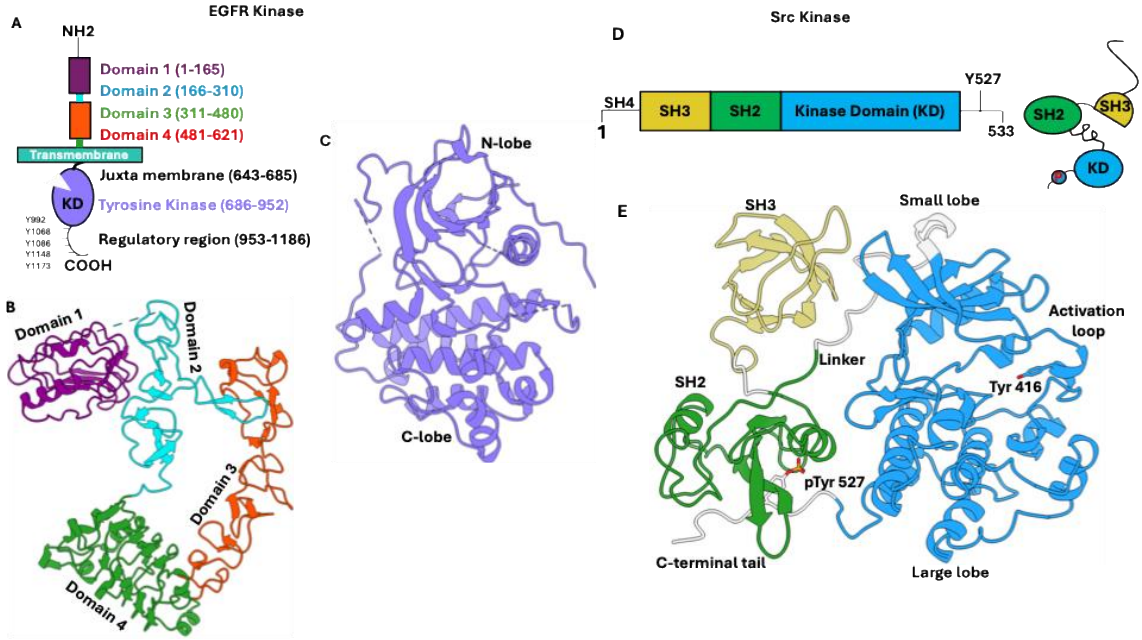

Figure 1. A. The domain architecture of EGFR. B. The Extracellular region of EGFR is composed of 4 domains I-IV, domain I (red), domain II (cyan), domain III (green), and domain IV (orange). C. The EGFR kinase domain is displayed in medium purple D. The domain architecture of Src. The boundaries of domains are based on the chicken numbering system. E. Ribbon diagram displaying the overall structure of Src (PDBID: 2SRC). The SH3 (pale yellow) and SH2 (green) domains coordinate the linker and C-terminal tail regions, respectively. The kinase domain is colored in blue.

3.2. Src Structure and Regulatory Mechanism

The primary function of the Src is to transmit the external signal to the cell interior by phosphorylating tyrosine residues on substrates, mainly downstream of RTKs and integrins[186]. Src kinases are crucial in various cellular processes, such as cell proliferation, adhesion, migration, and more[91]. The Src kinase's complicated regulation is due to its complex structure. The structures of SH3, SH2, and SH1 kinase domains of Src kinases have been extensively studied and reviewed elsewhere[33,96,99,185,187]. The Src kinase domain features a characteristic bilobed architecture comprising a small N-terminal lobe and a large C-terminal lobe. The residues 267-337 and 341-520 make up these lobes, respectively[188–191]. The N-lobe predominantly anchors and orients ATP, featuring a G-rich loop, which is a part of the nucleotide-phosphate binding site. The N-lobe is mostly composed of antiparallel β sheet structures[192]. The C-lobe is predominantly composed of α helix, responsible for binding the protein substrates and contributing to the ATP-binding site. The catalytic site of Src is situated in a cleft between these two lobes; they open and close during ATP hydrolysis[187]. The dynamic conformational switch regulates ATP binding and ADP release; the open form is required to allow ATP to bind to its catalytic pocket and release ADP; the closed form is important to bring residues into the catalytically active form. The Src kinase regulation is precisely involved in the coordination of non-regulatory SH2 and SH3 domains and a regulatory kinase domain[145,187]. In the autoinhibitory conformation, the SH2 domain binds to phosphotyrosine-containing motifs, precisely phosphorylated Tyr527 in the C-terminal tail of Src, and stabilizes the conformation[145,187]. The SH3 domain interacts with a polyproline-rich motif situated between the SH2 and Kinase domains. This interaction positions SH2-SH3 domains as a compact structural unit, which further prevents the movement of the kinase domain and, consequently, locks the Src in its inactive state. The activation loop (residues 404-418) conformations in the kinase domain dictate the active and inactive state of the Src kinase. In the inactive Src kinase, the activation loop forms a short α-helix between the N and C-lobes known as the A-loop helix[145,187]. As a result, the Tyr416 residue side chain is buried between the N and C-lobes; this conformational switch leads to the prevention

