Chapter 7
Receptors for Targeting Growth Factors for Treatment of Cancers

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Abstract Growth factor receptors (GFR) are expressed on cell membranes or in the cytoplasm and play a major role in cell growth, survival, angiogenesis, and metastasis. Tumor growth and cell survival are composed of dodging apoptotic signals in cancer cells. The growth of cells is further supported by angiogenesis and metastasis to distant organs. Elevated expression of growth factor receptors contributes to the development of drug resistance. Therefore, therapeutics to target GFRs is a potentially attractive molecular approach to treat cancer more effectively. In this review, we have discussed the contribution of growth factor receptors to cancer development and thereby their subsequent molecular targets for novel drugs developed leading to inhibition of growth factor receptor-mediated pathways.

Keywords Receptor-ligand interaction · Recognition domain · Extracellular domain · Transformation · Drug target

Abbreviations

BMP	Bone morphogenetic protein
CDK	Cell cycle-regulated kinases
CRC	Colorectal cancer
DOX	Doxorubicin

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ECD	Extracellular domain
EMT	Epithelial-mesenchymal transition
FGF	Fibroblast growth factor
FST	Follistatin
GAB	Grb2-associated binding protein
GAS	Growth arrest specific protein
GBM	Glioblastoma multiforme
GH	Growth hormone
GMP	Gemcitabine monophosphate
HUVEC	Human umbilical vein endothelial cells
IL	Interleukin
ILGF	Insulin-like growth factor
IONP	Iron oxide nanoparticles
IPT	Immunoglobulin-like plexin-transcription
IR	Insulin receptor
JMD	Juxtamembrane domains
JNK	Jun N-terminal kinase
mAbs	Monoclonal antibodies
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
MSN	Mesoporous silica nanoparticles
mTOR	Mammalian target of rapamycin
MVD	Microvessel density
NFkβ	Nuclear factor kappa-light-chain-Enhancer of activated β cells
NMOF	Amino-triphenyl dicarboxylate-bridged Zr4+ metal-organic framework nanoparticles
PDGF	Platelet-derived growth factor
PEI	Polyethylenimine
PI3k	Phospho-inositol 3 kinase
PlGF	Placental growth factor
PSI	Plexin-semaphorin-integrin
PTK	Protein tyrosine kinase
RSK2	Ribosomal protein S6 kinase 2
RTK	Receptor tyrosine kinase
SCF	Stem cell factor
SEMA	Structural domain of semaphorins
SH2	Src-homology-2 domain
SHC	Src-homology-2 domain
SPARC	Secreted protein acidic and rich in cysteine
SPIO	Superparamagnetic iron oxide
STAT	Signal transducer and activator of transcription
TGF	Transforming growth factor
TMD	Transmembrane domain
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

1 Introduction

The dynamics of cell growth and commitment to a specific lineage is generally governed by the growth factors [1]. As the name suggests, these factors are responsible for determining the fate of the cells, with regard to their division and differentiation. Apart from differentiation, these proteins also have a crucial impact on normal cellular processes, their transformation, regulation, as well as the programmed cell death (apoptosis). Imbalance and overexpression of growth factors can thus modulate a normally dividing cell into an unconditionally dividing cancerous cell, causing further dysfunction in the human body. Such cellular transformations occur due to transcriptional upregulation or via ligand overproduction and signaling through autocrine or paracrine model. These changes impact morphological and mechanical attributes, such as the membrane strength of the cells to form junctions and altered cytoskeletal arrangements, thereby affecting their motility [2]. The transformation of a healthy cell into a highly malignant cell occurs due to the genetic changes caused by the intracellular and extracellular factors, which further leads to invasion and metastasis. The root cause of such transformations can be traced back to the loss of tumor suppressor genes and the gain of oncogenic genes that synthesize oncogenic proteins, in an un-regulated manner [3]. Eradication of cancer cells has been conducted using chemotherapy and radiation therapy, both of which are associated with numerous side effects, especially due to their nonspecificity. The targeted therapy focuses on directing the therapeutics to specific sites or molecules on the cell membrane, thereby inhibiting cellular proliferation. While the traditional therapies generally exert cytotoxic effects, the targeted therapies are predominantly cytostatic (inhibiting the cell proliferation), which provides them with greater specificity, by overcoming resistance toward cytokines and growth factors, and safety [4]. These specific therapies halt cellular proliferation by affecting particular signaling cascades involved in this process and targeting drugs toward receptors to block the downstream signaling pathways and leading to collapse in the growth cone [5].

In this chapter, we have described various cancers that arise due to alteration in normal cell signaling pathways. Further, the downstream signaling molecular targets that are hampered due to this mutational change have also been elaborately discussed. Further, this manuscript also focuses on the predominant targets of specific growth factor receptors that exhibit the potential to form transformed cells that exhibit uncontrolled proliferation, invasion, and metastasis. Finally, the discussion also emphasizes on the structure and mediation of the signaling pathways and their interaction with various natural and synthetic ligand molecules that may present a significant therapeutic role. The structure and downstream signaling pathways of receptors have been depicted in Figs. 7.1 and 7.2. The list of endogenous ligands and malignancies associated with misfunction of these receptors are stated in Table 7.1.

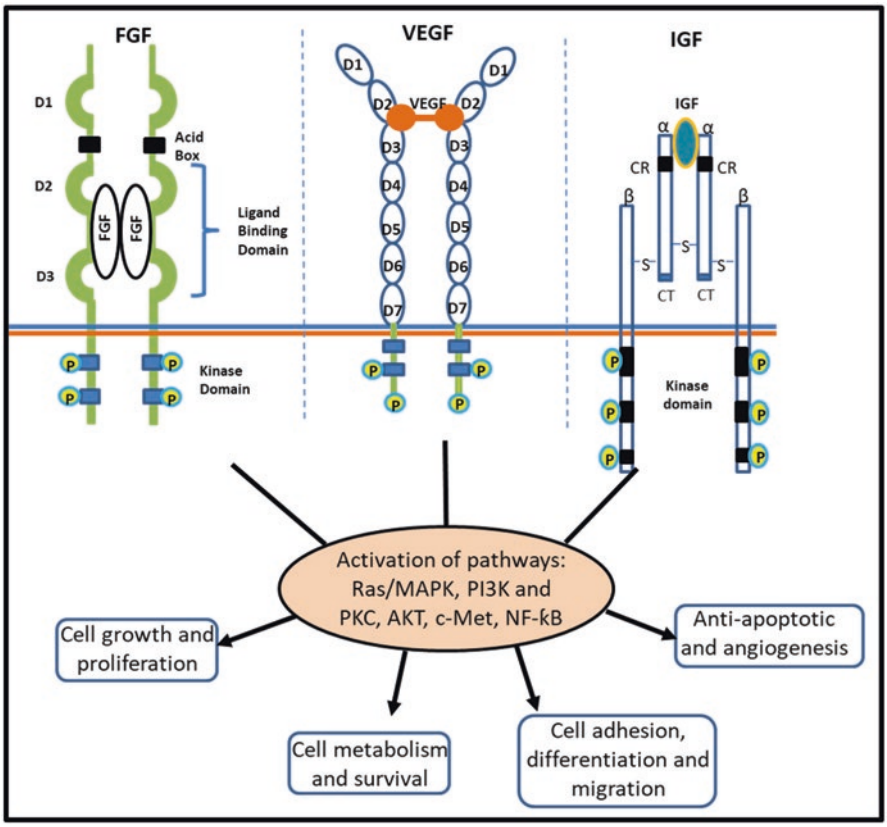

Fig. 7.1 Pictorial depiction of the receptor structure of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF). Ligand-binding domains and phosphorylation sites have been shown encompassing the downstream activation signal for various cellular processes. Constitutively, active pathways can lead to malignancies and various deleterious effects

2 Hepatocyte Growth Factor Receptor (HGF)

Hepatocyte growth factor, also known as scatter factor (SF), by is released the mesenchymal cells, like fibroblasts and some smooth muscle cells. HGF secretion activates Met protein signaling in a paracrine fashion. The receptor responsible for triggering the response mediated by HGF is the c-Met tyrosine kinase receptor, a single-pass heterodimer transmembrane receptor. Overexpression of HGF and its binding to the receptor has been reported to lead to oncogenesis and tumor propagation, thereby causing liver cancer, colorectal cancer, gastric cancers, and various other solid tumor malignancies. The receptor also accounts for cellular processes like mitogenesis, morphogenesis, survival, and motility in various cell types like the endothelial cells, neurons, epithelial cells, hematopoietic cells, and the hepatocytes [16, 17].

