

Something old, something new and something borrowed: emerging paradigm of insulin-like growth factor type 1 receptor (IGF-1R) signaling regulation

Leonard Girnita · Claire Worrall ·
Shin-Ichiro Takahashi · Stefan Seregard · Ada Girnita

Received: 11 May 2013 / Revised: 17 October 2013 / Accepted: 7 November 2013 / Published online: 26 November 2013
© The Author(s) 2013. This article is published with open access at Springerlink.com

Abstract The insulin-like growth factor type 1 receptor (IGF-1R) plays a key role in the development and progression of cancer; however, therapeutics targeting it have had disappointing results in the clinic. As a receptor tyrosine kinase (RTK), IGF-1R is traditionally described as an ON/OFF system, with ligand stabilizing the ON state and exclusive kinase-dependent signaling activation. Newly added to the traditional model, ubiquitin-mediated receptor down-regulation and degradation was originally described as a response to ligand/receptor interaction and thus inseparable from kinase signaling activation. Yet, the classical model has proven over-simplified and insufficient to explain experimental evidence accumulated over the last decade, including kinase-independent signaling, unbalanced signaling, or dissociation between signaling and receptor down-regulation. Based on the recent findings that IGF-1R “borrows” components of G-protein coupled receptor (GPCR) signaling, including β -arrestins and G-protein-related kinases, we discuss the emerging paradigm for the IGF-1R

as a functional RTK/GPCR hybrid, which integrates the kinase signaling with the IGF-1R canonical GPCR characteristics. The contradictions to the classical IGF-1R signaling concept as well as the design of anti-IGF-1R therapeutics treatment are considered in the light of this paradigm shift and we advocate recognition of IGF-1R as a valid target for cancer treatment.

Keywords IGF-1R · GRKs · IRS · Beta-arrestins · Serine phosphorylation · Cancer · Ubiquitination · GPCR · RTK

Abbreviations

aa	Amino acid residues
A-loop	Activation loop
ATP	Adenosine triphosphate
CH2	Collagen homology 2 domain
C-terminal	Carboxyl terminal
cAMP	Cyclic adenosine monophosphate
CREB	cAMP response element binding-protein
ECM	Extra-cellular matrix
EGFR	Epidermal growth factor receptor
ERK	Extracellular-related kinase
ES	Ewing sarcoma
GDP	Guanosine diphosphate
GSK 3 β	Glycogen synthase kinase
GTP	Guanosine triphosphate
G proteins	Guanine nucleotide binding-proteins
GPCRs	G-protein coupled receptors
Grb2	Growth factor receptor bound protein
GRKs	GPCR-related kinases
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding-protein
IGF-1R	Insulin-like growth factor-type 1 receptor
IR	Insulin receptor
IRR	Insulin receptor-related receptor

L. Girnita (✉) · C. Worrall · A. Girnita
Department of Oncology and Pathology, Cancer Center
Karolinska, CCK R8:04, Karolinska Institute, Karolinska
University Hospital, 171 76 Stockholm, Sweden
e-mail: leonard.girnita@ki.se

S.-I. Takahashi
Departments of Animal Sciences and Applied Biological
Chemistry, Graduate School of Agriculture and Life Sciences,
The University of Tokyo, Tokyo, Japan

S. Seregard
Ophthalmic Pathology and Oncology Service, St Erik's Eye
Hospital and Karolinska Institute, Stockholm, Sweden

A. Girnita
Dermatology Department, Karolinska University Hospital, 171
76 Stockholm, Sweden

IRS	Insulin receptor substrate
MAPK	Mitogen-activated protein kinase
Mdm2	Mouse double minute 2 homolog
MEK	Mitogen-activated protein kinase
N-terminal	Amino terminal
PDK	3-Phosphoinositide-dependent protein kinase
PH	Pleckstrin homology
PTB	Phosphotyrosine binding domain
PI3K	Phosphoinositol 3 kinase
PIP3	Phosphoinositol triphosphate
PPP	Picropodophyllin
RTKs	Receptor tyrosine kinases
TK	Tyrosine kinase
TKI	Tyrosine kinase inhibitor
SAPK	Stress-activated protein kinase
SH2/3	Src homology 2/3 domain
Shc	SH2 domain-containing-protein
SOCS	Suppressor of cytokine signaling
Sos	Son of sevenless
SRF	Serum responsive factor
TKI	Tyrosine kinase inhibitor

Introduction

Transmission of extracellular signals across the plasma membrane by receptors is a fundamental, evolutionary conserved cellular process. These signals are in part generated by specialized plasma membrane receptors, grouped into different families based on their specific structural characteristics. Although different receptor families utilize common intracellular signaling-proteins and activate common signaling pathways, each cell surface receptor family leads to specific biological outcomes in the cell [1].

The receptor tyrosine kinases (RTKs) represent one major cell surface receptor family, containing around 60 members, subdivided into at least 13 receptor families [2, 3]. The RTKs are defined by the presence of an intracellular tyrosine kinase domain and typically a large glycosylated extracellular ligand binding domain, separated by a single transmembrane pass [4]. Traditionally defined by their ligands and hence ligand binding domains, the cytoplasmic kinase regions, juxtamembrane domain and carboxyl (C)-terminal tail also differ significantly among the individual receptors.

The canonical, binary model still in use today, describes the RTKs as having an OFF/ON mechanism. The agonist stabilizes the “ON” state through autophosphorylation of tyrosine residues within the kinase domain, followed by exponential increase in its kinase activity and subsequent activation of the intra-cellular signaling pathways [1]. In most cases, the ligand-induced activation of the kinase domain is mediated by receptor oligomerization (for reviews, see [4–6]). This event favors interactions between

cytoplasmic kinase-partners and induces kinase transactivation. Dimerization can take place between two identical receptors (homodimerization), between different members of the same receptor family, or, in some cases, between a receptor and an accessory protein (heterodimerization) [6–9]. How ligands bind to receptors and induce oligomerization seems specific for each class of RTK [7, 10].

Over the last decades, RTKs have received particular attention, not only as essential regulators of normal cellular processes but also as key factors involved in the development and progression of human cancers. From the first discovery of a mutated RTK in cancer in 1984 [11], a huge amount of information about aberrant RTK signaling in cancer has built up, leading to incontestable recognition of various forms of RTK hyper-activation in cancer: gene amplification, overexpression, mutation, or autocrine growth factor loops that are responsible for the cancer-promoting potential of RTKs [12].

Among RTKs, the insulin-like growth factor type-1 receptor (IGF-1R) is one of the most important players in cancer development. The fundamental evidence for this is the demonstration that IGF-1R knock-out mouse embryonic cells are refractory to transformation by several oncogenes, viruses, or over-expression of other RTKs [13]. Subsequently, IGF-1R and its natural ligands were demonstrated to regulate multiple cellular functions essential for the malignant phenotype including cellular proliferation, survival, anchorage-independent growth, tumor neovascularization, migration, invasion, and metastasis [14–17]. Confirming this critical role, in preclinical settings, a large amount of experimental data clearly demonstrates that inhibition of IGF-1R would be beneficial for cancer treatment [18–24]. In vivo and in vitro studies using IGF-1R antibodies, small molecule inhibitors, and antisense technology have shown that IGF-1R is functionally essential for tumor cell growth and proliferation in most if not all forms of cancer [23, 25–28]. On the other hand, in clinical settings, no clear mechanism of aberrant IGF-1R could be recognized: IGF-1 or IGF-1R over-expression is not a general rule [17, 29], the receptor does not show intrinsic receptor abnormalities [30], and therefore other regulatory pathways and quantitative changes are likely to be involved and have to be considered.

This review will follow the development of our understanding of IGF-1R signaling and relate it to the models considered when different IGF-1R-targeting strategies are designed, in particular with regards to cancer therapy.

Something old: classical signaling pathways through IGF-1R phosphorylation

The IGF family includes ligands, receptors, and IGF-binding-proteins (IGFBPs). Classically, there are three ligands

Table 1 IGF-1R structure–function relationship

Residues	Binding partners	Functions
V922		M [267]
Y943		I [50]
Y950	IRS-1/2/3/4 [268–271], Shc [268, 269, 272], CrkII, CrkL [82, 273, 274]	I [49, 50], M, T [89, 275–278], pY
Y957		I [50]
Kinase domain 969–1236	RACK1 [87], Vav [80], Vav3 [81], p125FAK [83]	
976–981		ATP binding site, A [89]
K1003		A, M, T, K [89, 279]
K1025, K1100, K1120		Su [151]
K1081		[280]
G1125		K [281]
K1138, K1141		Ub [141]
Y1131, Y1135, Y1136		Auto-phosphorylation, M, T, K [89, 234, 276, 282, 283], pY
W1173		A, T [284]
Y1221		K [276]
1229–1245	Grb10 [78]	
S1248	RACK1 [88], β arr1 [124, 134]	pS
Y1250		T [128], M/I, A [89, 276, 285]
Y1251		I [286], K [276], A, T [89]
S1252		pS, I [280]
S1272	14.3.3 [287]	
S1280–S1283	14.3.3 [268, 288, 289]	T [290, 291]
S1291	β arr1 [124, 134]	pS
H1293–K1294		A [89, 292]
F1310		A [277]
Y1316	Grb10 [79], p85 [293], SHP2 [84, 145], PI3K [63]	[89, 292]
Undetermined	p38, JNK [294], TIMP2 [295], SOCS1/2 [86], SOCS3 [296], p55 γ [297], JAK1/2 [298]	

The key residues for posttranslational modifications, protein–protein interaction and their functional outcome as determined by mutation-analysis are summarized

K kinase activity, *I* internalisation, *M* mitogenic, *T* transforming, *A* anti-apoptotic, *M/I* migration/invasion, *Su* Sumoylated residue, *Ub* ubiquitinated residue, *pY* phosphotyrosine, *pS* phosphoserine

cellular domain (196 aa), the transmembrane domain (aa 906–929), and the intracellular portion of the β -subunit, which itself is subdivided into three domains: a juxtamembrane domain, the tyrosine kinase (TK) domain, and C-terminal domain/tail. The juxtamembrane domain contains an NPXY motif, which may be important for receptor internalization [47–50]. The catalytic region of IGF-1R contains the ATP binding motif (GXGXXG) at positions 976–981, and a catalytic lysine in position 1003, which is critical for the Mg-ATP binding [51]. Within the TK domain, a cluster of three tyrosines, located at positions 1131, 1135, and 1136, is critical for receptor autophosphorylation [41]. The C-terminus of the IGF-1R (roughly the last 100 amino acids) contains several regulatory elements essential for IGF-1R function [52] (Table 1; Fig. 1).