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of the formation of a salt bridge between Lys295 and Glu310 required for enzyme activity. The autophosphorylation of Tyr416 disrupts the autoinhibitory state of the Src kinase, leading to an extended conformational switch in the activation loop and alignment of catalytic residues such as Asp386 and Asp404. Asp386 residue acts as a catalytic base for the tyrosine substrate, whereas Asp404 interacts with magnesium ions that stabilize ATP. Numerous studies on Src have revealed that SH2 and SH3 domains are critical for maintaining the autoinhibited state of Src. However, the kinase domain is involved in severe conformational changes to switch between active and inactive states. This structural equilibrium is disrupted when C-terminal Tyr527 is mutated. In the case of v-Src, a mutation at Tyr527 impaired the SH2-SH3 interaction between the kinase domain and resulted in constitutive kinase activity[7,145,186,187].

The Src protein-tyrosine phosphorylation levels are balanced by counteraction between C-terminal Src kinase (CSK) and protein-tyrosine phosphatases (PTPs). Okada and Nakagawa[193] were the first to demonstrate that CSK, a cytoplasmic PTK, controls the regulatory tyrosine phosphorylation in rat brains. They also highlighted its efficiency in phosphorylating Src at Tyr527, a key regulatory site for its activation. In contrast, PTPs such as PTPα and PTPε facilitate the dephosphorylation of phosphotyrosine 527 in the Src kinase domain, thereby displacing it, leading to Src kinase activation (Fig. 2C, D). Structural studies have revealed that the substrate recognition mechanism between Src and PTPs relies on the cysteine-dependent active site of PTPs and the phosphorylated tyrosine side chain of Src (Fig. 2C, D)[194]. Recent findings have identified two additional key charge-charge interactions between rPTPε and phospho-Src beyond the active site interactions [195]. These biochemical and structural insights are extremely important for the development of novel therapeutic strategies for targeting kinases, particularly in cancer treatment.

3.3. EGFR Structure and Regulatory Mechanism

EGFR regulates multiple functions involved in developmental, metabolic, and physiological processes[196]. When exposed to ligands like EGF, the receptor EGFR binds to EGF, undergoing a conformational switch from an inactive monomer to an active dimer (Fig. 2A). This conformational change leads to autophosphorylation of the receptor, which sequentially activates downstream signaling pathways to control cell proliferation and differentiation. EGFR, along with growth factor-α, amphiregulin, and other ligands, promotes either homodimerization of two EGFRs or heterodimerization of EGFR with other family members[197]. Upon activation of receptor TKs, there is a subsequent activation of downstream Ras/mitogen-activated protein kinase pathway, the pI3K/Akt pathway, and activation of transcription pathways[198].

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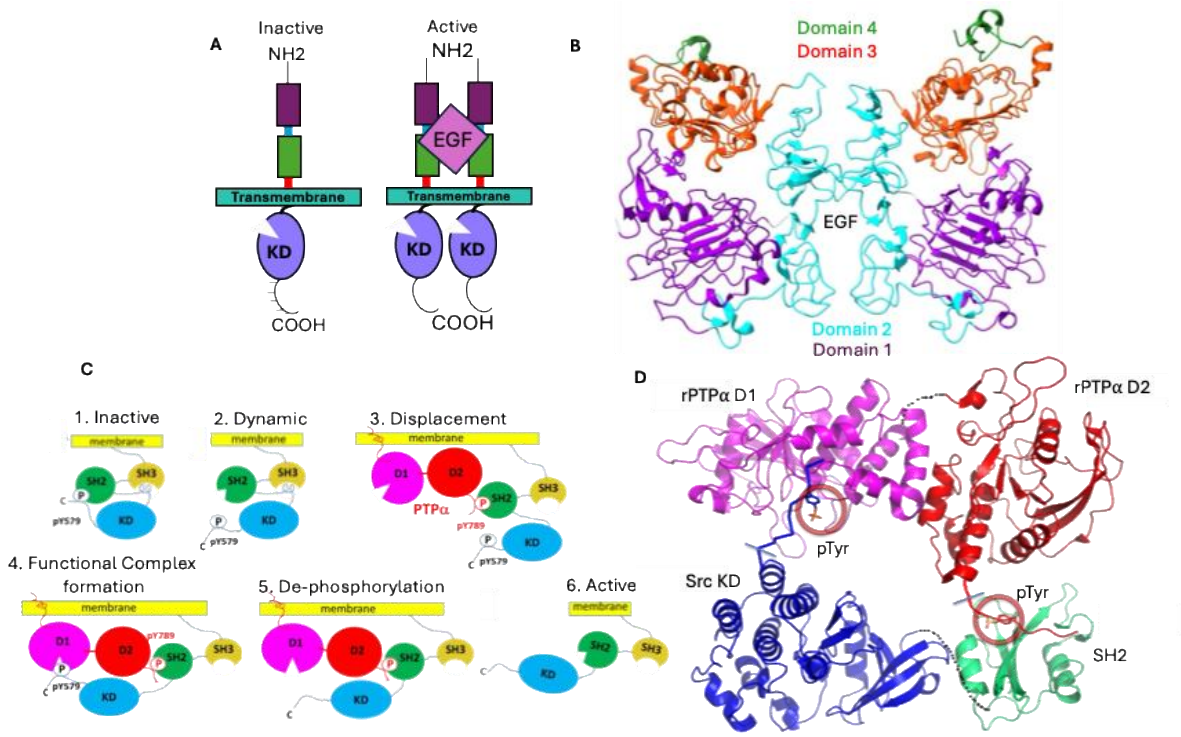