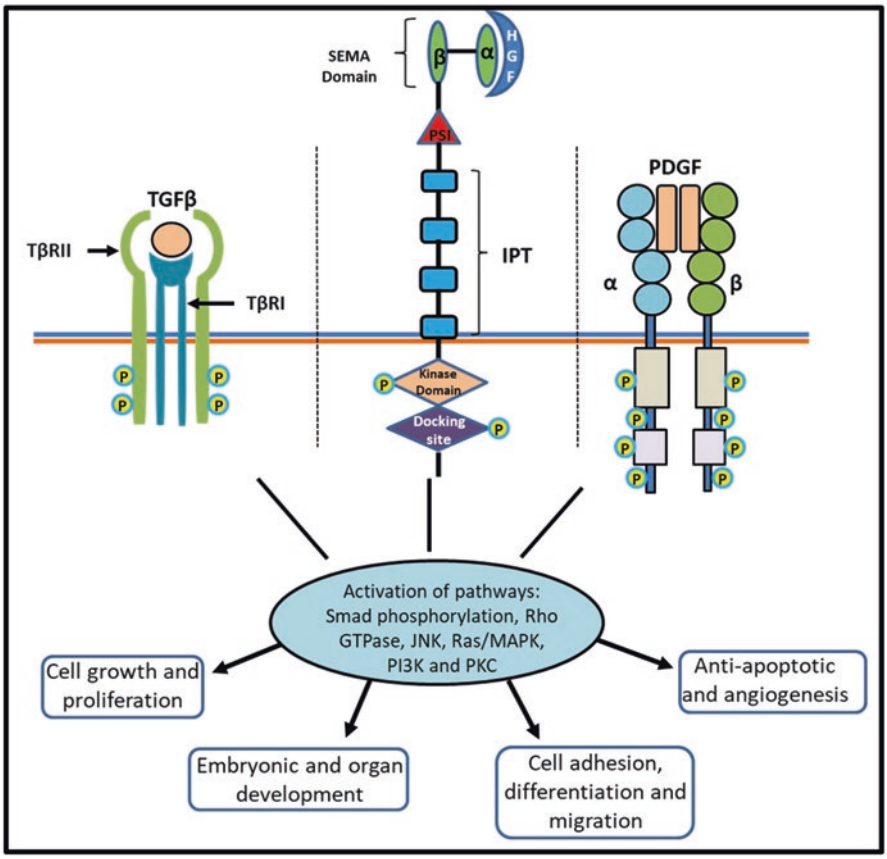

Fig. 7.2 Depiction of the receptor structure of transforming growth factor-B (TGFβ), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDFR). Ligand-binding domains and phosphorylation sites have been shown encompassing the downstream activation signal for various cellular processes. Constitutively, active pathways can lead to malignancies and various deleterious effects

2.1 Recognition Domain of HGFR

Ligand delivery in case of the receptor Met tyrosine kinase (TK) is controlled in a paracrine manner, allowing activation of the binding site and leading to receptor internalization and degradation. Receptor c-Met is transcribed from chromosome 7 into a fully functional 120 kb in length with molecular weight of 190 kDa (50 kDa α chain and a 140 kDa β chain connected together by a disulfide bond). The extracellular region of c-Met contains SEMA domain (structural domain of semaphorins) that directly binds to the ligand, a PSI domain (plexin- semaphorin-integrin) and four immunoglobulin-like plexin-transcription (IPT) domains [18].

Table 7.1 List of growth factors and their respective receptors, physiological functions, along with the endogenous ligands which trigger their intracellular signaling for causing various cancer types

Growth factor	Growth factor receptors	Endogenous ligands	Antagonist		Associated cancer
			Natural	Synthetic	
Hepatocyte growth factor receptor	c-MET receptor	Hepatocyte growth factor (HGF), scatter factor (SF)	NK2, NK3, NK4, SR 144782	MetMab, m224G11, hz224G11, ABT-700, AMG-337 [6]	Neuroblastoma, glioblastomas, osteosarcomas, brain cancer [7] Oesophageal, gastric, colorectal cancer [8], multiple myelomas, and T-cell leukemia [9]
Insulin-like growth factor – 1	IGF2R, IGF2R	Insulin IGF-I (Somatomedin C), IGF-II	GH, Somavert (R), pegvisomant	Linsitinib	Breast, melanoma, squamous cell carcinoma of lung [10]
Platelet-derived GF	PDGFRα, PDGFRβ	PDGF-A, PDGF-B, PDGF-C, PDGF -D	GAS1, SPARC, BM40, osteonectin	Imatinib, sorafenib, dasatinib, sunitinib, neutralizing PDGFR antibodies, GFB-111	Gastrointestinal tumors, chronic eosinophilic leukemia, prostate cancer, nonsmall-cell lung cancer [11]
Transforming growth factor-β	TGF-β1, TGF-β2, TGF-β3	Bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), anti-Mullerian hormone (AMH), activin, nodal, and TGF-β, TGF-β includes - TGF-β1, TGF-β2, TGF-β3	FST, noggin, chordin, BMP-3	Galunisertib	Colon, gastric, neck, and pancreatic cancer [12]
Vascular endothelial growth factor	VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4), Neuropilin1, Neuropilin-2 (nonenzymatic Receptors)	VEGF-A, VEGF-B, VEGF-C, VEGF-D, placental growth factor, thrombospondins (TSPs)	sFlt-1, AUF1-RGG peptides	Axitinib, cabozantinib, lenvatinib, sorafenib, sunitinib, pazopanib, bevacizumab, nivolumab	Bladder, brain, breast [13], colon, gastric, lung, ovarian, prostate cancer
Fibroblast growth factors	FGFR1b, FGFR1c, FGFR2b, FGFR2c, FGFR3b, FGFR3c, FGFR4	FGF1 (acidic FGF), FGF-2 (basic FGF), FGF-6, FGF-8	FGF23	Dovitinib (TKI258), AZD4547, Ki23057, E7080, brivanib alaninate, nintedanib, ponatinib, MK-2461, and E-3810 [14]	Chronic myeloid leukemia (CML) [15], metastatic prostate cancer, breast cancer, glioblastoma [15]

2.2 Binding of Ligand with HGFR

The receptor binding primarily includes the N-terminal domain of a 69-kDa HGF ligand comprising of α chain. The N-terminal of the ligand contains a hairpin loop, comprising α and β sheets, and has two disulfide linkages that are responsible for proper conformation of the protein. The innermost residues of the ligand, Cys74–Cys84, constitute the disulfide linkage, connecting the C-terminal of the α helix and the N-terminal of the β sheet. On the other hand, the outermost disulfide linkage includes Cys70–Cys96 and joins the middle regions of the α helices and β strands [7]. The binding of the ligand to the receptor is not directly dependent on the N-terminal α sheet (amino acids 25–307) and β sheet (amino acids 308–519). The cysteine-rich domain, comprising amino acids 520–561 along with the C-terminal residues 562–932 (containing four Ig domains), participates in the proper binding of the ligand. The ligand-binding domain of c-Met consists of β-propeller fold. The amino terminal in the juxtamembrane (JM) region encompasses two protein phosphorylation sites, viz., S985 and Y1003. Phosphorylation of S985 negatively regulates the kinase activity [19, 20], while phosphorylation of Y1003 recruits c-Cbl, a ubiquitin protein ligase, which ubiquinates Met, thereby resulting in its internalization and degradation [20, 21].