The TK domain is highly homologous to that of the IR (84 %), the juxtamembrane domain shares 61 % of homology with the IR, whereas the C-terminal domain shares

only 44 % [40]. Despite this high degree of homology, it is largely accepted that the two receptors have distinct biological roles. The IR is known to be a key regulator of physiological processes such as glucose transport and biosynthesis of glycogen and fat [53], whereas the IGF-1R is a potent regulator of cell growth, proliferation, and differentiation [54, 55]. The structure–function relationship of the IGF-1R has been extensively investigated, with mutational analysis revealing residues crucial for the binding of signaling or adaptor proteins (Table 1; Fig. 1) or particular downstream bioactivities (Table 1).

IGF-1R tyrosine kinase activation

According to the classical model, IGF-1/2 binding induces a conformational change in the preformed dimeric receptor, leading to activation of the RTK [6]. In the unphosphorylated state, the receptor catalytic activity is very low

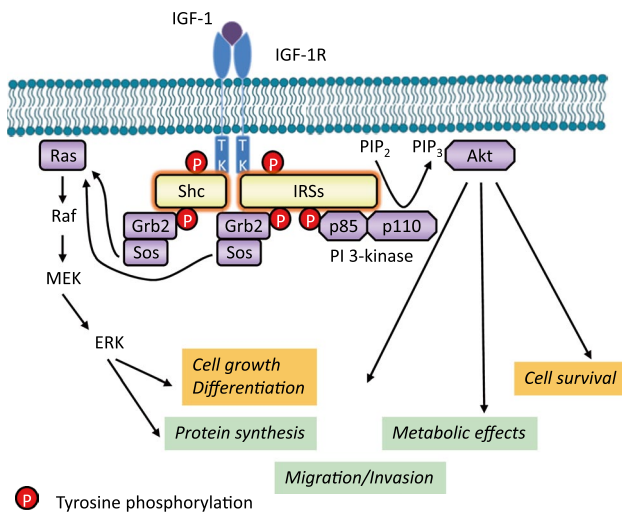


Fig. 2 IGF-1R kinase-dependent signaling pathways. IGF-1 (or IGF-2) binding to the IGF-1R promotes intrinsic tyrosine kinase activity and auto-phosphorylation. Activated receptor can recruit and phosphorylate substrates such as IRS and Shc. Tyrosine phosphorylation of IRS and Shc proteins leads to binding of signaling molecules such as Grb2 and PI 3-kinase. These associations induce downstream signaling activation, primarily through the MAPK and PI3K pathways coordinating downstream IGFs bioactivities

due to the inhibitory conformation of a specific domain in the kinase region, which interferes with ATP-binding and tyrosine phosphorylation. This domain, known as the activation loop (A-loop), behaves as a pseudosubstrate that blocks the active site [56]. The A-loop contains the critical tyrosine (Tyr) residues 1131, 1135, and 1136 and the activation of intrinsic protein kinase activity results in the autophosphorylation of them [56] (Fig. 1). Tyr 1135 (being the first tyrosine to be phosphorylated) in the A-loop is bound in *cis* position in the active site, thus preventing substrate access while simultaneously occluding the ATP binding site. The kinase activity is at a low basal level, but sufficient to induce trans-autophosphorylation, once stimulated. After ligand binding, the three tyrosines of the A-loop are *trans*-phosphorylated by the dimeric subunit partner. Phosphorylation of Tyr 1135 and Tyr 1131 destabilizes the auto-inhibitory conformation of the A-loop, whereas phosphorylation of Tyr 1136 and, to a lesser extent, Tyr 1135 stabilizes the catalytically optimized conformation of it [56]. Autophosphorylation of Tyr 1135 is necessary but not sufficient to destabilize the autoinhibitory A-loop conformation; full destabilization also requires autophosphorylation of Tyr 1131. Autophosphorylation also occurs outside the kinase domain and creates docking sites for downstream signal transduction molecules. According to the classical model, the two main downstream signaling pathways activated by IGF-1R kinase are the MAPK and PI3K pathways (Fig. 2).

Intracellular substrates of the IGF-1R tyrosine kinase

The docking sites outside the kinase domain bind signal transduction molecules and adaptor proteins to activate downstream signaling pathways. One important docking site of the IGF-1R to a substrate is the NPXY950 motif in the juxtamembrane domain. This motif is recognized by the phosphotyrosine binding (PTB) domain that the insulin receptor substrates (IRSs) and the src homology 2 (SH2)-containing-protein (Shc) possess (Figs. 1, 2). Extensive mutation analysis exposed the specific residues involved in binding these substrates as indicated in Fig. 1 and Table 1.

After phosphorylation of the tyrosine residues of the β -subunit of the IGF-1R, the first molecule that reaches full binding in 1–2 min is the IRSs. The IRS protein family consists of four proteins from IRS1 to IRS4. Among them, IRS1 and IRS2 are well known to play important roles in mediating metabolic effects of the IGFs as well as their cell growth and differentiation activities. Each IRS possesses high homology regions: a pleckstrin homology domain (PH) and a PTB domain at the N-terminal region. These regions are thought to be important for interaction with cell surface receptors. The C-terminal region of IRS proteins is poorly conserved, suggesting that this region mediates the different biological activities of each IRS. It also has a C-terminal domain with multiple potential phosphorylation sites (more than 20 in IRS1/2/4) that interact with high affinity to SH2 domain-containing-proteins in a manner dependent on the specific phosphotyrosine motif (YXXX) involved [57]. The SH2 domain-containing-proteins include a p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) class I, growth-factor-receptor-bound protein 2 (Grb2), SH-PTP2 (a tyrosine phosphatase), and other adaptor proteins like Crk and Nck. IRS1, as a docking-protein, has been involved in interactions with multiple molecules secondary to IGF-1R activation, and among them β 1 integrins seem to have an important role in cell adhesion to laminin after IGF-1 stimulation [58]. In addition, a recent study described that IRSs form high-molecular-mass complexes with a variety of proteins in a phospho-tyrosine-independent manner and modulate their availability to the IGF-1R [59, 60].

The second major pathway is through Shc, which reaches its maximal phosphorylation within 5–10 min of IGF-1R stimulation. Shc has been shown to consist of four unique members, ShcA, B, C, and D, and multiple splicing isoforms [61]. In general, Shc proteins possess a PTB domain at the N-terminal region and a SH2 domain at the C-terminal region. Between the PTB domain and SH2 domain, there are three tyrosine residues, possibly phosphorylated by IGF-1R, involved in Grb2 recruitment.

PI 3-kinase pathway

One of the downstream pathways activated by the IGF-1R involves IRS interaction with a p85 regulatory subunit of PI3K class I, leading to activation of the catalytic subunit p110 of PI3K and inducing phospholipid products activating the downstream signaling pathway [62].

It has also been shown that tyrosine phosphorylation of the IGF-1R (Y1316XXM) can also induce direct binding of PI3K to the cytoplasmic region of the receptor [63] (Figs. 1, 2).

One of the main functions of PI3K is to synthesize the second messenger phosphatidyl inositol (3, 4, 5) triphosphate (PIP3) at the inner side of the membrane. These phospholipids function as ligands for recruiting PH domain-containing-proteins to the inner surface of the cell membrane [39]. The Akt/PKB serine threonine kinase interacts with these phospholipids causing its translocation to the inner membrane and activation by the 3-phosphoinositide-dependent protein kinases (PDKs) located around the membrane. IGFs-mediated activation of the PI3K pathway induces phosphorylation of the Thr308 and Ser473 residues on Akt and activates this kinase [64, 65]. Active Akt in turn phosphorylates and inhibits several pro-apoptotic proteins such as Bad [66] and caspase 9 [67], plus at least three other Akt effectors: the survival transcription factor cyclic AMP response element binding-protein (CREB), the pro-apoptotic effector protein glycogen synthase kinase-3 β (GSK-3 β), and the winged-helix family of forkhead transcription factors FKHRL1, FKHR, and AFX. Akt activation can also induce stimulation of mTOR that will lead to phosphorylation of the 40S ribosomal S6 protein by the p70S6 kinase, thereby enabling efficient translation of 5' terminal oligopyrimidine tract (5'TOP) mRNA [68]. This class of mRNA is critically involved in the control of the protein synthesis machinery regulating the transition from G0 to G1 of the cell cycle. Activated mTOR can also induce phosphorylation of eukaryotic initiation factor 4E (eIF-4E) binding-protein (4E-BP), thus regulating cell cycle proteins like cyclin D1 [69]. mTOR is also involved in activation of matrix metallo proteinase (MMP)2 with effects on cell migration and metastasis potential [70]. Another effect of Akt activation is phosphorylation of Mdm2 on serine 166 and serine 186. Phosphorylation on these sites is necessary for translocation of Mdm2 from the cytoplasm into the nucleus, where Mdm2 decreases p53 transcriptional activity and hence diminishes cellular levels of p53 [71].

MAP kinase pathway

The second major pathway is mediated by tyrosine-phosphorylated IRSs or Shc through another

SH2-domain-containing signaling molecule, Grb2 (Figs. 1, 2). Grb2 is an adapter protein which possesses one SH2 and two SH3 domains. Grb2 can be activated by binding directly to phosphorylated IRS or Shc via its SH2 domain, while the SH3 domains interact with son of sevenless (Sos), a guanine nucleotide exchange protein. Sos stimulates the release of GDP and subsequent binding of GTP to the membrane-bound, low-molecular-mass GTP-binding-protein Ras. GTP-bound Ras interacts with and translocates the serine/threonine protein kinase Raf to the plasma membrane, where Raf becomes activated. Activated Raf phosphorylates and activates the dual specificity mitogen-activated protein kinase (MAPK) MEK, which in turn stimulates the ERK subset of MAPKs: extracellular-related kinase (ERK)1 and ERK2. ERK1/2 are translocated into the nucleus where they are involved in spindle formation. ERK also mediates transcriptional induction of the cyclin D1 gene, stimulating phosphorylation of the pRb protein and release of the E2F-1 transcription factor [72]. The free E2F-1 can activate in its turn the transcription of p14^{ARF} [73]. ARF was shown to inhibit the p53-Mdm2 association that maintains p53 in its inactive form [74]. In its turn, p53 can directly interfere with the Ras/MAPK cascade by inactivating ERK2/MAPK via caspase-mediated cleavage [75]. One of the other substrates of MAPK is p90Rsk. Both MAPK and p90Rsk translocate to the nucleus after phosphorylation, where they phosphorylate and activate transcription factors such as serum-responsive factor (SRF), T cell-specific transcription factor, and CREB, and, thus, alter gene expression. In the cytoplasm, MAPK regulates microtubule dynamics by phosphorylating microtubule-associated proteins. By regulating microtubule networks, MAPK regulates the CyclinB/Cdc2 complex: Cyclin B phosphorylation by MAPK is important for the translocation of the complex to the nucleus where it is activated by CDC 25C [76, 77].