Figure 2. A. A schematic diagram of inactive EGFR, ligand-bound active dimeric extracellular EGFR, and an asymmetric dimer of kinase domain B. Structure of TGFα dimer of sEGFR (PDB ID: 1MOX). An extracellular structure bound to EGF, a transmembrane helix dimer. C. Phosphotyrosine displacement by PTPα and activation mechanism of Src kinase. D. Haddock model of PTPα and Src kinase complex, displaying the phosphatase and tyrosine kinase interaction.

3.4. Extracellular Structure of EGFR

The extracellular structural modules of all four EGFR members have been thoroughly studied both in the presence and absence of their respective ligands, as well as in complexes with antibodies[199,200]. Atomic structures reveal two key conformations that are important in the extracellular modules. One is an extended form that facilitates the conformation of one protomer in the active dimer, while the other is folded over or tethered conformation where dimerization elements are buried. Upon ligand binding, the extracellular domains display significant conformational change, transitioning the module from tethered to the extended state, resulting in dimerization and activation of the EGFR (Fig. 2B). This extended conformation is represented as a back-back dimer configuration, with the ligand positioning between domains I and III of each receptor subunit. The glycosylation of the EGFR extracellular region is critical for its activation; the sugar moiety, around 40 kDa is known to play a role in EGFR maturation and cell surface translocation. Mutation studies have identified that Asn579 is crucial for regulating receptor conformation and ligand binding affinity. Another mutation at Asn 579, located on a specific glycosylation site, influences the structural conformation of EGFR and ligand binding. Furthermore, a mutation N420D in EGFR was shown to display ligand-independent activation through spontaneous oligomer formation [200]. Together, the biochemical and structural details underscore the complexity of these receptors' regulation and offer a base for therapeutic strategies targeting EGFR family members.

3.5. EGFR Intracellular Kinase Structure Activation

The intracellular region of EGFR is mostly comprised of its kinase domain (KD), the KD adopts a canonical kinase fold that exists as both active and inactive conformations. EGFR (Unphosphorylated) structure in the presence of erlotinib from Genentech is the first atomic structure

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of the EGFR kinase domain; this structure provides its unique structural features and activation mechanism[178,198]. The structure features the conserved Asp-Phe-Gly (DFG) motif at the base of the activation loop, which is a key activation/regulatory motif. In inactive conformation, the aspartic acid residue flips out of the catalytic center, making the kinase inactive and preventing the entry of ATP. This is observed in several kinases[201]. In the EGFR: erlotinib complex, the DFG motif is found in the 'in' conformation; in this, the activation loop is open and properly configured to bind its ligands. Additionally, the active site element αC in the N-lobe switches inward and facilitates the ion pair interaction between Glu 738 and Lys, which is critical for catalytic activity[200]. In contrast to other kinases, EGFR does not require phosphorylation of its activation loop to transition to the active state. The atomic structure of EGFR in complex with lapatinib captured in its inactive state, surprisingly, this structure resembles the inactive states of Src-family kinases[200]. In the structure, the αC in the N-lobe switched outwards, and the activation loop formed a short helix, blocking its ATP binding. Mutations on the activation loop phosphorylation sites revealed that the phosphorylation is not an absolute requirement for EGFR's activation. Overall, the atomic details of these structures detailed the understanding of EGFR's regulatory flexibility and underlined its divergence from other RTKs, which rely on autoinhibitory interactions and activation loop phosphorylation for regulation[200].