2.3 Structure-Activity Relationship of Selective Binding of HGF with HGFR

The HGF contains mainly immunoglobulin-like domains. Here, two c-Met heterodimers dimerize, leading to the consecutive autophosphorylation of two tyrosine residues, one within the catalytic loop (Tyr1234–Tyr1235) and in the C-terminal domain (Tyr1349–Tyr1356), thereby providing a docking site for the recruitment of other downstream molecular interactions. A sequence consisting of 13 amino acids, in the binding region, mediates the interaction between Gab1 and c-Met, thus creating a docking platform [22]. This induces morphological changes in the receptor's intracellular protein tyrosine kinase (PTK) domain and thereby activating the receptor. The Grb2-associated binding protein 1 (Gab1) triggers autophosphorylation and provides binding platform for Src-homology-2 domain (SH2)-containing effectors, like the SH2-transforming protein (SHC), the phosphoinositide 3 kinase (PI3K), the SH2-domain-containing protein tyrosine phosphatase (SHP2), the phospholipase Cγ1 (PLCγ1), the signal transducer and activator of transcription 3 (STAT3), and the Ras GTPase p120 [23, 24]. Ras thereby activates Raf, MEK, MAPKs, ERK, JNK (Jun N-terminal kinase), and p38 (HOG). Thereafter, the activated MAPKs enter the nuclei to activate transcription factors Elk1, Etsl, and c-Myc, through further phosphorylation. Abnormal signal transduction, in turn, interferes with the cell cycle and induces cell transformation, consequently promoting carcinogenesis. MAPKs are known to induce degradation of proteins and matrix, thereby promoting cell migration and proliferation of solid tumors.

2.4 Strategies to Target HGFR

The current therapies directed against Met involve the use of kinase inhibitors, ligand- or dimerization-blocking antibodies that inhibit the tumor growth and eliminate the tumor. Thus, in order to improve anti-Met therapy, cross-linked albumin nanoparticles have been linked with anti-HGFR or Met nanobodies (anti-Met-NANAPs). In vitro studies indicated that these lysosome-targeted nanoparticles were designed to bind the cells expressing Met and were further internalized, resulting in lysosomal degradation and hence downregulation of the Met protein [25, 26]. Superparamagnetic iron oxide (SPIO) nanoparticles were combined with polyethylenimine (PEI) to form cationic complexes and bind with c-Met siRNA, *forming* nanoparticles. Galactose (Gal)-modified magnetic nanoparticles were used to target the asialoglycoprotein receptors. SPIOs modified with PEI and Gal were found to protect c-Met siRNA and mediate its cellular uptake and thus can be effectively targeted via Gal-modified PEI-SPIO to inhibit the tumor growth [27].

Human embryonic kidney 293T (HEK293T) cells-derived exosomes were used as delivery vehicles for anti-HGF siRNA. Human umbilical vein endothelial cells (HUVEC), co-cultured with SGC-7901 cells treated with exosomes, loaded with anti-HGF siRNA indicated effectively the delivery of anti-HGF siRNA, suppressing the cellular proliferation and vascular ring formation in HUVEC. The inhibitory effect of siRNA on tumor growth and angiogenesis in gastric cancer resulted in a marked downregulation of HGF expression [28].

3 Insulin-Like Growth Factor Receptor

The insulin-like growth factor (ILGF) receptor is a transmembrane protein tyrosine kinase receptor that transduces the signal through the MAPK and PI3K signaling pathway. The receptor-ligand interactions govern the cell growth and survival. In addition to regulating the normal cellular processes, the binding is also responsible for tumor formation and development and survival of malignant cells [29, 30]. The insulin receptor (IR) and insulin-like growth factor-1 receptor (IGF1R) are members of the RTK family of cell surface receptors [31].

3.1 Recognition Domain of IGFR

ILGF binds to two receptor types, with different affinities. The first type of receptor is the IGF-R1, which plays a crucial role in regulating the growth of normal and malignant cells. The second receptor, namely, the insulin receptor (IR), regulates the cellular differentiation and metabolism [32]. Both the receptors share sequences

and exhibit a high morphological similarity (about 70%). The receptors belong to the glycoprotein category, which comprise 2α- and 2β-subunits. These subunits are evenly spanned into the membrane. The α-subunits are exposed toward the extracellular region to enable binding to the ligand, while the β-subunits are distributed in the transmembrane and intracellular domains. Being protein tyrosine kinase receptors, binding of ligands like IGF-I or IGF-II induces a conformational change in the receptor, due to the autophosphorylation of the three tyrosine residues in the catalytic domain of the C-terminal in the β-subunit, thereby leading to the downstream signaling. The structure of receptor and pathway has been depicted in Fig. 7.2. The insulin receptor is flexible in structure, encoded by various exons, wherein alternative splicing of exon 11 results in two isoforms (A and B) of the insulin receptor that differ in the presence or absence of a 12-residue sequence (717–729). The two isoforms have slightly different affinity for insulin, but the A isoform has significantly higher affinity for IGF-I (40 nM vs. 350 nM) and IGF-II (close to that of insulin). The IGF-I receptor binds to IGF-II with a lower affinity than for IGF-I and to insulin with a 500-fold lower affinity [33].

3.2 Binding of Ligand with IGF Receptor

The C-terminal half of the receptors consists of three fibronectin type III (FnIII) domains, each comprising a seven-stranded β-sandwich structure. The second FnIII domain comprises the C-terminal part of the α-subunit and the N-terminal part of the β-subunit and contains a large insert domain of ~120–130 residues. The structure of this insert domain is largely unknown, but includes a site of cleavage between the α- and β-subunits. The intracellular portion of the β-subunit contains the kinase catalytic domain (980–1255), flanked by two regulatory regions. These comprise a juxtamembrane region involved in docking insulin receptor substrates (IRS), IRS 1–4, and Shc, as well as in receptor internalization. The regulatory regions also contain a C-terminal tail comprising two phosphotyrosine-binding sites. The detailed organization of the modular domains of the insulin receptor has been depicted in Fig. 7.2. The IGF-I receptor has a very similar organization, with the sequence homology varying between 41% and 84%, depending on the domain. Maximum sequence homology has been observed in the kinase domain [33].

3.3 Structure-Activity Relationship of Selective Binding of Ligand with IGFR

The QSAR analysis of the ILGF receptor depicts several carbon atoms being connected to one hydrogen atom and the presence of two aromatic bonds (SaaCHcount) that are detrimental to the receptor activity. The harmful heteroaromatic rings, containing multiple nitrogens, like triazine, were anticipated to result in better

inhibition, as compared to the aromatic rings, like benzene, pyridine, and pyrimidine. Further, the electrotopological state indices of NH_2, connected to one single bond, were conducive for bioactivity, leading to the speculation that primary amines resulted in effective inhibition. This was most likely due to the hydrogen bonding, either by donating or accepting hydrogen atoms, provided that the nitrogen atom was connected to the electropositive groups. The docking poses of the IGF molecule at e MET 1112 at the receptor active site (IGF-1R) exhibited interaction of the fragment R1 with MET 1126 and ARG 1128, and of the fragment R3 with MET 1052 and GLU 1050. The amide group of the fragment R1 donates hydrogen to Met 1126 and Arg 1128. No hydrophobic interactions were observed between R1 and the active site of IGF-1R. Nonhydrophobic substituents, having branching and low NH count, at the fragment R1, were found to be essential for enhanced activity. Scaffolds of the molecules that formed the fragment R2 could be modified by decreasing the number of oxygen atoms and increasing the number of hydrogen bond donors. GQSAR studies suggested that substituents at R3 that contained lower number of aromatic carbons and higher content of NH_2 groups were responsible for majority of the activity [34].