Other downstream signaling pathways

Previous studies to identify potential substrates of the IGF-1R and/or interactions with the receptor after its activation have revealed several additional possible downstream signaling molecules. One of these, Grb10, is a member of a super family of adaptor proteins, sharing a common overall structure, including an N-terminal region harboring a conserved proline-rich motif, a central PH domain, and a C-terminal SH2 domain. Ligand activation of the IGF-1R involves the Grb10 adaptor protein, which probably binds to autophosphorylated tyrosine residues located between amino acids 1229 and 1245 of IGF-1R [78] or tyrosine 1316 [79]. Activated Grb10 interacts with neuronal precursor cell-expressed developmentally downregulated 4

(Nedd4), and by doing this it has an important role in ubiquitination of the IGF-1R. Other substrates of the IGF-1R tyrosine kinase include the guanine nucleotide exchange factors Vav [80] and Vav3 [81], the adapters CrkII and CrkL [82], and focal adhesion kinase (FAK) [83]. Aside from IRS proteins, IGF-1R has been reported to interact *in vitro* and/or *in vivo* with numerous molecules, including Syp [39], GTPase-activating-protein [84], C-terminal Src kinase [85], and suppressor of cytokine signaling (SOCS)2 [86]. The IGF-1R can also activate the stress-activated protein kinases (SAPKs) including p38 and Jun N terminal kinase (JNK), a pathway associated with regulation of DNA damage responses and cell survival. It has been shown that RACK1, a small molecule that was identified as an IGF-1R interacting protein using a yeast two-hybrid interaction trap, regulates IGF-1R signaling and interaction of the IGF-1R with integrin signaling [87–89]. However, information regarding the roles of many of these molecules in IGF-1R signaling and biological functions is currently limited.

The classical IGF-1R signaling cascades are more complex, and they have been described in great detail in some dedicated reviews [1, 23, 33, 37, 41, 90–94].

Something new: posttranslational modification of IGF-1R controlling IGF-1R expression and function

Receptor internalization and signal attenuation

Owing to the presence of an intracellular tyrosine kinase domain, IGF-1R is classified as an RTK, and accordingly phosphorylation was considered to be the central process governing IGF-1R signaling [94, 95]. However, during the last decade, several laboratories have been investigating the mechanisms controlling the subsequent receptor downregulation and signaling desensitization. In this context, other posttranslational modifications including dephosphorylation, serine phosphorylation, ubiquitination, and sumoylation are increasingly recognized as modulating receptor levels and function.

Many cell surface receptors undergo endocytosis, being incorporated into clathrin- [96] or caveolin-coated vesicles. Some receptors are internalized constitutively and recycled (e.g., the transferrin receptor); however, with most RTKs and G-protein-coupled receptors (GPCRs), internalization is triggered by ligand binding [97, 98]. Ligand-activated receptors are normally downregulated by internalization [99–101], allowing the cells to return to an unstimulated, basal state. This internalization is considered to occur only for phosphorylated receptors [99–101], and is thus ligand-dependent.

Clathrin- or caveolin-mediated internalization then follows a process through early endosomes to late endosomes,

during which decisions regarding fate of the proteins are made: whether to recycle or degrade. Early endosomes are mildly acidic, allowing detachment of the ligand and hence attenuation of the signal [102, 103]. If the receptor is to be degraded, it will progress to the highly acidic, hydrolase-containing lysosomal compartments. Internalization of a receptor does not necessarily mean immediate cessation of the signal followed by receptor degradation. This ligand-mediated endocytosis appears to have a double function: although subsequently attenuating the signal from activated receptors, it is also facilitating the interaction between the internalized receptor and the downstream signaling molecules [103]. For example, signaling through the EGFR is maintained in endosomal compartments and determines the longevity of the signaling [104, 105].

The ubiquitin pathway is a regulatory system for endocytosis [99–101, 106], and RTKs are well recognized as targets of ubiquitination [107]. Ubiquitination is the covalent attachment of the 7-kDa ubiquitin polypeptide to lysine residues on target proteins through the sequential actions of E1, E2, and E3 ligase enzymes [108, 109]. E1 and E2 work to load the E3 ligase with the ubiquitin, while E3 transfers the ubiquitin to the target protein. The E3 provides the specificity for the substrate, binding directly or through adaptor proteins. Ubiquitination can either be mono- (single ubiquitin moiety) or poly- (where chains of ubiquitin are added). Proteins can be multi-ubiquitinated (at multiple lysine residues) and polyubiquitination can be straight or branched type, depending on which of the lysine residues within ubiquitin the subsequent ubiquitin is attached [101, 110–112]. The type of ubiquitination is increasingly being recognized as determining the fate of the substrate protein [113]. Old or damaged cytosolic proteins are labeled with a polyubiquitin chain, which is recognized by the proteasome, multi-subunit proteolytic enzymes situated in the cytoplasm.

In addition to the degradation of cytosolic proteins, ubiquitin is also implicated in the internalization and degradation of plasma membrane proteins. In mammalian cells, a number of membrane proteins which are ubiquitinated are degraded through both the proteasome and lysosomal pathways [100], including the IGF-1R [114–117] and other RTKs [118, 119]. In some cases (e.g., the RTK Met), cytoplasmic fragments are cleaved from the receptor and degraded by the proteasome, in a complementary mechanism to lysosomal degradation [119].

IGF-1R internalization, recycling, and degradation

The IGF-1R has been shown to be ubiquitinated [116, 117, 120] and internalized, through both clathrin and caveolin routes, in a ligand-dependent manner [116, 117, 120, 121]. Ubiquitination of the IGF-1R occurs prior to entry into

endocytotic vesicles [120]. IGF-1R internalization reaches an apparent plateau after 20 min at 37 °C in cultured hippocampal neurons, while half-maximal IGF-1R internalization was obtained after 5 min at 37 °C in NIH-3T3 fibroblasts [122]. After internalization, some receptors are sorted for recycling to the cell surface. In activated T lymphocytes, internalization of the IGF-1R from the cell membrane was accompanied by a reduction in its mRNA. This was followed by re-expression of IGF-1R on the cell surface and an increase in IGF-1R mRNA levels in the cytoplasm, reaching levels higher than those initially recorded. However, a slower increase in the mRNA levels indicated that the earlier recovery of IGF-1R results from receptor recycling, followed by de novo synthesis [123]. There are certainly internal routes where the IGF-1R is recycled back to the cell surface and where this balance between recycling and degradation can be manipulated [124]. After internalization, IGF-1R degradation is mediated by both the proteasome and lysosomal pathways or recycled to the plasma membrane [100, 114, 116, 117, 120], although the relative roles of each are not clear.

A key question is: what is the downregulation signal for IGF-1R? To answer this question, at least two models have emerged during the last decade: one postulating internalization by a tyrosine-based motif and another by an ubiquitin-based motif. However, these two are not mutually exclusive, with phospho-tyrosine motifs potentially modulating ubiquitination and hence mediating receptor internalization.

Tyrosine motif-based downregulation model

The ligand-activated receptors are targeted to clathrin-coated membrane invaginations [125], a process mediated by a specific internalization signal situated within cytoplasmic domain of the receptor [100]. There is evidence that internalization signals have a tyrosine-based motif usually located within the juxtamembrane region of the receptor [126, 127]. The human IGF-1R contains three tyrosine residues in the juxtamembrane region [50] that may be involved in internalization. However, contradictory results have been reported regarding the role of these tyrosine-based motifs as internalization signals. Prager et al. [50] demonstrated that the NPXY motif in IGF-1R is important for receptor internalization, whereas Miura et al. [128] demonstrated that tyrosine 1250 within the IGF-1R tail is the functional tyrosine-based internalization signal (Fig. 1).

Ubiquitin motif-based downregulation model

Degradation of the IGF-1R by a proteasome mediated route was originally described by Sepp-Lorenzino et al. [114] to explain the mechanism of Herbyomicin A-induced IGF-1R

downregulation. Herbyomicin A was demonstrated to only degrade IGF-1R in the presence of functional ubiquitin E1 ligase activity, and its action was prevented by proteasome inhibitors while being insensitive to lysosomal inhibitors. Since then, the IGF-1R has been demonstrated to be a substrate of three E3 ubiquitin ligases: Mdm2 [116], Nedd4 [120], and c-Cbl [117].

After Grb10 recognition as an IGF-1R interacting partner and negative regulator of IGF-1 signaling [78, 129], a yeast two-hybrid screen subsequently identified Grb10 as a binding partner of the Nedd4 E3 ligase [130]. Grb10 overexpression increased ligand-dependent IGF-1R ubiquitination, receptor internalization, and degradation. This ubiquitination did not occur with a catalytically inactive Nedd4 or with mutant Grb10 unable to bind Nedd4 [120]. This work identified Nedd4 as an ubiquitin E3 ligase and Grb10 as the key adaptor protein to recruit Nedd4 to the IGF-1R. Further work from the Morriane laboratory described Nedd4 ubiquitination of the IGF-1R as predominantly of monoubiquitination type [131]. In addition, the internalization mediated by Nedd4 was both clathrin- and caveolin-dependent as demonstrated by co-localization studies [131].

Following original research demonstrating a feedback mechanism of wild-type p53 negatively regulating IGF-1R at the transcriptional level [132], it was revealed that overexpressed mutant or wild-type p53 mitigate ligand-induced IGF-1R downregulation [29]. Analysis of IGF-1R mRNA levels discard an exclusively transcriptional mechanism, indicating a posttranslational p53-IGF-1R control mechanism [29]. Further work demonstrated that an inhibition of p53 caused ubiquitination and degradation of the IGF-1R, suggesting that p53 and IGF-1R might compete for the same ubiquitin ligase [116]. This possibility was validated in experiments in which inhibition of Mdm2 (the well-known p53 ubiquitin ligase) resulted in accumulation of IGF-1R [116]. This work confirmed the direct IGF-1R/Mdm2 interaction, identified Mdm2 as an ubiquitin ligase for the IGF-1R, promoting proteasome-inhibitor-sensitive IGF-1R degradation, and highlighted a positive posttranslational control mechanism between p53 and IGF-1R [116]. Subsequent research revealed the mechanism of Mdm2 binding to the IGF-1R by identifying that β -arrestins, otherwise known as master regulators of GPCR biology, serve as adaptors to bring the E3 ligase Mdm2 to the IGF-1R [133]. Both Mdm2 and β -arrestin co-immunoprecipitated with the IGF-1R and in an *in vitro* reaction β -arrestins enhanced Mdm2-mediated IGF-1R ubiquitination [133]. In a cell system, overexpression or depletion of β -arrestin 1 enhanced and decreased IGF-1R ubiquitination and degradation, respectively. Thus, β -arrestin 1 was proved to act as an essential component in the ubiquitination and downregulation of the IGF-1R [133]. Most

recently, β -arrestin 1 was recognized not only as an aid to IGF-1R internalization and signal cessation but to initiate its own second wave of signaling through the MAPK/ERK pathway [134], with IGF-1R stimulation also leading to ubiquitination of β arrestin 1 [134]. Intriguingly, this β -arrestin 1-mediated ERK activation occurs even when the classical IGF-1R kinase signaling is impaired (see below) [134]. Taken together, these studies demonstrated that β -arrestin 1 serves as an adaptor to bring the E3 ubiquitin-ligase Mdm2 to the IGF-1R, with a dual outcome on the IGF-1R: ubiquitination and receptor downregulation as well as IGF-1R/ β -arrestin 1-mediated activation of MAPK signaling.