The activation mechanism of EGFR was revealed through the determination of the homodimer KD structure. In this structure, one kinase domain (activator) allosterically interacts with its partner (receiver) to activate the EGFR. This dimerization interaction occurs at the N-lobe of the receiver and the C-lobe of the activator, resembling the cyclin-mediated CDK type of activation. Unlike other RTKs, the EGFR activation mechanism is driven by protein-protein interactions at the dimerization interface. This mechanism is also observed in other members such as Her2, Her3, and Her4 (Fig. 2A, B)[202–206]. Further MD simulation studies demonstrated how EGFR transitions between active and inactive conformations through local unfolding at the hinge region between N- and C-lobes. Together, these structural insights have significant clinical implications, helping in developing novel targeted antibodies like erlotinib and lapatinib, which exploit EGFR's conformational flexibility. For example, EGFR inhibitor Mig6 is known to block the asymmetric dimer interface and inhibit activation[207–216]. This understanding highlights the unique regulatory mechanism of EGFR and its critical role in cancer biology.

4. The Role of Tyrosine Kinases in Cancers

Tyrosine kinases, a large family of kinases that include both RTKs and NRTKs, serve as critical molecular switches in regulating various cellular processes such as growth, survival, development, and differentiation[108,217–219]. Several studies have highlighted the role of PTKs in various cancers and their potential for drug discovery. The current review focuses on EGFR and Src's role as therapeutic targets for developing treatments against cancer cell-specific pathways.

4.1. Role of EFGR-Tyrosine Kinase in Cancers

The EGFR family regulates developmental, metabolic, and physiological processes[196]. A key aspect of EGFR-driven cancers involves mutations in the tyrosine kinase domain of the *EGFR* gene (exon 18-21), categorized into three classes: class-I (In-frame deletions in exon 19), class II (single-nucleotide substitutions), class III (In-frame duplications and or insertions in exon 20)[220,221]. Class I accounts for approximately 44% of the activating EGFR-TK domain mutations, including deletion at LRE (Leu-747 to Glu-749, while class II mutations contribute ~41%, often affecting the kinase domain C-helix. Class III mutations, constituting ~5%, are less frequent but still play a role in tumor progression[198,220,222–224]. Lemmon and Schlessinger, in 2010, best characterized the function of EGFR in ligand- and kinase-dependent activation, also known as the canonical EGFR signaling pathway[225]. Several of these stress pathways are activated in cancer cells to induce survival advantage as well as resistance to cancer therapy[226,227]. Casanova et al. (2002) demonstrated that EGFR signaling is responsible for the Ha-ras-dependent activation in epidermal tumor cells[228].

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Recent publications support the activation of EGFR signaling pathways in epithelial cancers, including breast, ovarian, prostate, and NSCLC[229–231].

4.2. Role of Src-Tyrosine Kinase in Cancers

SFKs play a crucial role in various cellular processes, such as cell proliferation, adhesion, migration etc[91]. Their dysregulation is frequently implicated in tumors, where they are often overexpressed due to their role in cell-cell adhesion[232,233]. Particularly, Src is involved in activating STAT transcription factors, promoting tumorigenesis, and influencing cytokine signaling in hematopoietic cells[234]. It also plays a significant role in regulating the RAS/RAF/MEK/ERK MAPK and VEGF pathways in various tumors[235–238]. Additionally, Src plays a vital role in facilitating tumor cell invasion by phosphorylating target substrates, aiding in the translocation of tumor cells through matrix barriers and tissue compartments. Invasion is a complex process, and tumor Src activation leads to the phosphorylation of targeted substrates, influencing the activity of cellular proteins to carry out this entire cellular process[239–241]. SFKs are activated in tumors through mutations of the Src allele, leading to a disorganized negative regulatory pathway or by binding to activating partners such as growth factors (Her 2/Neu, PDGF, EGFR). Oncogenic Src (v-Src) can activate Ras by recruiting the Grd 2/Sos complex, thereby stimulating Ras-mediated tumorigenic signals[242,243]. Furthermore, p120RasGAP-mediated activation of c-Src is important for Ras-induced tumor invasion[244]. The tumor microenvironment plays a crucial role in Src upregulation, leading to enhanced Src activity during cancer progression[245]. Additionally, inhibitory phosphorylation of Tyr 530 is mediated by the kinase Csk, which acts as a crucial regulator of Src activity[246]. Given the importance of Src/EGFR in tumor progression, the review will explore tyrosine kinase therapeutic targets and also provide insights into potential strategies for overcoming therapeutic resistance.

5. Tyrosine Kinases as Therapeutic Targets

5.1. Development of TKIs

Cancer cell survival in the tumor microenvironment (TME) is challenging and highly influenced by external factors. Cancer treatment has advanced in developing tyrosine kinase inhibitors (TKIs). Discovery and development of imatinib (Gleevec, Inc) as the first effective TKI to treat chronic myeloid leukemia (CML), established as tumor-targeted therapy that acts specifically against Bcr-Abl fusion protein. Inhibitors such as Sorafenib and sunitinib served as early examples of TKIs approved for solid tumors and renal cell carcinoma[247–252]. Over the past 20 years, robust and specific TKIs with single or multiple targets have been identified, which include EGFR, ROS1, VEGFR, MEK, FGFR, and PDGFR[253,254]. The known FDA-approved TKI is listed in Table 3. IRIS trials (2000-2001) confirmed the long-term survival benefit of treating Imatinib[255]. However, there has been concern over the emergence of resistance to imatinib. Nilotinib and dasatinib, are two of the TKIs (second generation) approved worldwide for the treatment of chronic myeloid leukemia after imatinib failure[255]. Development of TKIs always challenging because of the resistance over a while, most patients developed acquired resistance against TKIs upon a median period of 10-15 months[256,257].