3.4 Strategies to Target the IGFR

Targeted co-delivery of IGF-1R-specific siRNA and docetaxel (DTX) to SKBR3 cells was performed using anti-mucin 1 aptamer (Apt)-conjugated chitosan nanoparticles. Augmentation pathways involved in tumorigenesis and metastasis of breast cancer were studied. The nanoparticles with size of 110–118 nm and zeta potential of 14 mV loaded siRNA and DTX. The Apt-conjugated nanoparticles enhanced the cellular uptake of siRNA into the SKBR3 cells and reduced the genetic expression of IGF-1R, activators of transcription 3 (STAT3), and matrix metalloproteinases [35]. Co-delivery of IGF-1R-specific siRNA and doxorubicin (DOX) using chitosan nanoparticles resulted in a synergistic effect on the DOX-induced cytotoxicity and apoptosis of the tumor cells, when compared with only DOX. This resulted in decreased migration and expression of MMP9, VEGF, and STAT3, in A549 lung cancer cell lines. The loaded chitosan nanoparticles exhibited a size of about 176 nm size, zeta potential of 11 mV, and polydispersity index of 0.3 and possessed the capacity to simultaneously deliver several therapeutic agents. It also favored a controlled release of drugs or siRNA at the acidic pH of the tumor microenvironment [36]. Magnetic resonance imaging (MRI)-guided and IGF-1R-targeted theranostic iron oxide nanoparticles (IONPs) were found to be effective as they overcame the stromal barriers in tumor microenvironment. Pancreatic cancer, featuring enriched tumor stroma intravenously administered IGF1-IONPs resulted in excellent tumor penetration with better inhibition of the growth of pancreatic tumors. The intratumoral nanoparticle delivery was detected by MRI [37]. In female A/J mice, picropodophyllin was administered via nasal inhalation, demonstrating a good bioavailability in lungs and plasma. In human lung cancer cell lines, it inhibited cell

proliferation and phosphorylation of IGF-1R downstream targets, resulting in increased apoptosis and reduced cellular invasion. It is suggested that picropodophyllin can be potential chemopreventive agent [38].

4 Platelet-Derived Growth Factor Receptor

The platelet-derived growth factor (PDGF) isoforms bind to two distinct class III receptor tyrosine kinases, PDGFRα and PDGFRβ. The binding of the ligand leads to autophosphorylation of the receptors on tyrosine residues and this event induces activation of several signaling molecules [39]. Individual PDGF chains have different affinities for the two receptors. PDGFRα has high affinity for PDGF-A, PDGF-B, and PDGF-C, whereas PDGFRβ has high affinity for PDGF-B and PDGF-D. These interactions can be demonstrated in vitro, but it is not known if all are effective in vivo [40]. Ligand binding to receptors induces receptor dimerization, which leads to activation of the intrinsic tyrosine kinase domain and subsequent recruitment of SH-2-domain-containing signaling proteins [41]. Finally, activation of these pathways leads to cellular responses like proliferation and migration. Expression of activated p21Ras in cells influences PDGFRβ signaling at multiple levels. Two separate mechanisms occur for defective PDGFRβ signaling, namely, the transcriptional downregulation of PDGFRβ expression and inhibition of ligand-induced PDGFRβ by a factor of the cell membrane, in p21Ras-expressing fibroblasts [42]. Reversion of the cell phenotype results in the recovery of the PDGFRβ kinase activity. Disruption of the fibroblast cytoskeleton leads to a loss of PDGFRβ function.

4.1 Recognition Domain of the PDGFR

The ligand dimer has a flat shape with β-strands forming a super sheet, leaving the inter-strand loops at the ends of these strands. These loops are not only used for propeptide binding but also for receptor binding. There is a significant steric incompatibility between binding of receptor to PDGFs and the binding of pro-peptides to PDGFs. When the PDGF-A/pro-peptide complex and the PDGF-B/PDGFRβ complex are superimposed, with the backbones of the growth factor domains overlaid, it is apparent that these two binding events are mutually exclusive. The same hydrophobic residues important for pro-peptide association are also used for receptor binding. Consequently, receptor binding can displace the pro-peptide that is bound at the same site [43]. The two arms of the ligand clamp the PDGFR perpendicularly near the receptor's D2–D3 boundary. For PDGFRβ, the D2–D3 linker uses an extended conformation to open a large cleft for contacting PDGF-B. The overall shape of the PDGF-B:PDGFRβ recognition complex resembles other class III RTKs, such as Kit and FMS [44–46]. The positions of the D3 domains are similar in the SCF/Kit complex, the M-CSF/FMS complex, and the PDGF-B/PDGFRβ

complex, despite the positions of the D1 and D2 domains being dramatically different [47]. The PDGF family consists of five members, viz., the disulfide-bonded dimers of homologous A-, B-, C-, and D-polypeptide chains, and the AB heterodimer [48]. The PDGF-α receptor binds to all the PDGF chains, except the D chain, whereas the β receptor binds to PDGF-B and PDGF-D; thus, the different PDGF isoforms can induce αα-, αβ-, or ββ-receptor dimers. The ligand-binding sites are located in Ig-like domains 2 and 3 [43, 49]; however, the ligand-induced receptor dimerization is stabilized by direct receptor-receptor interactions in Ig-like domains 4 and 5 [50]. The latter interactions are important because they orient the receptors so that their activation by autophosphorylation in *trans* is facilitated.

4.2 Binding of the Ligand with PDGFR

PDGF-induced receptor dimerization leads to the autophosphorylation of certain tyrosine residues in the intracellular parts of the receptors. Thus, the α and β receptors have 10 and 11 autophosphorylation sites, respectively [51]. The autophosphorylation serves two important functions, viz., it leads to changes in the conformation of the intracellular parts of the receptors, promoting their activation, and it provides docking sites for the SH2-domain-containing signal transduction molecules. There are at least three mechanisms involved in the activation of the PDGF receptor kinases. Like most tyrosine kinase receptors, the PDGF receptors are autophosphorylated in the activation loop of the kinases (residues Tyr849 and Tyr857 in the α and β receptors, respectively). Phosphorylation of this residue of the β receptor is necessary for the full activation of the receptor kinase [52]. Phosphorylation causes a change in conformation of the activation loop, which opens the active site of the kinase and allows access of ATP and the protein substrate. Moreover, truncation of the carboxy-terminal tail of the β receptor causes receptor activation. The C-terminal is folded over the kinase domain that keeps kinase inactive further leading to autophosphorylation. The juxtamembrane domain of several tyrosine kinase receptors inhibits the kinase domain, causing a change in conformation led by autophosphorylation [53].

4.3 Structure-Activity Relationship of Binding of the Ligand with PDGFR

Autophosphorylation of the PDGF receptors allows binding of the signaling molecules containing the SH2 domains, which recognize phosphorylated tyrosine residues. Different SH2 domains have different preferences regarding the three to six amino acid residues downstream from the phosphorylated tyrosine, and there is a certain specificity in binding. The PDGF receptors are known to bind to about 10 different families of SH2-domain-containing molecules, which initiate the activation

of different signaling pathways. Because the autophosphorylation pattern of the PDGF-α and PDGF-β receptors differs, depending on whether the receptors occur in homo- or heterodimeric complexes, each of the three dimeric PDGF receptor complexes has distinct signaling properties [48]. Much efforts have been dedicated toward elucidating the signaling pathways that mediate the effects of PDGF on cells (i.e., cell proliferation, survival, chemotaxis, and actin reorganization). In general, the PI3 kinase has been found to be important for the antiapoptotic and motility responses of the PDGF, though differences between various cell types have also been reported. Src, via activation of the transcription factor Myc and Ras via activation of the ERK MAP kinase pathway are important for the growth-stimulating effects. However, it should be noted that there is an extensive cross talk between different signaling pathways. Thus, each of the many signaling pathways, induced by the activated receptor, can contribute to most of the cellular effects of the PDGF to different extents and in a cell type-specific manner.