The identification of c-Cbl as an ubiquitin-ligase for IGF-1R [117] was based on the observation that suppression of Mdm2 did not completely abolish ligand-induced IGF-1R ubiquitination, suggesting that there are other ligases contributing to this process. Previously recognized as an E3 ligase of other RTKs, including EGFR and PDGFR [135], c-Cbl was demonstrated to associate with and to ubiquitinate the IGF-1R [117], especially at higher doses of IGF-1, indicating complementary roles for the different E3 ligases [117]. In vitro experiments using ubiquitin with mutated lysine residues suggested that Mdm2 polyubiquitinates IGF-1R with K63 type chains, whereas c-Cbl polyubiquitinates IGF-1R with K48 type chains. Co-localization between caveolin and an early endosome marker showed that higher doses of IGF-1 increased caveolin-mediated endocytosis, whereas low doses did not. In agreement with IGF-1 concentration determining ligase and endocytotic processes, overexpression of Mdm2 could reduce caveolin-mediated endocytosis.

The E3 ligases Mdm2 and Nedd4 have been demonstrated to bind to the IGF-1R through the adaptor proteins β -arrestin 1 [133] and Grb10 [131], respectively, suggesting that adaptor proteins determine substrate specificity. For Nedd4, there is evidence of the ligase determining appropriate localization of the receptor [136], and that it directs towards a proteasome-independent pathway of degradation [137]. The ligases c-Cbl and Mdm2 appear to have complementary roles, with c-Cbl recruited following high dose IGF-1 and initiating caveolin-dependent endocytosis and Mdm2 recruited at low dose IGF-1 and initiating clathrin-dependent endocytosis [117]. It is clear, however, that different ligases associated with the IGF-1R as well as their adaptor proteins have redundant as well as complementary roles: they keep the balance between recycling, relocalization, and proteasome/lysosomal degradation of the receptor [117, 120, 121, 131, 133, 134]. The complexity is further increased by the fact that receptor signaling relies on its localization: signaling still occurs from endosomes and prevention of endocytosis is even inhibitory to signaling [84, 124, 134, 138].

Through IGF-1R mutation analysis, the interplay between phosphorylation and ubiquitination has been demonstrated [121]. According to this study, a completely kinase-dead IGF-1R (with mutation of the ATP pocket) cannot be ubiquitinated after ligand stimulation, while a kinase impaired IGF-1R (Y1136A) is ubiquitinated, activates ERK signaling, but fails to phosphorylate Akt. Deletion of the C-terminal tail (Δ 1245) had no effect on IGF-1R phosphorylation but completely abolished its ubiquitination [121], confirming the previous findings that IGF-1R ubiquitination is essentially dependent on its β -arrestin binding domain [133, 134].

Although initially described as a modification initiated by the natural ligand binding to the receptor, recent studies suggest that IGF-1R ubiquitination is a more complex process that can also be activated kinase-dependent (activation-loop phosphorylation) by IGF-2 [139], insulin [139], LL-37 [140], or kinase-independent by anti-IGF-1R antibodies [140–142]. In addition, IGF-1R ubiquitination can be activated in a ligand and kinase-independent manner, from inside the cell, by adaptor proteins recruited to the intracellular domain of the IGF-1R [124, 143, 144]. The mechanism of IGF-1R ubiquitination is different depending on the ligand, adaptor protein, or ubiquitin-ligase employed; however, the common theme is that a specific receptor conformation may trigger ubiquitination with divergent effects on receptor signaling, trafficking, and biological outcomes. It should be noted here that such conformation-activating ubiquitination is not essentially dependent on ligand–receptor interaction or activation-loop phosphorylation.

IGF-1R dephosphorylation

Direct IGF-1R dephosphorylation has been little investigated. Rocchi et al. [145] described the association of activated IGF-1R with the phosphatase SHP2, with binding between phospho-tyrosines on IGF-1R and SH2 domains in SHP2 being critical. Subsequently, it was demonstrated that SHPS2 is critical for recruitment of SHP2 to the plasma membrane for this purpose [146]. Cross-talk with the α V β 3 integrin, through SHP2, was later described, with blockage of this integrin reducing IGF-1-induced IGF-1R phosphorylation [146, 147]. The phosphatase PTP1B also negatively regulates the IGF-1R [148], associating with it in a ligand-dependent manner [149].

Nuclear IGF-1R

Recently, there has been greater appreciation of plasma membrane receptor relocalization to the nucleus. Nuclear localization has been demonstrated for the IGF-1R and shown to be mediated by sumoylation, the addition of a small protein similar to ubiquitin [150, 151]. The direction

to the nuclear compartment and the role of nuclear IGF-1R is currently little investigated, but studies suggesting that IGF-1R nuclear localization can alter transcription [152], and that nuclear localization of IGF-1R predicts better outcome in patients treated with IGF-1R antibody [153], suggest that further research would have therapeutic relevance. One study has demonstrated that transfer to the nucleus is mediated at least in part by clathrin, and confirmed that both the α - and β -chains of IGF-1R translocate to the nucleus [154], suggesting a more complex function to IGF-1R internalization than solely degradation versus recycling. The emerging role of nuclear IGF-1R has recently been thoroughly reviewed by Sarfstein and Werner [155].

Something borrowed: IGF-1R transactivation by other plasma-membrane receptors

It is generally accepted that several posttranslational modifications control the IGF-1R levels at the cell surface, with direct impact on receptor signaling. Are these modifications, in combination with the “two-states” model, sufficient to explain the differential signaling efficacy of the IGF-1R? Described by terms such as “cross-talk” or “transactivation”, it is increasingly recognised that IGF-1R signaling and efficacy is modified by interactions with other plasma-membrane molecules. This group include other RTKs, integrins, and GPCRs. Among the RTKs, probably the best studied is the IGF-1R interaction with the IR, resulting in formation of hybrid receptors [187, 188] which are able to respond to insulin, IGF-1, and IGF-2 but with different affinities. The mechanism of hybrid receptor activation, signaling, as well as their biological effects, have been extensively investigated [37, 139, 156–158]; however, it should be highlighted here that simple interaction of the IGF-1R with IR modified IGF-1R signaling and trafficking to various ligands [139] and anti-IGF-1R antibodies [156]. The IR is not the only RTK associated with the IGF-1R; for example, a direct interaction between the IGF-1R and EGFR was identified in cancer cells, with EGFR depletion affecting IGF-1R ubiquitination, degradation, and signaling [159]. In a similar manner, another group of plasma-membrane molecules were demonstrated to modify the IGF-1R response to IGF-1. Integrins, as transmembrane receptors, transfer information from the extracellular matrix (ECM) to signaling pathways inside cells (outside–in signaling) or from within the cell (inside–out signaling) [160]. They form part of cell surface signaling complexes known as focal adhesions which link the cytoskeleton to the ECM and are intimately linked with cell adhesion and migration [160]. There has been increasing recognition of the bi-directional cross-talk between integrins and the IGF-1R signaling pathway [161]: integrin activation modulating the

IGF-1R signaling and vice versa. Studies identifying the overlap between IGF-1R and integrin signaling pathways from the Clemmons group demonstrated IGF-1R-dependent “transactivation” of $\alpha\beta3$ integrin [162]. The mechanism was described as due to the recruitment and phosphorylation/dephosphorylation of adaptor proteins. IGF-1 stimulation causes phosphorylation of the transmembrane adaptor protein SHPS1 and subsequent SHP2 recruitment [146]. Along with SHP2, IGF-1R through IRS1 recruits the focal contact adaptor protein paxillin and FAK, which are involved in the turnover of focal contacts. IGF-1 stimulation leads to SHP2 dephosphorylation of paxillin and FAK, as part of an integrin deactivation mechanism crucial for migration [163]. In further evidence, the DOK1 protein, itself an IRS protein, brings SHP2 to the $\alpha\beta5$ integrin [164]. IGF-1 is well known to initiate cell migration, IGF-1 triggers integrin activation and binding to ECM [165], and evidence showed that $\alpha\beta3$ and $\alpha\beta5$ are involved in the migratory/invasive IGF-1 response [165, 166].

However, there is also increasing recognition that the activation state of integrins alters IGF-1R signaling. The SHP2 protein is not only recruited to integrins and dephosphorylates paxillin and FAK but regulates IGF-1R dephosphorylation, curtailing the IGF-1R signaling response [146], identifying it as a common molecule between the integrin and IGF-1R signaling pathways. The Clemmons group also describe integrin modulation of the SHPS1-IGF-1R association, with activation and tyrosine phosphorylation of $\alpha\beta3$ integrin determining the recruitment to the IGF-1R of SHPS1 and subsequently SHP2, and involving IAP [167, 168]. Overall, ligand occupancy of at least some integrins is required for a sustained IGF-1 response [169], and transmembrane integrin complexes including SHPS1 and IAP modulate IGF-1R signaling through determining SHP2 recruitment (reviewed in [170]), with subsequent effects on cell behavior [171].

The O’Connor group described a further common node between integrins and IGF-1R. The RACK1 scaffolding-protein, known to interact with $\beta1$ integrin, was identified as also binding IGF-1R affecting Shc/Grb2 downstream signaling [87, 172]. Further, the O’Conner group demonstrated that RACK1 associated mutually exclusively with phosphatase PP2A or $\beta1$ integrin, controlled by IGF-1 stimulation [173]. However, this complex differed between transformed and non-transformed cells, with only transformed cells showing direct RACK1–IGF-1R interaction [174].

Recent work by the Takada group posited an extracellular determinant of cross-talk between the two pathways, in agreement with the co-localization of IGF-1R and integrins together at focal adhesion complexes. They demonstrated direct binding of IGF-1 ligand to $\alpha\beta3$ integrin and $\alpha\beta4$ integrin and direct effects on IGF-1R signaling [175],

including sustaining of cells during anchorage independence [176] [177]. In their further work, an IGF-1 mutant unable to bind integrins but able to bind IGF-1R had a dominant negative effect on IGF-1R-mediated tumorigenesis in vivo [178], suggesting further functional effects of such ternary complexes.

Together, these data show that integrin activation is able to modify the phosphorylation and signaling from the IGF-1R and subsequently the biological outcomes. As mentioned before, in addition to RTKs and integrins, members of the GPCR family, can modify the IGF-1R responsiveness to different ligands.