Table 3. TKI inhibitors are used in research studies.

TKIs from clinical studies, research studies result from patients with EGFR-mutated lung cancer drug target	TKI	Clinical phase	#pts (%EGFRm+)	RR%	Reference
1st/2nd-generation EGFR TKI	Neratinib	II	91 (100)	3	[258]
	XL647	II	33 (53)	3	[259]

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	Afatinib (A) vs. placebo (P)	IIB/III)	585 (16)	7 (A)<1 (P)	[260]
	Afatinib	II	62 (73)	8	[261]
	Dacomitinib	II	62 (73)	8	[262]
	MM-121 + erlotinib	II	50 (48)	9	[263]
	AP26113	I	32 (35)	3[a]	[264]
Mutant-specific TKI	CO-1686	I	40 T790M+ (100)	58	[265]
	AZD9291	I	107 T790M+ (100)	64	[266]
	HM61713	I	48 T790M+ (100)	29	[267]
EGFR antibodies	Cetuximab + erlotinib	II	19 (84)	0	[268]
	Cetuximab + afatinib	IB	126 (98)	29	[269]
Chemotherapy	Carboplatin/paclitaxel	III	52 (100)	28.8	[270]
	Chemo/erlotinib (CE) vs. chemo (C)	Retro	78 (100)	41 (CE); 18 (C)	[271]
	Pemetrexed + gefitinib or erlotinib	II	27 (100)	25.9	[272]

RR: Response rate, #pts: Patients.

Two main approaches to therapeutically targeting EGFR rely on using mAbs and small molecules of EGFR-tyrosine kinase Inhibitors (EGFR-TKIs). mAbs specific to EGFR target the extracellular domain, whereas EGFR-TKIs block the binding of ATP to the intracellular catalytic domain of EGFR[273]. For example, Panitumumab and cetuximab are the two approved mAbs widely used in the treatment of colorectal cancer where EGFR with KRAS expressions[274–276]. Erlotinib and gefitinib are two selective TKIs used in combination with mAbs in the treatment of NSCLC. Several preclinical and clinical studies were conducted to study the effect of these EGFR inhibitors alone and in combination with mAbs/chemotherapies[277–279]. Cetuximab and panitumumab have been studied in combination with anthracycline/texane-based chemotherapy through pilot studies of multicentric neoadjuvant TNBC[280–282]. Studies reported that using cetuximab in combination with either gefitinib or erlotinib has proven to enhance apoptosis and growth inhibition of neck cancer cell lines over using them alone in the treatment[283]. Additionally, it is suggested that cetuximab and gefitinib showed a synergistic effect on EGFR downstream signaling pathways[284,285]. Trastuzumab, in combination with lapatinib, is used to treat HER2-overexpressed breast cancer, these two develop resistance in patients when treated alone[286]. One of the strong reasons to use combinational therapy on mAbs and selective EGFR-TKIs was to target different molecular domains of the EGFR. However, selective targeting of EGFR was limited to EGFR-driven cancers, in the case of EGFR-and KRAS or STKs driven cancers, one needs to be more selective in choosing combinational therapies.

5.2. Resistance to TKIs and Strategies to Overcome Resistance

TKIs are the most common and successful strategies for targeting cancer cells[287–289]. However, eventually, cancer cells develop resistance to these drugs. Multi-drug resistance (MDR) in cancer arises when tumors become nonresponsive to chemotherapeutic agents. Many factors contribute to MDR, including enhanced drug efflux caused by overexpressed ABC transporters[290], genetic mutations, the activation of specific signaling pathways, and intracellular-extracellular ATP, which also promotes drug resistance[291–293]. Mutations in the EGFR and Src also contribute to drug

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resistance in cancers. To overcome MDR, researchers have developed strategies emphasizing the use of monoclonal antibodies (mAbs) that target specific receptors or signaling components of the pathway, or any protein that specifically promotes tumor oncogenesis. Here, we highlight the use of mAbs alone and in combination to achieve effective treatment against cancers.