4.4 Strategies to Target PDGFR

The PDGF receptors expressed in cervical cancer cells have been targeted using mesoporous silica nanoparticles (MSN), loaded with the anticancer prodrug cisplatin (cis-DDP) with an affinity probe of poly-acrylic acid (PAA). These PAA-MSNs are specifically taken up by the endothelial cells. The mean particle sizes and zeta potential ranged between 60 and 100 nm and −26.4 to +20.3 mV, respectively [54]. NH3+ groups present on MSNs-cis-DDP complexes interacted with the –OH group of the PAA; thus, the unreacted carboxylic groups had affinity to bind with the receptor [55].

Insufficient therapeutic agents are available to glioblastoma multiforme (GBM) tumor, after crossing the blood-brain barrier. The chemotherapeutic temozolomide is converted to 5-(3-methyltriazen-1-yl) imidazole4-carboxamide [56]. pH-responsive micelles loaded with TMZ and composed of distearoyl phosphoethanolamine-PEG-2000-amine and N-palmitoyl homocysteine and functionalized with PDGF peptide and Dylight 680 fluorophore showed uptake and increased cytotoxicity in glial cells. In vivo studies in orthotopic gliomas implanted in mice demonstrated selective accumulation of PDGF-micelles containing TMZ, with reduced systemic toxicity [57].

Ultrasound-mediated delivery using thermosensitive polymer (TSP)-based liposomes, modified with DNA aptamers, was targeted to PDGFR ligands on cancer cells (APT/TSP liposomes). These liposomes were formulated for breast cancer, using copolymer of N-isopropylmethacrylamide (NIPMAM) and N-isopropylacrylamide (NIPAM) forming TSP liposomes. The APT/TSP liposomes had binding affinity toward the MDA-MB-231 human breast cancer cells due to the presence of PDGFR aptamers. Cancer cell injury assay showed that using DOX-loaded APT/TSP liposomes and ultrasound irradiation, cell viability was 60%, which was lower than that with ultrasound irradiation and DOX-loaded TSP liposomes or with DOX-loaded

APT/TSP liposomes alone [58]. In aptamer-assisted targeting, inhibitory PDGF aptamers and PDGF β-receptors antagonist enhanced antitumor effect of Taxol on subcutaneous KAT-4 tumors in SCID mice and increased the antitumor effects of 5-fluorouracil on subcutaneous PROb tumors in BDIX rats [59].

5 Transforming Growth Factor Receptor

Transforming growth factor-β (TGF-β) regulates various cellular processes like mitotic inhibition or stimulation. The TGFβ pathway, by itself, controls various cellular functions that may lead to differing cellular phenotypes. The receptor model of TGF-β consists of heteromeric complexes of type I and II receptors. Upon ligand binding, the type I receptor is phosphorylated in the GS domain, located upstream in serine/threonine kinase domain and acts as an important regulatory domain for TGF signal transduction. This phosphorylation activity is assisted by the serine/threonine kinase of the type II receptor [60]. Phosphorylation of the GS domain is proposed to activate the type I receptor, resulting in signal propagation to the downstream effector molecules. In addition, specific residues in the nearby regions have also been suggested to have both positive and negative regulatory functions [60–63].

5.1 Recognition Domain of the TGF-β Receptor

The TGF-β family members facilitate signal transduction via binding to the dual specificity kinase receptors, at the surface of the target cells. The receptor family has similar structural characteristics for both serine/threonine and tyrosine kinases. Even though literature refers the receptor family as serine/threonine kinase receptors, they carry dual specificity kinases [64]. There are seven type I human receptors and five type II receptors; individual members of the TGF-β family bind to characteristic combinations of type I and type II receptors. The receptors have a rather small cysteine-rich extracellular domain, a transmembrane domain, a juxtamembrane domain, and a kinase domain; however, except for the BMP type II receptor and in contrast to tyrosine kinase receptors, parts of the carboxy terminal of the kinase domains are very short. Ligand-induced oligomerization of type I and type II receptors promotes phosphorylation of the type II receptors with the help of type I receptors, in a region of the juxtamembrane domain that is rich in glycine and serine residues (GS domain), thus *causing* activation of its kinase [64, 65]. The three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) bind to a single type II receptor (TβRII). Prior to ligand binding, TβRI and TβRII are in form of monomers, homodimers, and heterodimers.

5.2 Binding of the Ligand with TGF-β Receptor

Binding of ligand to receptor forms heterotetrameric complex of two TβRI and two TβRII molecules [63, 66, 67]. Structural studies have reported that the ligand-receptor binding occurs in the ratio of 2:2:2, as a complex, wherein a dimeric TGF-β binds to two TβRI and two TβRII molecules. The TGF-β molecule resembles a hand-like structure, containing a disulfide linkage and the finger-like TβRI and TβRII receptors. The receptor-receptor interaction enhances the stability of the ligand-receptor complex [68]. Binding induces phosphorylation in the GS domain. Phosphorylation of the GS domain, furthermore, enhances interaction with R-Smads, which promotes their phosphorylation [69]. Reports suggest TβRII is tyrosine phosphorylated that leads to the possibility of binding to SH2- or the PTB-domain-containing signaling molecules. The phosphorylation of Tyr284 has been shown to promote binding of the adaptors Shc and Grb2; Grb2 forms a complex with Sos1, a nucleotide exchange factor for Ras, which in turn activates the Erk1/2 MAP kinase pathway.

5.3 Structure-Activity Relationship for Selective Binding of Ligands with TGF-β Receptor

Activation of receptors TβRI and TβRII are regulated via various phosphorylation events. Upon ligand-receptor binding, structural alignment of the complex is induced, wherein TβRII phosphorylates TβRI in the GS domain located in the upstream region of the kinase domain [70]. The phosphorylation occurs on several closely located residues (i.e., Thr186, Ser187, Ser189, and Ser191); wherein no single residue is of crucial importance for activation, but phosphorylation needs to reach to a certain threshold for activation of the TβRI kinase. This phosphorylation leads to a conformational change that causes release of the 12 kDa-immunophilin FK506-binding protein (FKBPI2), which binds to the GS domain and inhibits the TβRI kinase [71–73]. TβRI can be phosphorylated at the Ser165, in the juxtamembrane domain. Interestingly, this phosphorylation modulates TGF-β signaling; growth suppression and matrix production are enhanced after mutation of Ser165, whereas the pro-apoptotic effect is decreased. Similar to TβRII, the kinase domain of TβRI has structural elements similar to both serine/threonine and tyrosine kinases [64]. Like TβRII, TβRI undergoes autophosphorylation on the serine/threonine residues, as well as on the tyrosine residues. The phosphorylated tyrosine residue(s) form docking site(s) for the adaptor molecule Shc via its PTB-domain, followed by its phosphorylation and the recruitment of the Grb2/Sos1 complex, and activation of Ras and the Erk MAP kinase pathways. The phosphorylation of TGF-β receptors is counteracted by several phosphatases. Thus, GADD34, a regulatory subunit of the protein phosphatase 1 (PP1) was found to bind to Smad7, which in turn binds to TβRI; the PP1 catalytic activity is thereby recruited in TβRI and dephosphorylates the receptor [63, 74].