Something borrowed: IGF-1R utilize components of GPCR signaling

It is generally accepted that RTKs share signaling pathways with the larger class of the GPCRs [3, 179]. Over the last decades, at least two mechanisms of receptor cross-talk between RTKs and GPCRs have been described: receptor transactivation and RTK signaling through GPCR components.

RTK transactivation is typically explained as the GPCR-dependent increase in phosphorylation, kinase activity, and signaling of the RTK. One example is represented by the lysophosphatidic acid (LPA) transactivation of the EGFR [180]: LPA, a classical GPCR agonist, triggers EGFR autophosphorylation and MAPK signaling activation, effects that are sensitive to EGFR-kinase inhibitors or expression of EGFR kinase-defective mutants [180]. Although not completely understood, the mechanism of LPA-induced transactivation of EGF receptors is likely to involve the release of an EGFR ligand by a GPCR-activated metalloproteinase. Several other RTKs, including the ones for PDGF, VEGF, and NGF, were reported to be transactivated by GPCR agonists such as LPA, angiotensin, endothelin, and bradykinin (for review, see [179]). It is noteworthy that RTK transactivation by GPCRs required tyrosine phosphorylation and kinase activity of the growth factor receptor and is sensitive to inhibitors of receptor kinases [179, 181]. The IGF-1R is not an exception from this rule: transactivation and phosphorylation of the IGF-1R with subsequent MAPK activation was reported following thrombin stimulation of aortic smooth muscle cells [182]. Several other GPCR-agonists were described as IGF-1R transactivators, including neurotensin [183] and vasopressin [184].

The second mechanism used to explain RTK/GPCR cross-talk is the employment of the GPCR signaling components, G-proteins and β -arrestins, by the RTKs for their signaling activation. Characteristic of this type of signaling is the response to G-protein signaling inhibitors (e.g., pertussis toxin which uncouples $G_{\alpha i}$ from an activated

receptor), G-protein sequestration [185], or β -arrestin downregulation [144, 186]. Most if not all RTKs, including PDGFR, EGFR, and VEGFR, utilize G-proteins as signaling mediators (for in depth reviews, see [3, 179, 181, 187]). In this context, it is worth mentioning that the members of the IR family were the first described to engage the G-proteins signaling: the IR signaling was demonstrated to be sensitive to pertussis toxin treatment [186], with subsequent decrease of the insulin-induced inhibition of adenylyl cyclase in isolated hepatocytes [188]. Several other studies have demonstrated the $G_{\alpha i}$ involvement in IR signaling and their effects on insulin signaling biological outcomes [186, 189]. Given the high structural similarities between IR and IGF-1R, it is not surprising that some G-proteins have also been identified to physically associate and mediate IGF-1R signaling. Almost 20 years ago, a study from Robert Lefkowitz's laboratory reported that IGF-1R-dependent activation of the MAPK signaling pathway was inhibited by the $G_{\alpha i}$ -inhibitor pertussis toxin or by sequestration of G-protein $\beta\gamma$ subunits by a peptide derived from GRK2 [185]. This study clearly demonstrated that IGF-1R signaling depends on heterotrimeric G-proteins containing the $G_{\alpha i}$ and $G\beta\gamma$ subunits. Consistent with these observations, subsequent studies demonstrated that, in rat neuronal cells or mouse fibroblasts, $G_{\alpha i}$ and $G\beta$ subunits were associated with IGF-1R while $G_{\alpha s}$ was not associated with the IGF-1R in any cell type. More importantly, this study demonstrated the IGF-1 induced release of the $G\beta$ subunits from the IGF-1R with no effect on IGF-1R/ $G_{\alpha i}$ association, indicating a discrete pool of $G\beta\gamma$ subunits available for IGF-1R downstream signaling [190]. The IGF-1R/ $G_{\alpha i}$ association was also reported in a separate study investigating the roles of heterotrimeric G-protein signaling components in insulin and IGF-1 signaling. In 3T3L1 adipocytes, in basal state, $G_{\alpha i}$ and $G\beta$ were associated with the IGF-1R, while IGF-1 stimulation increased the IGF-1R/ $G_{\alpha i}$ association, releasing the $G\beta$ subunits [144].

While RTK transactivation by GPCRs has long been recognized and is today considered a "classical" signaling pathway, the complexity of the RTK/GPCR cross-talk was further demonstrated by recent findings of bidirectional cross-communication between RTKs and GPCRs (for in depth reviews, see [181, 187]). In these processes, the GPCR signaling is activated by RTKs by the same two mechanisms described above: production of a GPCR ligand stimulated by RTK activation or a ligand-independent cross-activation of the signaling network [181, 187]. In the case of the IGF-1R, such GPCR-transactivation has been demonstrated to be essential for migratory and pro-survival functions controlled by IGF-1 [181, 187]. Taken together, these studies indicate a common signaling platform between IGF-1R and GPCRs to differentiate between

the responses upon combined growth factor/GPCR agonist stimulation from single stimulation by either ligand.

The corollary of these studies is that IGF-1R (and IR) can activate signaling as a GPCR, using different G-protein partners for downstream signaling. The $G\alpha_i$ subunit is constitutively associated with the IGF-1R while IGF-1 treatment leads to GTP loading of $G\alpha_i2$ [144] and $G\beta\gamma$ dissociation. Yet, these findings raise another critical question: if IGF-1R and IR can activate G-protein signaling, what is the system desensitizing this pathway? In the case of GPCRs, the signaling is terminated by β -arrestin recruitment to the activated receptor. Early studies demonstrated the involvement of β -arrestin in IGF-1R signaling and trafficking: a dominant negative mutant of β -arrestin 1 was demonstrated to impair IGF-1R internalization, whereas overexpression of wild-type β -arrestin 1 or β -arrestin 2 increases IGF-1R internalization [191]. Moreover, G-protein signaling activated by IGF-1R was demonstrated to be sensitive to β -arrestin inhibition [144].

The discovery of the dual regulatory role of β -arrestin 1 in the case of IGF-1R, downregulation [133], and signaling activation [134], pointed towards a remarkable parallel with the role of β -arrestins in the case of the larger GPCR family. While internalizing the GPCR and ending G-protein signaling, β -arrestins activate the MAPK pathway [192–194].

Recognized as a universal mechanism of GPCR regulation, β -arrestin 1 binds to the receptor and desensitizes G-protein signaling only after phosphorylation of specific serine residues by the G-protein-coupled receptor kinases (GRKs) [193, 195, 196]. Therefore, a legitimate question was whether the same mechanism is in place for the IGF-1R.

Investigating this scenario, we found that activated IGF-1R allows recruitment of the GRK proteins, specifically with contrasting effects between GRK2 and GRK6 [124]. Subsequent GRK2- or GRK6-dependent phosphorylation of IGF-1R C-terminal serine residues 1248 or 1291, respectively, allows β -arrestin 1 recruitment, with the residue that is phosphorylated controlling the duration and strength of the β -arrestin/IGF-1R association.

Identification of GRK-dependent phosphorylation of IGF-1R serine residues as the underlying mechanism for β -arrestin/IGF-1R interaction not only revealed another connection to the complex cross-talk between the IGF-1R and GPCR but in fact provided the missing link to functionally identify IGF-1R as a GPCR. This paradigm shift is founded on the accepted universal model of GPCR activation and desensitization delineated by six distinct processes [195, 197], all of which have been identified to occur for the IGF-1R (Fig. 3): (1) ligand binding to the IGF-1R, in addition to the classical kinase signaling cascade triggers signaling through heterotrimeric G-proteins [144, 185],

(2) subsequent GRK2- or GRK6-dependent phosphorylation of IGF-1R C-terminal serine residues 1248 or 1291, allowing β -arrestin binding to these specific phosphorylated serine residues [124], with (3) β -arrestin recruitment [124, 133, 134], (4) subsequent kinase/G-protein signaling desensitization, (5) activation of a β -arrestin-dependent second signaling wave through MAPK [124, 134], and (6) receptor endocytosis with the GRK isoform determining receptor degradation [124, 133] or recycling [124]. In this model, one uncertainty is whether, for the IGF-1R, GRK-mediated β -arrestin binding initiates a desensitization process (Fig. 3, IV) and whether the desensitization process affects both G-protein and kinase signaling. While for GPCRs the key role of GRKs in desensitization is well recognized, for RTKs few studies have investigated the contribution of GRKs [3]. In all published studies, GRK2 is reported to desensitize or modulate RTK signaling. For instance, GRK2 is recruited and co-localizes with the ligand-activated EGFR or PDGFR, leading to receptor serine phosphorylation and increased ERK activation in the case of EGFR [198], or inhibiting the kinase activity in the case of PDGFR [199]. The insulin receptor (IR), closely related to the IGF-1R, is a special case: GRK2 was shown to have inhibitory effects on IR-mediated signaling and glucose uptake, though the observed effects were demonstrated to be mediated in a kinase-independent manner through GRK2 sequestration of $G\alpha_q/11$ [200].

For GPCRs, the desensitization requires the membrane localization of GRKs. In a palmitoylated state, GRK6 has been found to be exclusively associated with the membrane; however, it is not yet clear whether this reversible posttranslational modification is induced by activated receptor [3]. On the other hand, a clear regulatory negative feedback, induced by activated receptor, was demonstrated for GRK2: $G\beta\gamma$ subunits, generated by the agonist-occupied receptor, interact with GRK2 and serve to target this enzyme into proximity with its membrane receptor substrate [201]. Consequently, β -arrestin recruitment to GRK-phosphorylated receptors physically prevents the coupling of receptor to its cognate G-protein. Thus, a true comparison of IGF-1R desensitization with GPCR desensitization would make sense only if IGF-1R utilizes heterotrimeric G-proteins for its signaling. Our results showing that GRK2 is involved in limiting the ERK response to IGF-1 fully support the GRK2 desensitization function in the case of IGF-1R. For GRK6, our interpretation is that prolonged ERK activation is due to the second wave of signaling activated by the stable β -arrestin recruitment to the IGF-1R (Fig. 3, V), masking the desensitization of the first signaling wave. Taken together, the proposed model represents IGF-1R as not only “borrowing” components of GPCR signaling but to operate as a functional hybrid RTK/GPCR (Fig. 3).