Resistance to TKIs in EGFR-mutants NSCLC remains a challenge in cancer therapy. Studies have identified that, on average, 50% of resistance to first- and second-generation EGFR-TKIs is due to EGFR T 790 M mutation. This amino acid substitution in EGFR leads to an increased affinity to ATP caused by a conformational change, resulting in steric hindrance and reducing drug efficacy[294,295]. Osimertinib, a third-generation EGFR-TKI, inhibits both EGFR T 790 M and EGFR-sensitizing mutations, demonstrating increased efficiency over gefitinib and erlotinib[296–298]. However, patients developed resistance to long-term usage of third-generation EGFR- TKIs, particularly EGFRC 797 S on exon 20, as the main cause for this acquired resistance[299,300]. Patients responded to a combination of first- and third-generation EGFR- TKIs when harboring C 797 S in trans with T 790 M, whereas those with C 797 S in cis with T 790 M did not respond to this combination[301,302]. EGFR T790 and Src-mediated resistance are two distinct mechanisms where tumor cells develop resistance to therapies, especially EGFR-targeted therapies. Most of the TKIs that target EGFR were less sensitive because of the specific mutation in the EGFR gene. Whereas Src-mediated resistance, on the other hand, involves the activation of Src kinase, which can also bypass the effects of EGFR inhibitors and drugs that target NTKIs[303,304]. To overcome this evolving resistance, researchers are developing fourth-generation EGFR-TKIs and also exploring combination therapies. For instance, EGFR-TKIs combined with programmed death ligand 1 (PD-L1) antibodies with chemotherapy have shown significant survival benefits to patients suffering from EGFR mutation-driven drug resistance in cancers[305]. The FDA-approved TKI and NTKI inhibitors used in cancer therapy are listed in Tables 4 and 5.

Table 4. US FDA approved TKI and NTKi inhibitors for use in cancer therapy.

TKI	Family targeted	Inhibitor name	Application	Adverse effects (Cardio related)	Extra-cardio adverse effects
TKI-first generation	EGFR/ERBB family	Gefitinib[306]	NSCLC	MI	Skin rashes, nausea, diarrhea, anorexia, stomatitis, nausea,
TKI-first generation	EGFR/ERBB family	Icotinib[307]	NSCLC	HTN	Diarrhea, nausea, skin rashes, loss of appetite
TKI-first generation	EGFR/ERBB family	Lapatinib[308,309]	Breast cancer	HF, LVD	Skin rashes, diarrhea, nausea
TKI-first generation	EGFR/ERBB family	Erlotinib[308, 309]	NSCLC and prostate cancer	Edema	Skin rashes, diarrhea, nausea, loss of appetite, fatigue, neuropathy, alopecia
TKI-second generation	EGFR/ERBB family	Afatinib[310, 311]	NSCLC	HTN	Severe diarrhea, loss of appetite, paronmychia, dry skin, rashes
TKI-second generation	EGFR/ERBB family	Neratinib[312,313]	Breast cancer	Low rates and decline in LVEF and QT prolongation	GI related disorders, headache, fatigue, diarrhea
TKI-second generation	EGFR/ERBB family	Dacomitinib[314,315]	*EGFR*-mutated NSCLC	HTN	Dry Skin, appetite loss, diarrhea, Weight Loss, Alopecia, Cough, Hemorrhoids, Wound, Back pain, Headache

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TKI-third generation	EGFR/ERBB family	Osimertinib[316]	NSCLC	MI, pericardial effusion, LVD, HF	Diarrhea, nausea, fatigue, stomatitis
TKI-third generation	EGFR/ERBB family	Pyrotinib[317,318]	HER2-postive		Diarrhea, Hand-foot syndrome, Leukopenia, Neutropenia, GI disorders, Increased ALT, Anemia, Asthenia
TKI-third generation	EGFR/ERBB family	mobocertinib[319,320]	*EGFR*-mutation driven NSCLC		Dermatitis acneiform, GI Disorders, Rash, Dry skin, Stomatitis, Fatigue, Rash, Paronychia, Anemia

Table 5. List of approved mAbs targeting EGFR.

mAbs	Nature of molecule	Binds to	Antibody dependent cell mediated cytotoxicity (ADCC)	Type of cancer tested	Mechanism	Side effects	Clinical approved
Nimotuzumab[321]	Humanized, mouse mAb	Extracellular domain of EGFR,	-	Squamous cell carcinoma of head and neck (SCCHN), glioma and nasopharyngeal cancer	Prevents binding of EGF	Reported	Yes, III (approved for treating HNSCC in non-USA countries)
Zalutumumab[322]	Humanized IgG1	Extracellular domain of EGFR		Squamous cell carcinoma of head and neck (SCCHN)	preventing the binding of ligands like EGF and TGF-alpha, thereby inhibiting EGFR signaling		YES, III
Trastuzumab[323,324]	Humanized IgG1	Juxtamembrane domain IV	Yes	Several HER2-postive cancers including Breast and gastric cancer	Inhibits HER2 homodimers and ligand-independent HER2–HER3 dimers	Reported	YES
Pertuzumab[325]	Humanized IgG1	Heterodimerization domain II	Yes	HER2-postive cancer such as breast cancer	Inhibits ligand-induced HER2-containing heterodimers	Reported	YES
Cetuximab[326,327]	Humanized IgG1	Extracellular domain of EGFR	preventing the binding of ligands like EGF and TGF-alpha, thereby inhibiting EGFR signaling	Certain advanced colorectal, head and neck cancer	EGFR	Reported	Approved
Panitumumab[328]	Humanized IgG1	Extracellular domain of EGFR	preventing the binding of ligands like EGF and TGF-	Certain types of metastatic colorectal cancer (mCRC)	EGFR	Reported	Yes, III