5.4 Strategies to Target the TGF-β Receptor

Administration of TβR-I inhibitor (LY364947) [75] alters the tumor microenvironment along with an enhanced EPR (enhanced permeability and retention) effect. Doxil and a polymeric micelle incorporating ADR have demonstrated the effect of low-doses of the TβR-I inhibitor in xenografts in nude mice, developed using BxPC3 human pancreatic adenocarcinoma cell line [76]. This TβR-I inhibitor has exhibited success in pancreatic adenocarcinoma and gastric cancer, characterized by hypovascularity and thick fibrosis in the tumor microenvironment. Low dose decreased the pericyte coverage of the endothelium and promoted the accumulation of anticancer nanocarriers [77]. Heterogeneous drug distribution-induced regional insufficient chemotherapy accelerates the process of epithelial-mesenchymal transition (EMT) and thus accelerates tumor metastasis. Since TGF-β plays an essential role in EMT to eliminate the insufficient chemotherapy promoted metastasis [12], a combination of DOX and TGF-β receptor inhibitor LY2157299 was investigated in an in vivo study incorporating TGF-β receptor inhibitor along with hydroxyethyl starch-polylactide HES-PLA nanoparticles. The co-delivery of DOX and LY2157299, using HES-PLA nanoparticles, was found to be effective [78]. N-terminal of TTB, TGF-β receptor blocker, was fused with the RGD (arginine-glycine-asparagine) is a peptide of amino acids Arginine-Glycine-Asparagine, to target the tumor. In xenograft models, TTB resulted in distinct neutralization of TGF-β and inhibited cancer cell migration. TTB also attenuated the TGF-β1-induced Smad2 phosphorylation and EMT and suppressed breast cancer metastasis, indicating blocking of the TGF-β-induced pathogenesis [79]. An alternative approach to avert TGF-β signaling was the employment of recombinant Fc-fusion proteins, containing the soluble ectodomain of either TβRII (TβRII-Fc) or the type III receptor, betaglycan [80]. Zebin et al. developed recombinant oncolytic adenoviruses as a potential new class of antitumor agents [81]. These have been hypothesized to kill the tumor cells and simultaneously target the TGF-β pathways, to treat bone metastasis of prostate cancer. Further, Hu et al. also evaluated systemic administration of the TβRII-Fc coupled with an oncolytic adenovirus (Ad.sTβRII-Fc), in a nude mouse model of breast cancer bone metastases. Their study demonstrated that intravenous delivery of Ad.sTβRII-Fc resulted in viral replication and expression of TβRII-Fc in skeletal tumors, as well as a signification reduction of primary tumor growth and osteolytic bone destruction [82].

6 Vascular Endothelial Growth Factor Receptor

The VEGF binds to VEGFR to induce receptor homodimerization or heterodimerization, leading to the activation of tyrosine kinase and autophosphorylation of the tyrosine residues in the intracellular domains of the receptor. The phosphor-tyrosines and the surrounding amino acid residues constitute the binding sites for the adapter molecules, which initiate various intracellular signaling pathways. These pathways mediate immediate responses, such as vascular permeability and long-term responses

that require gene regulation, such as endothelial cell survival, migration, and proliferation. Noncanonical VEGFR signaling is initiated by non-VEGF-dependent activation of VEGFRs [83, 84]. The VEGFR signaling is tightly regulated at numerous different levels, including at the receptor expression level, with respect to the availability and affinities of binding to its different ligands, the presence of VEGF-binding co-receptors, non-VEGF-binding auxiliary proteins and inactivating tyrosine phosphatases, the rate of receptor cellular uptake, the extent of degradation, and the speed of recycling. VEGFR-mediated endocytosis and trafficking regulate the specificity, as well as the duration and amplitude of the signaling output. Once they are in the cytoplasm, the VEGFRs are either shuttled to lysosomes for degradation or recycled back to the membrane, via fast or slow recycling pathways. In case of VEGFR2, activation of ERK1/2 signaling, which is essential for the biology of VEGFR2, is dependent on the speed of the receptor's intracellular trafficking.

6.1 Recognition Domain of the VEGFR

The positively charged domain of VEGF, encompassing the Arg82, Lys84, and His86 residues and located in a hairpin loop, is responsible for the receptor-ligand binding, while the negatively charged residues like Asp63, Glu64, and Glu67 are associated with VEGFR-1. The VEGFR-1 binds to VEGF with 50-fold higher affinity than VEGFR-2 [85], which governs its angiogenic response [86] and is therefore of great therapeutic interest. Only a small number of VEGF residues are important for its binding to the VEGF receptors [87, 88]; thus, several molecules are able to modulate the biological activities of VEGF [89]. Many VEGF mimetic peptides, having antiangiogenic activity, have been described, while only a few of these molecules are known to exhibit a pro-angiogenic activity [90]. Copper stimulates VEGF [91, 92] and is required for the activation of the hypoxia-inducible factor-1, a major transcription factor regulating the expression of VEGF [93]. The activity of copper is VEGF-dependent; the metal ion perturbs the distribution of VEGF receptors, switching the signaling pathways from VEGFR-2 to VEGFR-1, which is associated with the inhibition of the growth of cardiomyocytes and regression of hypertrophy [94].

6.2 Binding of the Ligand with VEGFR

The binding of ligands to the VEGFR is thought to induce receptor dimerization. However, in vitro studies show pre-formed VEGFR2 dimers, with a certain level of kinase activity. The dimer, upon ligand binding, is stabilized by the receptor via binding at specific points. Moreover, binding of the ligands induces a slight conformational change in the transmembrane domains, which is accompanied by rotation of the dimers that is of critical importance for the full activation of the kinase

functions. Different ligands can influence the degree of rotation of the receptor molecules to different extents, and thereby the extent of receptor activation. For example, VEGFβ has been shown to lack the ability to optimally rotate its receptor, VEGFR1, as compared to PlGF. Thus, VEGFβ is a weaker activator of VEGFR1 signaling. In addition to the classical VEGF ligands, the alternatively spliced VEGFβ contains a unique exon 8b that confers it with antiangiogenic effect. However, the VEGFβ variants are also weak VEGFR2 agonists; thus, the mechanism of their antiangiogenic effect is still unclear.

6.3 Structure-Activity Relationship of Binding of the Ligands with VEGFR

The VEGFRs are related to the fibroblast growth factor (FGF) receptors, the colony-stimulating factor 1 (CSF-1) receptors, the stem cell factor (SCF) receptor, c-Kit, and the platelet-derived growth factor (PDGF) receptors. The extracellular domain (ECD) of classes III, IV, and V of the RTKs consists of several Ig-like subdomains and the linkers connecting them. The extracellular Ig-like subdomains have been attributed with three distinct *functions as follows:* (i) they form the ligand-binding domain, (ii) they participate in receptor dimerization, after or concomitant with ligand binding, and (iii) they maintain receptors in the monomeric state in the absence of the ligand. The ligand-binding ability of ECD is documented at the biochemical and the structural level. The VEGFR-1 subdomain 2 is sufficient for binding to the VEGF, while binding to VEGFR-2 involves subdomains 2 and 3 [95–97]. Subsequent to ligand binding, the receptor monomers form dimers that are further stabilized by homotypic interactions of the domains that are in proximity of the plasma membrane Ig-like domains 4 and/or 5, as surveyed for PDGFR-b and Kit [45, 50]. The domains immediately adjacent to the lipophilic membrane, in which the receptors are anchored, the so-called juxtamembrane domains (JMDs), have been shown to regulate the kinase activity in multiple ways [98, 99]. Evident in both extracellular as well as the intracellular JMD [100, 101], which played essential roles in kinase activation, either by properly positioning the kinase monomers relative to each other or by direct interaction with the activation loop. Phosphorylation of the JMDs at specific tyrosine residues disrupts this interaction thereby promoting reorientation of the activation loop and inducing an enzymatically active conformation [101, 102]. The RTKs are activated upon ligand-mediated dimerization or *higher-order* multimerization. Receptor multimerization is not only mediated via ligand binding but also requires additional homotypic interactions in the ECD, the JMD, and the TMD. Dimerization results from interaction between specific epitopes in the N- and the C-lobe of kinase monomers. RTK activation is suggested to rely on mechanisms as deduced for soluble intracellular kinases, for example, cell cycle-regulated kinases (CDKs). Activation of CDKs requires their binding to a regulatory subunit, the cell cycle-regulated cyclins. It remains to be shown whether this model also applies to the other RTK family members [101].