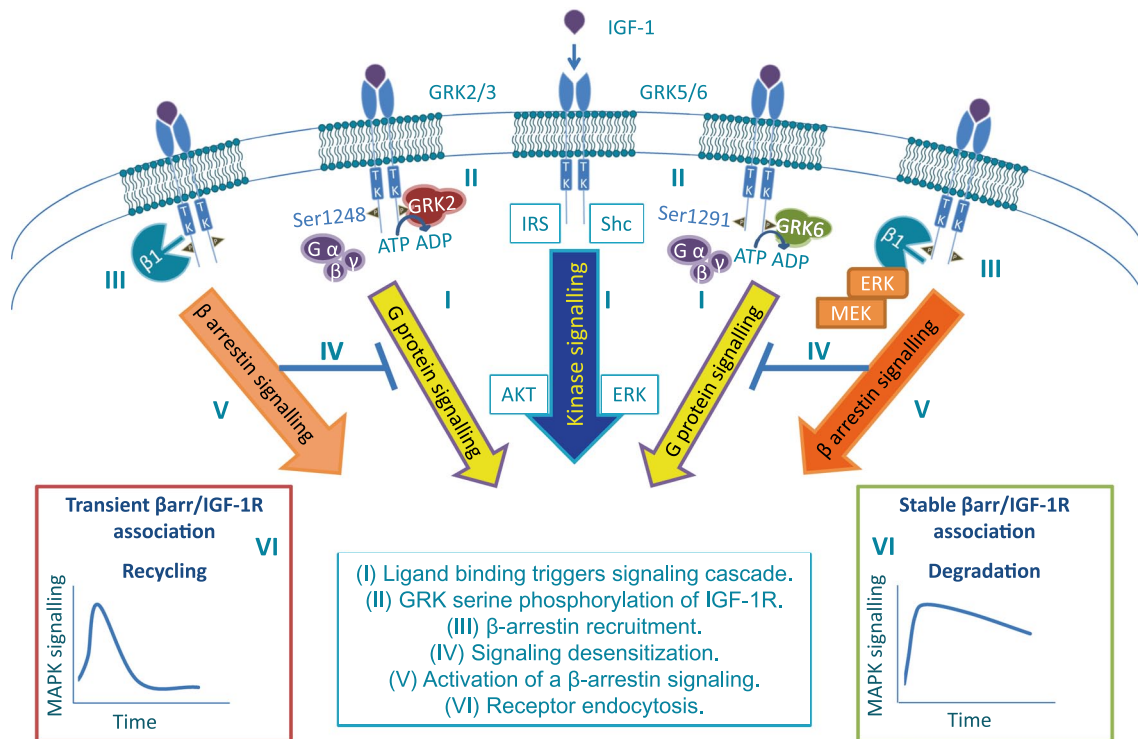


Fig. 3 IGF-1R as a RTK/GPCR functional hybrid. Signaling: agonist-stimulation of the IGF-1R triggers the classical RTK signaling leading to downstream kinase-cascade signaling activation. In addition, agonist-stimulation of IGF-1R leads to non-canonical GPCR signaling through heterotrimeric G-proteins ($G\alpha, \beta, \gamma$) (I), following which the receptors are rapidly phosphorylated by G-protein-coupled receptor kinases (GRKs) at serine residues within the C-terminus (II). Desensitization: Serine-phosphorylated receptors present high affinity binding sites to recruit the multifunctional adaptor protein β -arrestin 1 ($\beta 1$) (III). Steric binding by β -arrestin to the IGF-1R C-terminal prevents further G-protein coupling, leading to the desensitization of G-protein-dependent signaling (IV). β -arrestin signaling: β -arrestin acquires an active conformation upon binding the IGF-1R and scaffolds components of the MAPK pathways leading to the activation of a second wave of IGF-1R kinase independent signaling through

β -arrestin 1 (V). The dynamics of this signaling activation is determined by the strength of the β -arrestin 1/IGF-1R interaction, dependent on GRK-isoform (VI) Such β -arrestin-dependent MAPK activity has been shown to regulate multiple IGF-1R biological effects including proliferation, apoptosis, cell migration, and cancer metastasis. Endocytosis: agonist-stimulation promotes rapid endocytosis of the IGF-1R. This internalization is enabled by β -arrestin binding, connecting the receptor to the endocytic machinery efficiently. The interaction between β -arrestin and the E3 ubiquitin ligase Mdm2 promotes IGF-1R and β -arrestin ubiquitination that facilitates IGF-1R endocytosis, followed by post-endocytic sorting of internalized IGF-1R. The strength of the β -arrestin 1/IGF-1R interaction GRKs-isoform-dependent determines the fate of internalized receptor: recycling (transient β -arrestin 1 binding) or degradation (sustained β -arrestin 1 binding) (VI)

The emerging paradigm for IGF-1R signaling

A major limitation of the two-state model for IGF-1R signaling is the explanation of the two main components of the IGF-1R activity: the signaling activation and receptor downregulation as separate events. In this paradigm, all receptors are equal and the receptor activity is exclusively and directly related to kinase activation by ligand–receptor interaction. Signaling, and downregulation triggered simultaneously by the ligand-activated receptor. Both involve phosphorylation, ubiquitination, and probably other post-translational modifications. The logical conclusion is that receptor signaling and degradation never dissociate from each other or from ligand-induced receptor (kinase) activation. Moreover, in the same cellular context, the MAPK/ERK and PI3K/Akt pathways are described as being

activated in a balanced manner following IGF-1R stimulation. Yet, there is experimental data that do not support this model, including some major contradictions (Table 2) such as kinase-independent signaling activation [124, 134, 202] or unbalanced signaling in the same cellular background [34, 124, 134], signaling–degradation dissociation, or receptor downregulation in the absence of the ligand or activation-loop phosphorylation [37, 124, 203]. Beyond this, there is the major paradox of the IGF-1R inhibitors (antibodies and small molecules inhibitors, see below), which are able to activate IGF-1R downregulation and signaling despite clear inhibitory effects on receptor tyrosine phosphorylation.

The appreciation of the dual functions of β -arrestin, as a mediator of IGF-1R signaling [124, 134, 191, 204] as well as mediator of receptor downregulation [124, 133], provide

Table 2 The new paradigm for IGF-1R signaling

Classical paradigm and its contradictions	Emerging paradigm
Receptor activity is exclusively and directly related to kinase activation by ligand–receptor interaction Contradiction: kinase-independent signaling activation: IGF-1R can trigger MAPK signaling in the absence of the ligand or without kinase domain activation [124, 134, 202]	In addition to classical RTK signaling, IGF-1R operates as a functional GPCR In this model, IGF-1R can initiate kinase-independent signaling such as β -arrestin signaling and heterotrimeric G-protein signaling
Signaling and receptor downregulation are triggered simultaneously by the ligand-activated receptor in a balanced manner Contradictions: signaling–downregulation dissociation-specific point-mutations outside the kinase domain can activate signaling or receptor downregulation in the absence of the ligand or activation-loop phosphorylation [124]	The receptor conformation activating the kinase signaling can be distinct from that which interacts with β -arrestins facilitating receptor ubiquitination and endocytosis in the absence of kinase activity This model can explain the dissociation between kinase activation and receptor degradation as well as ligand- or kinase-independent signaling or receptor downregulation triggered by kinase inhibitors (e.g., targeting antibodies) or IGF-1R partners
IGF-1R downregulation induced by ligands other than IGF-1 (e.g., antibodies)	
Unbalanced signaling downregulation induced by IGF-1R transactivation (e.g., hybrid receptors, integrins, other GPCR or RTKs)	
Equal receptors with balanced activation of the downstream signaling pathways (MAPK/ERK and PI3K/Akt pathways) Contradictions: unbalanced signaling: in the same cellular background, IGF-1R can preferentially activate either the MAPK or PI3K/AKT pathways [34, 124, 134]	The receptor conformation activating the kinase signaling can be distinct from that which interacts with β -arrestins or with other partners In this model, not all receptors are equal and their activity can be modulated from inside the cell by particular posttranslational modifications (e.g., serine phosphorylation, ubiquitination, etc.) or by interacting proteins (e.g., β -arrestins, IR, integrins, etc.)
The paradox of the IGF-1R inhibitors (antibodies and small molecules inhibitors) which are able to activate IGF-1R signaling despite clear inhibitory effects on receptor tyrosine phosphorylation	The same model would also accommodate the unbalanced IGF-1R signaling, activated in a “biased manner” via β -arrestin by IGF-1R inhibitors as well as by natural “biased” agonists

The contradictory evidence against the two-state model is described and how the new paradigm can explain these data

the basis for the emerging paradigm of IGF-1R signaling. In this model, IGF-1R can initiate classical kinase signaling, β -arrestin signaling, and heterotrimeric G-protein signaling as well as β -arrestin-mediated receptor desensitization (Fig. 4). However, the receptor conformation activating the kinase signaling can be distinct from that which interacts with β -arrestins, as demonstrated by the IGF-1R mutants constitutively binding β -arrestin, that are degraded even in the absence of the ligand [124] (Fig. 4). This scenario can explain the dissociation between kinase activation and receptor degradation as well as kinase-independent signaling. The same model would also accommodate the unbalanced IGF-1R signaling, activated in a “biased manner” via β -arrestin by IGF-1R inhibitors as well as by natural “biased” agonists [22, 143]. In this emerging model, not all receptors are equal and their activity can be modulated from inside the cell by particular posttranslational modifications (e.g., serine phosphorylation, ubiquitination, etc.) or by interacting proteins (e.g., β -arrestins, integrins, other RTKs).

Implications for treatment

Owing to its essential role in maintaining the malignant phenotype, IGF-1R-targeted therapy was considered a very promising strategy for cancer treatment [205–214].

Although several approaches targeting the IGF-1R were employed to prove the concept, the common aim for all these strategies is the inhibition of the classical kinase signaling cascade (for reviews, see [26, 27, 91, 215]). This can be accomplished either by preventing ligand–receptor interaction (e.g., using blocking antibodies) or silencing the effects of this interaction (e.g., tyrosine kinase inhibitors, TKIs).

Strategies aimed at blocking the ligand–receptor interaction

Receptor downregulation was among the first approaches used to prevent ligand–receptor interaction and to validate IGF-1R as a therapeutic target [216]. This was achieved by antisense oligonucleotides, plasmids expressing IGF-1R antisense cDNA or triple helix-forming oligodeoxynucleotides [23]. The same outcome was obtained with drugs interfering with IGF-1R glycosylation, folding and expression at the cell surface (tunicamycin or lovastatin) [17–19, 217] or by directly increasing receptor degradation [114, 218]. The majority of these systems were unsuitable for transfer to the clinic, either because they were unable to be delivered or because they were too unspecific, so the next work in this area focused on clinically suitable methods for blocking the ligand–receptor interaction.