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	alpha, thereby inhibiting EGFR signaling	

5.3. Resistance and Mechanism of Developing Resistance to Therapy

Trastuzumab (Herceptin), a drug used to treat breast cancer, is resistant in patients. Trastuzumab binds to an epitope in the juxtamembrane region of the HER2 RTKs. Upon binding, trastuzumab induces uncoupling of ligand-independent HER2-HER3 heterodimers and inhibits downstream signaling as well as antibody-dependent cell cytotoxicity[329]. The main reasons reported for resistance of the trastuzumab in patients were as explained in decreased interactions with HER2 due to blockage by cell surface proteins like mucin-4 (MUC4)[330]. Consistent treatment with trastuzumab leads to decreased expression of the tumor suppressor PTEN gene and activation of the Akt signaling pathway. Another main reason for developing resistance was the activation of the phosphatidylinositol 3-kinase/Akt pathway, which can lead to decreased sensitivity to trastuzumab[331]. Another potential explanation for developing trastuzumab resistance is its ability to bind to hyaluronan and CD44 a transmembrane receptor that can hinder trastuzumab access to HER2[332]. A clinical study was conducted to analyze sensitivity to trastuzumab treatment and reported in the study on 46 patients with breast cancer, 11.1% of patients responded to trastuzumab (expressing p95HER2), 51.4% with expressing p185HER2 achieved clinical response[333]. Lapatinib, a small molecule that can inhibit both HER2 and EGFR kinase, was tested in p95HER2 preclinical studies to prevent Her2 signaling loss of the trastuzumab binding site[333]. When lapatinib is coupled with trastuzumab, clinical studies have shown it to be progressive in patients with stage IV HER-overexpressing breast cancer[334].

Cetuximab is a mAb that treats metastatic colorectal cancer and squamous cell cancer (head and neck squamous cell cancer-HNSCC). The use of cetuximab and panitumumab in colorectal cancer patients is successful[335,336]. However, treatment with cetuximab and panitumumab as single agents was only 10% effective in clinical significance. This clearly explains the development of resistance to the therapy. Most patients develop resistance within 3-12 months of starting therapy[337]. The most probable explanation for developing resistance is but not limited to RAS mutations (these mutations prevent patients from having a response with therapy). Acquired resistance is another important reason when using EGFR-targeted mAbs. Preclinical and molecular profiling of clinical specimens developed resistance to EGFR-targeted mAbs are having genetic alterations of genes in EGFR-RAS-RAF-MEK signaling pathway and of RTKs are the mechanism of acquired resistance to anti-EGFR mAbs[338–340]. Mutations in the codons 12 and 13 of KRAS were the first identified mechanism of primary resistance to anti-EGFR therapy; later, patients screened for KRAS mutations prior to mAbs. Researchers also reported that oncogenic Ras and wildtype p53 stimulate STAT non-cell autonomously and promote tumor radioresistance[238]. However, in some instances, RAS wild-type patients can be non-responders to anti-EGFR therapy, as it was well understood that additional mechanisms of intrinsic resistance have been attributed to mutations in PI3KCA/BRAF[341,342]. The above genetic mutations leading to acquired resistance and escape from anti-EGFR blockade appear to converge on the activation of MEK-ERK/AKT signaling pathways. Considering each mAbs has its advantages over disadvantages in therapy.

Pertuzumab (Omnitarg, 2C4) is an anti-HER2 mAb that binds to the domain II epitope of Her2 and is able to block a binding pocket essential for receptor dimerization and signaling. Pertuzumab is suggested to be a potential synergism with trastuzumab in HER2 overexpression cell lines[343]. Phase II clinical trials of pertuzumab in combination with trastuzumab have shown disease progression over trastuzumab in patients with HER2-overexpressing metastatic breast cancer[344]. Currently, clinical trials in different stages testing pertuzumab in combination with trastuzumab in different settings and as well as pertuzumab with chemotherapy are ongoing[345]. Toxicity profiles of these new antibodies (mAbs) are comparable to that of Cetuximab, even though they are associated with less hypersensitive reactions. Mostly, mAbs administrations needed frequent clinical visits due

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to their mode of administration (intravenous infusions). Also, the proposed resistance to cetuximab can be applied to most EGFR-targeted mAbs. From these studies, it is well understood that mAbs targeting specific signaling molecules or receptors have shown progress in combination with other mAb or chemotherapy to overcome resistance in cancers.