6.4 Strategies to Target VEGFR

Antiangiogenic drugs, in particular those focusing on blocking the VEGF pathway, are a part of the standard therapy for which various drug delivery strategies are applied to target VEGFR. Some of the strategies are discussed in this section.

Liposomal drug delivery systems with the use of highly soluble cisplatin analogue, cis-diammine dinitrato platinum (II), demonstrated a high binding affinity to the glioma cells. Pharmacokinetic study on glioma C6-bearing rats revealed prolonged blood circulation time of the liposomal formulation, due to reticuloendothelial bypass [103]. VEGF-targeted siRNA and gemcitabine monophosphate (GMP) were co-formulated into a single cell-specific, targeted lipid/calcium/phosphate (LCP) nanoparticle. This delivery system enforces eightfold reduction in tumor cell proliferation and a significant decrease of tumor microvessel density (MVD) as compared to therapy with either anti-VEGF siRNA or GMP alone. Further, anisamide (AA) was added to the LCP surface to specifically target the sigma receptors that are overexpressed in many human cancer cells [104]. A novel nanocomposite comprising bevacizumab (Bev) modified SiO_2@LDH nanoparticles (SiO_2@LDH-Bev) loaded with DOX was explored to exhibit an improved cellular uptake and demonstrated targeting of DOX to the brain tumors. This resulted in enhancement of both antineuroblastoma and antiangiogenesis efficiency and also reduced the side effects caused by DOX [105]. Amino-triphenyl dicarboxylate-bridged Zr^{4+} metal-organic framework nanoparticles (NMOFs) were modified with a nucleic acid complementary to the VEGF aptamer. The nucleic acid-functionalized NMOFs were loaded with the anticancer drug, DOX, and were capped by hybridization with the VEGF aptamer that yielded the VEGF-responsive duplex nucleic acid gates. In addition, conjugation to the AS1411 aptamer sequence that binds to nucleolin receptors resulting in the construction of cancer cell-targeted VEGF-responsive DOX-loaded NMOFs. The system demonstrated selective permeation with a twofold enhanced uptake along with the selective apoptosis of the MDA-MB-231 cancer cells, as compared to the normal MCF-10A breast cells [106]. Further, combination of Ang2 inhibitor (recombinant peptide-Fc-fusion protein called peptibody) and VEGF inhibitor (humanized mAb bevacizumab) permitted vascular normalization at significantly reduced doses and avoided excessive vessel regression [107].

7 Fibroblast Growth Factor Receptor

Fibroblast growth factor family comprises 22 identified molecules, of which only 18 function as FGF ligands (exceptions are FGF 11–14). FGF are secreted glycoproteins and they carry strong affinity for the cell surface proteoglycans, which include glycosaminoglycan side chains. Thus, due to their ability to adhere, they are trapped on the surface of the cells which secrete them or the cells in proximity, enhancing their action to mediate short-range signal transduction [108, 109].

The FGF receptor is a transmembrane tyrosine kinase receptor that belongs to the immunoglobulin (Ig) superfamily. Humans consist of four genes encoding for the FGF receptors, a family of receptors responsible for the expression of transmembrane RTKs (FGFR1–4). The FGFR monomers consist of an extracellular domain, including a ligand-binding site, three immunoglobulin loops coded by alternative splicing, an acidic box containing glutamic acid and aspartic acid residues in the IgI–IgII linker region, a transmembrane domain, and a split tyrosine kinase domain constituting the C-terminal cytoplasmic domain. The first Ig-like domain and the acid box forming the N-terminal are reported to play a role in receptor autoinhibition. The second and the third Ig-like domains are known for FGF ligand binding and are responsible for binding to the FGFR subtypes [110–112]. The intracellular portion consists of a juxtamembrane domain, a split tyrosine kinase domain, and a carboxy-terminal tail [113].

7.1 Recognition Domain and Binding of Ligands with the FGFR

The extracellular ligand-binding domain has a hydrophobic signal peptide-containing region and two or three immunoglobulin (Ig)-like domains (D1–D3). The bridge between D1 and D2 comprises 30 serine residues. Signal transduction from extracellular region to the cytoplasmic domain is facilitated by the transmembrane domain. The C-terminal lies in the juxtamembrane region, which emerges from the cytoplasmic membrane and has a split tyrosine kinase domain [114, 115]. This receptor system contains heparan sulfate proteoglycans and the related heparin-like molecules necessary for FGF-FGFR binding and receptor activation. Binding of FGF to the FGFRs induces receptor dimerization, leading to conformational changes within the FGFR structure, thereby leading to trans-phosphorylation of the tyrosine residues in the intracellular part of the receptor, including the kinase domain and the C-terminus [108, 116, 117]. There are seven autophosphorylation sites in FGFR1, Y463 (juxtamembrane), Y583/Y585 (kinase insert), Y653/Y654 (the activation loop), Y730 (kinase domain) and Y766 (C-terminal tail) [118]. Transphosphorylation of the tyrosine residue Y653, in the activation loop, leads to the activation of the kinase by 50–100-fold, thereby autophosphorylating the tyrosine residues in the juxtamembrane (Y463), the split kinase insert (Y583/Y585), and the C-terminal (Y766). These autophosphorylations induce structural changes, thereby presenting the cytoplasmic domain as a docking site for the downstream signaling molecules. Finally, phosphorylation of tyrosine in the activation loop (Y654) leads to further enhancement in the kinase activity by tenfold [119]. The binding of the docking proteins to the FGFRs leads to activation of multiple signal transduction pathways, including the four main downstream pathways, Ras-Raf-MapK, PI3K-Akt, Stats, and PLCγ [108].

7.2 Structure-Activity Relationship for Binding of Ligands with the FGFR

Upon ligand binding, the tyrosine residue 463 in the juxtamembrane is phosphorylated, followed by Crk phosphorylation, to instigate formation of a complex between FGFR and Crk. SOS (activated by Crk) further 42 activates JNK, via Ras [116] and Rac pathway [120]. In addition to Rac, cdc42, a cell cycle regulator has also been reported as an intermediate to the JNK and p38 activation cascades [116]. Direct interaction of DOCK180 with Rac1 occurs, thereby activating JNK in a manner that is dependent on factors like Cdc42Hs and SEK and increasing the amount of GTP-bound Rac1 [121]. Receptor activation also phosphorylates the docking protein, FGFR substrate 2 (FRS2), which further employs Shp2 and enhances association between the growth factor receptor-bound 2 (Grb2) and SOS, thus triggering the induction of Ras/MEK/MAPK signaling pathways [74, 122, 123]. In addition, activation of the PI3-kinase pathway takes place via tyrosine phosphorylation in FRS2α and recruitment of Grb2 and Gab1 [124]. Additionally, interaction of the accessory proteins (SH2 domain-containing adaptor protein B (Shb) and SH2 domain-containing collagen (Shc)) with the FGFRs facilitates the signal transduction [120, 125]. Binding of Shb2 induces tyrosine phosphorylation (Y766), thereby activating the Ras/MEK/MAPK pathway. FGFR binds to the signal transducers and activators of transcription (STAT) and ribosomal protein S6 kinase 2 (RSK2). Further, STAT3 binds to the phosphorylated Tyr^{677} of the FGFR1. In addition, tyrosine activation of STAT3 requires overexpression of FGFR1 or FGFR2 [122]. In cancer cells, when the FGFRs bind to different FGF ligands, the FGFRs can cause abnormal upregulation of the Ras-dependent mitogen-activated protein kinase (MAPK), Ras-independent phosphoinositide3-kinase-protein kinase B/Akt (PI3K-PKB/Akt) pathway, and signal transducer and activator of transcription (STAT)-dependent signaling pathways, which are closely associated with the development of multiple cancers.