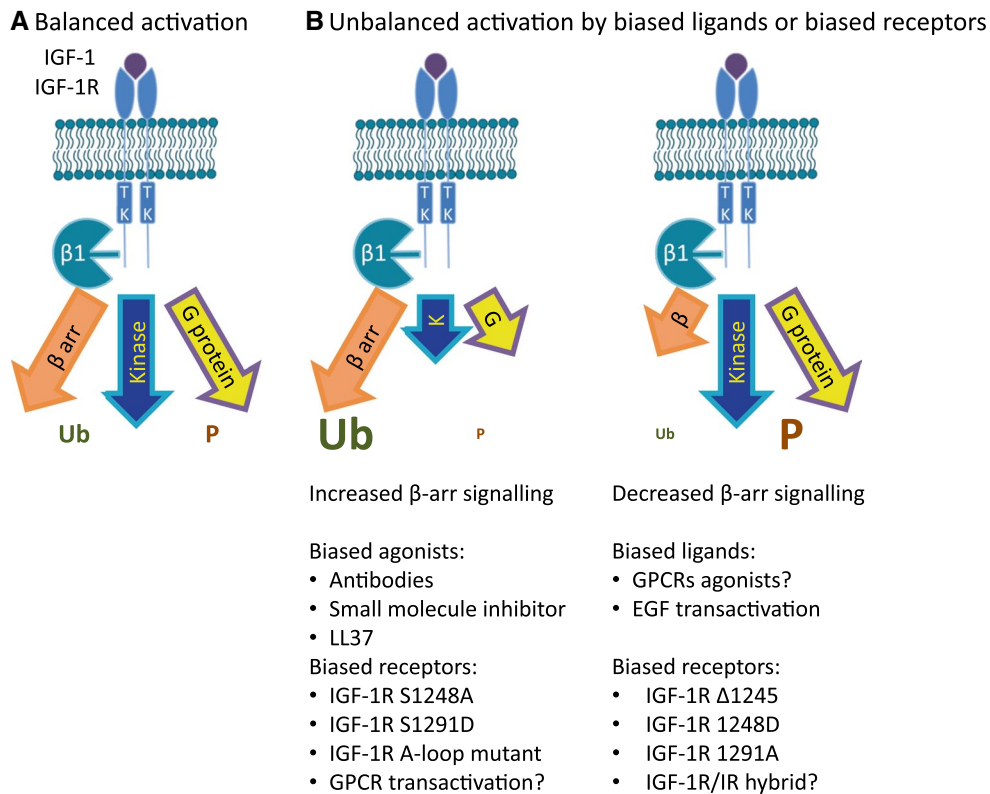


Fig. 4 New and old paradigms of IGF-1R signaling: balanced and biased signaling at the IGF-1R. **a** Classical model: IGF-1R activation is triggered exclusively by ligand-binding and signaling is mediated by kinase cascade through phosphorylation. Ligand-activated IGF-1R leads to balanced phosphorylation-dependent Akt/ERK signaling and balanced signaling/downregulation. **b** New paradigm. In the current model for IGF-1R activation, in addition to the classical RTK-cascade, binding of a ligand results in activation of signaling by G-proteins and β-arrestins, as well as desensitization and internalization by β-arrestins. In a system with balanced signaling, ligand-binding results in balanced activation of kinase signaling, signaling

by G-proteins and β-arrestins, as well as desensitization and internalization by β-arrestin. In a system with biased signaling, two alternative outcomes of receptor activation are depicted: kinase/G-protein-biased with enhanced IGF-1R phosphorylation and β-arrestin-biased with enhanced IGF-1R ubiquitination. Biased signaling is a feature of the ligand–receptor complex so either the receptor or the ligand could be biased. An IGF-1R biased ligand elicits one response over another compared with the classical ligand, (e.g., anti-IGF-1R favors β-arrestin signaling). A biased IGF-1R is only efficient at activating a restricted subset of downstream signaling pathways (e.g., mutant receptors)

Competition with the ligand–receptor interaction

In this category are included strategies that involve upregulation of the IGFFBPs, the natural IGF inhibitors [219], IGFs peptide analogues [219, 220], or ligand or receptor neutralizing antibodies [206, 221]. There are seven different IGFFBPs, most of them have been shown to inhibit the actions of IGFs. In vitro and in vivo experiments indicate that increasing IGFFBPs could be an alternative to IGF-1R targeting, particularly due to the lack of interference with insulin signaling [222]. There is also evidence that free IGFFBPs have anti-tumor activity independent of their IGF-binding capacity [223]. Anti-ligand antibodies, developed to mimic IGFBP action, have a high affinity against both IGF-1 and IGF-2, and do not cross-react with insulin [224–226]. Several antibodies targeting IGF-1 or IGF-2 have been reported, with one reaching clinical trial [225].

They prevent signaling through the IGF-1R and the hybrid receptors, but, importantly, they do not affect insulin-stimulated phosphorylation of the insulin receptor (IR) and its downstream signaling. While most of the ligand-targeting antibodies bind to both IGFs, a few have higher affinity for IGF-2 [224, 226, 227]. Notably, antibody sequestration of ligand can lead to high levels of free IGFFBPs, a consequence that might improve their intended effect [93].

Antibodies against the extracellular ligand binding domain of the IGF-1R were designed to block ligand–receptor interaction [27, 93]. This strategy was considered to be very promising, and therefore most of the large pharmaceutical companies developed anti-IGF-1R antibodies and those demonstrating significant activity in preclinical settings were taken forward for clinical evaluation [27, 93]. However, in clinical settings, treatment with anti-IGF-1R antibodies induced clinical responses only in some cases

of Ewing's sarcoma (ES) [228–230] and selected cases of lung carcinoma [93, 231, 232]. Although not chosen by design, all anti-IGF-1R antibodies used in clinical trials, besides inhibiting IGF-1-induced receptor phosphorylation, also trigger receptor downregulation ([140] and below).

Strategies aiming to inhibit IGF-1R signaling

The proof of mechanism for this approach is represented by IGF-1R kinase-dominant negative mutants, successfully tested in preclinical settings by several laboratories [211, 233]. Cells transfected to express IGF-1R cDNA with kinase-inactivating [234] or kinase-limiting mutations [235, 236], lose their malignant phenotype as well as their invasive and metastatic potential [237]. The promising strategy of inhibiting IGF-1R kinase activity is complicated by IR cross-reactivity issues, as the kinase domain of the IGF-1R shares 85 % homology with that of the IR, with the ATP binding cleft 100 % conserved [40]. Nevertheless, several small inhibitors have been developed (for review [238, 239]) and despite some of them having shown signs of cross-reactivity with the IR, they are still considered for use in clinical settings. Additionally, there is recent evidence and debate in the literature as to whether co-inhibition of the IR is beneficial for anti-IGF-1R therapy [240, 241]. While all small molecules targeting IGF-1R prevent kinase activation, they can be further divided in two subgroups, based on their mechanism of action: inhibitors of the ATP binding cleft or inhibitors of the kinase–substrate interaction, including inhibitors of the A-loop. Among the first category, NVP-AEW541 and NVP-ADW74 inhibit IGF-1R kinase activity and appear to be equipotent for both IGF-1R and IR inhibition *in vitro*, yet they show selectivity for the IGF-1R in intact cells. The two drugs were tested in various models such as fibrosarcoma, myeloma, and ES [242, 243]. Another small molecule, OSI-906 is a dual IGF-1R/IR kinase inhibitor with a strong anti-tumoral efficiency in an IGF-1R-driven xenograft model [244]. There have been several such inhibitors tested in clinical trials [27], and what they have in common is that inhibition of the ATP-binding also prevents IGF-1R downregulation. This is not surprising as *in vitro* experiments clearly demonstrated that IGF-1R ATP-defective mutants are not ubiquitinated [121].

Among the second category of the IGF-1R kinase inhibitors (TKIs), the cyclolignan picropodophyllin (PPP) demonstrates a special feature for this class. PPP was originally described to inhibit the IGF-1R without altering the IR, reducing Akt phosphorylation [20] and inducing tumor regression in xenografted mice. Rather than competing with ATP, PPP differs from other IGF-1R TKIs by interfering with phosphorylation of the kinase domain A-loop. PPP specifically blocked phosphorylation of the tyrosine (Y) 1136 residue, while sparing the two others (Y1131 and

Y1135), suggesting that it might act as a substrate inhibitor [245]. Since its discovery [20], PPP has been proven to inhibit IGF-1R biological activities in a very large number of experimental models as well as in clinical settings [14, 16, 24, 90, 246–258], has been demonstrated to produce very limited resistance [22, 259, 260] to anti-IGF-1R therapy, and has proven efficacy in clinical trials [261]. The remarkable feature of PPP that differentiates it from ATP-inhibitors is that, similar to anti-IGF-1R antibodies, PPP also triggers IGF-1R downregulation [143]. This feature deserves special attention because it supports the logical but still contentious [90] assumption that receptor downregulation is desirable in addition to inhibition of its TK activity [15, 95].

Yet, the debate on how important receptor downregulation is in relation to kinase inhibition is hiding a paradox: how is the receptor downregulated by inhibiting its kinase activity?

The classical, binary ON/OFF model for IGF-1R signaling and degradation implies that the only way to maintain the receptor in an 'OFF' state is by preventing kinase activity while degradation would only occur with an 'ON' state receptor. Evidently this model is insufficient, because it cannot account for the contradiction that anti-IGF-1R antibodies (or PPP) trigger receptor degradation in the absence of kinase activation. This contradiction was recently investigated for the case of anti-IGF-1R antibody (Figitumumab)-induced receptor degradation [140]. Although the antibodies were primarily designed to block the ligand–receptor interaction thus preventing kinase activity, it has been demonstrated that targeting antibodies act as “biased” IGF-1R agonists, activating β -arrestin recruitment to the receptor, with subsequent receptor ubiquitination, internalization, ERK signaling activation, and IGF-1R degradation [140]. In agreement with this, anti-IGF-1R therapy efficacy was proven to be highly dependent on β -arrestin 1 expression and modulated by co-targeting β -arrestin 1-mediated signaling [140]. Notably, a similar mechanism of β -arrestin 1-mediated receptor ubiquitination and downregulation with activation of the β -arrestin 1/ERK second signaling wave has also been demonstrated in the case of the PPP paradox [143].

The experimental data provided by these studies fully substantiate and support the new paradigm for IGF-1R signaling which allows for unbalanced signaling and kinase activation/receptor downregulation dichotomy (Fig. 4). According to this model, the conformation of IGF-1R, which activates the kinase cascade, can be distinct from that which interacts with β -arrestins or other signaling molecules. Receptor modification induced by ligands, small molecule inhibitors, or intra-cellular interacting proteins can further be interpreted as alterations to the IGF-1R conformation, there being more than just two

(ON/OFF) conformations. Different conformations can be mutually exclusive and can determine the quality, quantity, and duration of output signaling from the receptor as well as the fate of the receptor (Fig. 4). Recognizing the dual role of β -arrestin in controlling receptor downregulation as well as kinase-independent signaling, the new model we propose (Fig. 4) can accommodate all experimental data, demonstrating that IGF-1R signaling is not exclusively dependent on its kinase activity and can be activated and downregulated in a “biased” manner via β -arrestin 1 by IGF-1R inhibitors or by natural “biased” agonists [143, 253] (Table 2). In the emerging model of IGF-1R signaling, the question of “how important receptor is downregulation in relation to kinase inhibition?” is getting a new meaning: “Is it possible to downregulate the IGF-1R without activating signaling?” As downregulation without signaling has never been recognized, a simpler solution would be to identify the biased signaling pathways and target them separately [140].