5.4. Combined Targeting EGFR and Src as a Potential Therapeutic Approach

Triple-negative breast cancers (TNBC) are an aggressive subtype of breast cancer with limited therapeutic options. It is characterized by the absence of estrogen and progesterone receptors and lack of EGFR2 (HER2) gene amplification and protein expression[346]. Notably, overexpression of EGFR is highlighted in TNBC, attracting significant research interest in evaluating EGFR-TKIs as potential treatments[347]. Despite overexpression of EGFR in TNBC, the EGFR-specific TKIs have shown limited efficacy due to their intrinsic or acquired resistance mechanisms[348]. Studies identified a key factor that contributes to EGFR resistance is the association of Src family kinases, which have been shown to increase HER-family receptor expression[349,350]. The overexpression of Src enhances HER2/HER3 dimerization, consequently delaying receptor internalization and hence prolonging its downstream oncogenic signaling[351–353]. This crosstalk between EGFR and Src kinases suggests that targeting EGFR alone may not be sufficient, suggesting a dual-targeted approach that can inhibit Src signaling.

Src inhibitor dasatinib exhibits initial sensitivity in TNBC cells; however, later, it develops resistance[354,355]. However, combining both EGFR and Src inhibitors has shown promising results[356]. For instance, an irreversible pan-HER inhibitor, afatinib, and Src inhibitors have shown synergistic effects in MDA-MB-468, TNBC cell lines. Additionally, the combination of afatinib and dasatinib has also been shown to enhance apoptosis and growth suppression of NSCLC *in vitro* and *in vivo*[357,358].

These preclinical research studies have progressed to phase-I clinical trials evaluating the efficacy of these combination therapies (ClinicalTrials.gov identifier: NCT01999985). The cooperative interactions between these Src-tyrosine kinases and HER-family members in acquired resistance reveal the significance of developing novel combination drug therapies targeting both pathways. This combination of therapies may hold significant potential in overcoming MDR, improving treatment response, and increasing clinical benefits in TNBC and other EGFR-driven cancers.

5.5. Therapeutic Challenges and Limitations

The development of TKIs against cancer has significantly advanced in recent years. However, their clinical utility is often diminished by adverse effects related to the heart due to toxicity. It is well documented that older generations of TKIs can cause a wide range of cardiovascular issues such as hypertension, atrial fibrillation, and heart failure. These adverse effects highlight a critical therapeutic challenge: the necessity to design novel TKIs that maintain therapeutic efficacy while reducing off-target toxicities. In addition to TKI toxicity, another limitation is the development of drug resistance, which can cause postmenopausal symptoms, muscle and joint pains, and osteoporosis—common issues with prolonged use of TKI therapy[359,360]. Both drug toxicity and the emergence of long-term treatment drug resistance necessitate the development of novel TKIs that balance specific target inhibition with favorable safety profiles. In general, the therapeutic design must prioritize both efficacy and toxicity reduction to improve patient outcomes and ensure long-term treatment sustainability.

6. Summary and Conclusion

This review highlights the crucial role of PTKs, with special emphasis on EGFR and Src, in regulating important cellular functions like growth, differentiation, survival, and dysregulation that often lead to cancer. Furthermore, this review addresses the structural mechanism of EGFR and Src kinases that provides valuable insights into designing novel cancer therapies. Besides that, this review emphasizes the development of tyrosine kinase inhibitors (TKIs), including gefitinib and

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erlotinib, and the challenges posed by resistance in cancer treatment. We also outline and evaluate the existing clinician trials of combination therapy targeting EGFR and Src kinases, particularly in aggressive cancers like triple-negative breast cancer (TNBC). In conclusion, EGFR and Src kinases are significant players in tumor development and therapeutic resistance. Hence, the development of inhibitors/combination treatment is extremely promising in overcoming multi-drug resistance (MDR) and in augmenting therapeutic response in a broad spectrum of cancers.

Abbreviations

EGFR	Epidermal growth factor receptor
Src	Sarcoma (proto-oncogene tyrosine-protein kinase Src)
TKI	Tyrosine kinase inhibitors
TNBC	Triple-negative breast cancer
MDR	Multi-drug resistance
PTK	Protein tyrosine kinase
FDA	Food and Drug Administration
RTK	Receptor tyrosine kinase
NRTK	Non-receptor tyrosine kinase
KD	Kinase domain
IDR	Intrinsically disordered regions

Literature search and Methodology: For structural analysis, we used the AlphaFold models, the protein data bank (PDB) to retrieve structures, and used Chimera to analyze and generate figures. For protein-protein interaction studies, we used the HADDOCK online portal (https://rascar.science.uu.nl/haddock2.4/). For the chemo and immunotherapy drug search, we used already published literature, Drugs.com, antibodysocity.org, and cancerresearch.org.

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