7.3 Strategies to Target the FGFR

This section gives an overview of different drug delivery strategies like prodrug complex, nanoparticle delivery to target FGFR for anticancer therapies. Conjugation of truncated human FGF1 (residues 21–154) (FGF1V) with monomethyl auristatin E (MMAE) was developed for potent and specific cytotoxic effect. FGF1V contains three-point mutations (Q40P, S47I, and H93G) and an N-terminal four-amino acid linker (CGGG), which increases its stability. The FGF1V-valine-citrulline-MMAE conjugate showed targeted and efficient release of MMAE at lower concentration than the native MMAE [126]. Further, brivanib alaninate is an L-alanine ester prodrug of brivanib with enhances aqueous solubility of drugs and enables their oral administration. It is a selective dual inhibitor of FGF and VEGF signaling.

Brivanib alaninate has reduced 76% tumor cell proliferation and tumor vascular density in xenograft models [127]. Further, disulfide-stabilized diabody (ds-Diabody) against antibasic fibroblast growth factor (bFGF) was constructed by site-directed mutation and overlap extension PCR (SOE-PCR), at VH44 and VL100, in single-chain variable fragment (scFv) antibody. It inhibited the bFGF-induced activation of the downstream signaling regulators and also decreased the densities of microvessels and lymphatic vessels in the tumor tissue.

8 Clinical Development of Growth Factor Receptor Antagonists

An overview of the clinical development of various growth factor receptor antagonists for cancer treatment has been concisely presented in Table 7.2.

9 Conclusion

Cancer possesses numerous growth alteration mechanisms and compromised cell surface receptors that govern and regulate the cellular functions, enhancing malignant behavior. These cell surface receptor families consist of tyrosine kinases and serine/threonine kinases, which control the cellular expression of the growth factors. There are eight types of growth factors that participate in the controlled development normal cells. In this chapter, seven of the growth factor receptors (HGF, ILGF, PDGF, TGFβ, VEGF and FGF) have been elaborated, while the growth factor receptor activation and signal transduction of the epidermal growth factor receptor has been explained in Chap. 7. This document contains elaborate details about the growth factor receptors, with respect to their structure, binding with the ligands, their binding domains, the signal transduction pathway triggered upon ligand binding, and their downstream signaling mechanisms. Their respective receptors are majorly responsible for the transduction of downstream signaling pathways. These pathways, upon interaction with abnormally expressed ligands, exert continued signaling cascades that transform normal cells to cancerous ones. The factors dictating the transformation of cells into a cancerous can be controlled via regulations in the signaling of growth factor receptors upstream. The conventional drugs used in chemotherapy can be conjugated with various newly designed molecules like small peptides and monoclonal antibodies. Advancements in these molecules are their mode of delivery. Organ specificity of the drugs can be achieved via entrapment in different carrier molecules like liposomes or polysaccharide-based nanoparticles. The drugs and their targeting strategies developed are currently being tested for their stability and preventing the metastasis. Certain genetically engineered variants of ligand have also been studied for the receptor regularization. These ligand variants, drug complexes, and delivery techniques are still in their clinical trial phases.

Table 7.2 Overview of the recently completed clinical trials studies that have been conducted to target the cancer therapy

Drug/drug combination	Phase	Targeted cancer	Study outcome	Clinical trial identifier
HGFR				
Rilotumumab	Phase II	Ovarian epithelial cancer, fallopian tube cancer, primary peritoneal cancer	Well tolerated in the early phase clinical trials and inhibited the HGF-/MET-driven activities in recurrent and resistant cancer cells	NCT01039207 [128]
Crizotinib	Phase II	Solid tumors or anaplastic large cell lymphoma (ALCL) in children	The pharmacokinetics of oral crizotinib in children is similar to that in adults	NCT00939770 [129]
Cabozantinib	Phase III	Hepatocellular carcinoma	Longer overall survival and progression-free survival with cabozantinib than with placebo	NCT01908426 [130]
IGFR				
AMG 479	Phase I	Chemorefractory ewing sarcoma	Absence of severe toxicities and demonstrated promising single-agent activity	NCT00562380 [131]
R1507	Phase II	Chemorefractory ewing sarcoma	Active as monotherapy, but acquired resistance was commonly observed upon continued therapy	NCT00642941 [75]
Ridaforolimus + Dalotuzumab	Phase I	ER+/highly proliferative breast cancers	Inhibited (mTOR), prevented the activation IGFR signaling	NCT01234857 [132]
PDGFR				
Imatinib mesylate	Phase II	Recurrent meningioma	CYP3A4 inducers decreased the plasma concentration of imatinib	NCT00045734 [133]
Olaratumab + doxorubicin	Completed	Soft tissue sarcoma	Improved progression-free survival and overall survival	NCT02326025 [134]
Sunitinib (SU11248)	Phase I/II	Gastrointestinal stromal tumor (GIST)	Overall survival was improved	NCT00457743 [135]
TGF-β receptor				
LY2109761	Preclinical	Colon adenocarcinoma cells	Reduced oncogenic effects of TGF-β on cell migration, invasion, and tumorigenicity in CT26 cells	NA

(continued)

Table 7.2 (continued)

Drug/drug combination	Phase	Targeted cancer	Study outcome	Clinical trial identifier
Galunisertib (LY2157299 monohydrate)	Preclinical	Glioblastoma, pancreatic cancer	Downregulates the phosphorylation of Smad2, abrogating the activation of the canonical pathway	NA [136]
Belagenpumatucel-L	Phase III	Squamous cell carcinoma, large-cell lung cancer	Increased overall survival	NCT00676507
Fresolimumab (GC-1008)	Phase I	Metastatic melanoma, renal cell carcinoma	Safe and well tolerated	NCT00356460 [137]
VEGFR				
Bevacizumab + 5-fluorouracil, oxaliplatin, leucovorin or 5-fluorouracil, irinotecan, leucovorin	Phase III	Metastatic colorectal cancer	Reduced febrile neutropenia incidence	NCT00911170 [138]
Apatinib (YN968D1)	Preclinical	Advanced gastric adenocarcinoma	Improved safety and efficacy	NA [139]
Apatinib (YN968D1)	Phase III	Metastatic gastric carcinoma	Improved the progression-free or overall survival, who failed second-lines of chemotherapy	NCT01512745 [140]
Vatalanib + gemcitabine	Phase I/II	Advanced pancreatic cancer	Combination is well tolerated with antitumor response	NCT00185588 [141]
FGFR				
Dovitinib (TKI258)	Phase III	Metastatic renal cell carcinoma	Lesser activity than sorafenib in patients with progressed on VEGF-targeted therapies/ mTOR inhibitors	NCT01223027 [142]
AZD4547 + fulvestrant	Phase II	Breast cancer	Improved safety in efficacy in combination therapy	NCT01202591 [143]
Lucitanib (E3810)	Phase II	Breast cancer	Daily dosing provide durable clinical responses	NCT02202746 [144]
Lenvatinib	Phase II	Endometrial cancers	Tolerable and clinical benefits in advanced endometrial cancer	NCT01111461 [145]

The combinatorial therapies need a lot of in vitro research base with respect to proving its synergism and effect up on the linked signaling pathways. The pharmacodynamic studies reveal that these drugs carrying specific targets can be useful in designing personalized medicine. These therapies are currently under various phases of clinical trials. Their clinical success will lead to the availability of more specific, effective, and safer therapies for the treatment of a variety of cancers. Multiple clinical trials described in the document have passed phase II depicting the success ratio of the upcoming promising therapy with a drug delivery system. Research attempts directed toward these receptors are anticipated to provide interdisciplinary insights on their implication in the development, progression, and treatment of the cancers caused by overexpression of growth factor signals.

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