Although oversimplified and insufficient to explain several outcomes of IGF-1R activation, the classical paradigm is currently in use when selecting the agents targeting the IGF-1R. The best example is the case of anti-IGF-1R antibody: during their development, antibodies were designed to achieve the maximum binding to IGF-1R, to compete with the natural agonists [140]. In the subsequent drug screening for clonal selection, the assays were limited to confirmation of their inhibitory effects on IGF-1-induced receptor signaling [142, 262–265]. As long as the canonical IGF-1R model does not acknowledge kinase-independent signaling, it is not surprising that targeting antibodies were rarely evaluated in IGF-1-independent conditions to estimate their intrinsic agonistic potential: most measuring receptor phosphorylation, a few checking IRS/Akt activation, and none checking MAPK/ERK activity.

Despite big expectations regarding the use of anti-IGF-1R for cancer treatment, almost all clinical trials were stopped due to futility, whereas some pharmaceutical companies closed their programs for developing IGF-1R inhibitors [93]. Furthermore, the value of IGF-1R as a target for cancer therapy has been questioned [90, 93]. While several reasons for the failure of the anti-IGF-1R have already been discussed in detail [90, 93], the new paradigm we propose for the IGF-1R signaling highlights another potential cause for these unsatisfactory results: biased-signaling activation by anti-IGF-1R antibodies. The best example is again Figitumumab, designed to prevent the IGF-1 binding to its receptor and kinase-dependent signaling. In Ewing sarcoma, at least two resistance mechanisms have been reported, developed after Figitumumab treatment: formation of the IGF-1R/IR hybrid receptors [266] and biased IGF-1R signaling activation [140]. As described before, the IGF-1R/IR hybrid formation could be appreciated as a form

of receptor bias. Challenging the classical two-state model in which the antibodies were designed, the new paradigm in which a biased-receptor (IGF-1R/IR hybrid) is activated by a biased-agonist (Figitumumab) provides an explanation as to why such a strategy did not work in clinical settings and advocates recognition of IGF-1R as a key target for cancer therapy and a clear choice for the baby rather than the bathwater [91].

Concluding remarks

Today, targeting the IGF-1R and components of its signaling pathway in different forms of cancer is a major research area. Yet, the design of such targeting agents is based mostly on the classical ON/OFF model of IGF-1R activation. The present review highlights the fact that, in addition to the classical kinase pathway, IGF-1R activity and its biological effects are controlled by a variety of adaptor/signaling-proteins through IGF-1R posttranslational modifications, including tyrosine and serine phosphorylation, de-phosphorylation, ubiquitination, and sumoylation. The complexity of IGF-1R behavior following exposure to IGF-1R inhibitors reinforces the need to understand the relationships between different signaling pathways and between signaling and degradation. Only this can lead to the rational design and testing of IGF-1R inhibitors and the successful combining of these therapeutics with others targeting parallel pathways. Searching for therapies inhibiting only IGF-1R kinase activity may appear to invalidate the IGF-1R as a target for cancer therapy, while many potential drugs that modify alternative downstream effects, the “biasing agonists”, are not considered.

Acknowledgments Research support was received from the Swedish Cancer Society, Swedish Research Council, The Swedish Childhood Cancer Foundation, Crown Princess Margareta's Foundation for the Visually Impaired, Welander Finsen Foundation, King Gustaf V Jubilee Foundation, Vinnova, Stockholm Cancer Society, Stockholm County, and Karolinska Institute. To S. -I. T., Grants-in-Aid for Scientific Research (#25221204), a Grant-in Aid for International Joint Research and Core-to-Core Program from the Japan Society for the Promotion of Science.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. *Cell* 141(7):1117–1134
2. Aaronson SA (1991) Growth factors and cancer. *Science* 254(5035):1146–1153

3. Hupfeld CJ, Olefsky JM (2007) Regulation of receptor tyrosine kinase signaling by GRKs and beta-arrestins. *Annu Rev Physiol* 69:561–577
4. Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. *Cell* 61(2):203–212
5. Weiss A, Schlessinger J (1998) Switching signals on or off by receptor dimerization. *Cell* 94(3):277–280
6. Heldin CH (1995) Dimerization of cell surface receptors in signal transduction. *Cell* 80(2):213–223
7. Heldin CH, Ostman A (1996) Ligand-induced dimerization of growth factor receptors: variations on the theme. *Cytokine Growth Factor Rev* 7(1):3–10
8. Lemmon MA, Schlessinger J (1994) Regulation of signal transduction and signal diversity by receptor oligomerization. *Trends Biochem Sci* 19(11):459–463
9. Carraway KL 3rd, Cantley LC (1994) A new acquaintance for erbB3 and erbB4: a role for receptor heterodimerization in growth signaling. *Cell* 78(1):5–8
10. Schlessinger J (1997) Direct binding and activation of receptor tyrosine kinases by collagen. *Cell* 91(7):869–872
11. Ullrich A et al (1984) Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 309(5967):418–425
12. Roberts PJ, Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* 26(22):3291–3310
13. Sell C et al (1994) Effect of a null mutation of the insulin-like growth factor I receptor gene on growth and transformation of mouse embryo fibroblasts. *Mol Cell Biol* 14(6):3604–3612
14. Girnita A et al (2006) The insulin-like growth factor-I receptor inhibitor picropodophyllin causes tumor regression and attenuates mechanisms involved in invasion of uveal melanoma cells. *Clin Cancer Res* 12(4):1383–1391
15. Baserga R, Peruzzi F, Reiss K (2003) The IGF-1 receptor in cancer biology. *Int J Cancer* 107(6):873–877
16. Economou MA et al (2008) Inhibition of VEGF secretion and experimental choroidal neovascularization by picropodophyllin (PPP), an inhibitor of the insulin-like growth factor-1 receptor. *Invest Ophthalmol Vis Sci* 49(6):2620–2626
17. All-Ericsson C et al (2002) Insulin-like growth factor-1 receptor in uveal melanoma: a predictor for metastatic disease and a potential therapeutic target. *Invest Ophthalmol Vis Sci* 43(1):1–8
18. Girnita L et al (2000) Inhibition of N-linked glycosylation down-regulates insulin-like growth factor-1 receptor at the cell surface and kills Ewing's sarcoma cells: therapeutic implications. *Anticancer Drug Des* 15(1):67–72
19. Wang M et al (1999) Regulatory role of mevalonate and N-linked glycosylation in proliferation and expression of the EWS/FLI-1 fusion protein in Ewing's sarcoma cells. *Exp Cell Res* 246(1):38–46
20. Girnita A et al (2004) Cyclooligans as inhibitors of the insulin-like growth factor-1 receptor and malignant cell growth. *Cancer Res* 64(1):236–242
21. Ulfarsson E et al (2005) Expression and growth dependency of the insulin-like growth factor I receptor in craniopharyngioma cells: a novel therapeutic approach. *Clin Cancer Res* 11(13):4674–4680
22. Vasilcanu D et al (2006) The insulin-like growth factor-1 receptor inhibitor PPP produces only very limited resistance in tumor cells exposed to long-term selection. *Oncogene* 25(22):3186–3195
23. Baserga R (2005) The insulin-like growth factor-I receptor as a target for cancer therapy. *Expert Opin Ther Targets* 9(4):753–768
24. Yin S et al (2010) Targeting the insulin-like growth factor-1 receptor by picropodophyllin as a treatment option for glioblastoma. *Neuro Oncol* 12(1):19–27
25. Furukawa J et al (2010) Antisense oligonucleotide targeting of insulin-like growth factor-1 receptor (IGF-1R) in prostate cancer. *Prostate* 70(2):206–218
26. Gualberto A, Pollak M (2009) Clinical development of inhibitors of the insulin-like growth factor receptor in oncology. *Curr Drug Targets* 10(10):923–936
27. Gualberto A, Pollak M (2009) Emerging role of insulin-like growth factor receptor inhibitors in oncology: early clinical trial results and future directions. *Oncogene* 28(34):3009–3021
28. Tornkvist M et al (2008) Differential roles of SS18-SSX fusion gene and insulin-like growth factor-1 receptor in synovial sarcoma cell growth. *Biochem Biophys Res Commun* 368(3):793–800
29. Girnita L et al (2000) Increased expression of insulin-like growth factor I receptor in malignant cells expressing aberrant p53: functional impact. *Cancer Res* 60(18):5278–5283
30. Beauchamp MC et al (2010) Targeting insulin and insulin-like growth factor pathways in epithelial ovarian cancer. *J Oncol* 2010:257058
31. Jones JJ, Clemmons DR (1995) Insulin-like growth factors and their binding-proteins: biological actions. *Endocr Rev* 16(1):3–34
32. Nakae J, Kido Y, Accili D (2001) Distinct and overlapping functions of insulin and IGF-I receptors. *Endocr Rev* 22(6):818–835
33. Adams TE et al (2000) Structure and function of the type I insulin-like growth factor receptor. *Cell Mol Life Sci* 57(7):1050–1093
34. Girnita A et al (2011) Identification of the cathelicidin peptide LL-37 as agonist for the type I insulin-like growth factor receptor. *Oncogene* 31(3):352–365
35. Petrenko AG et al (2013) Insulin receptor-related receptor as an extracellular pH sensor involved in the regulation of acid-base balance. *Biochim Biophys Acta* 1834(10):2170–2175
36. Baillyes EM et al (1997) Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. *Biochem J* 327(Pt 1):209–215
37. Belfiore A et al (2009) Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev* 30(6):586–623
38. Evdokimova V et al (2012) IGFBP7 binds to the IGF-1 receptor and blocks its activation by insulin-like growth factors. *Sci Signal* 5(255):ra92
39. Sepp-Lorenzino L (1998) Structure and function of the insulin-like growth factor I receptor. *Breast Cancer Res Treat* 47(3):235–253
40. Ullrich A et al (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J* 5(10):2503–2512
41. LeRoith D et al (1995) Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr Rev* 16(2):143–163
42. Andersen AS et al (1990) Changing the insulin receptor to possess insulin-like growth factor I ligand specificity. *Biochemistry* 29(32):7363–7366
43. Gustafson TA, Rutter WJ (1990) The cysteine-rich domains of the insulin and insulin-like growth factor I receptors are primary determinants of hormone binding specificity. Evidence from receptor chimeras. *J Biol Chem* 265(30):18663–18667
44. Kjeldsen T et al (1991) The ligand specificities of the insulin receptor and the insulin-like growth factor I receptor reside in different regions of a common binding site. *Proc Natl Acad Sci USA* 88(10):4404–4408

45. Schumacher R et al (1991) Insulin and insulin-like growth factor-I binding specificity is determined by distinct regions of their cognate receptors. *J Biol Chem* 266(29):19288–19295
46. Zhang B, Roth RA (1991) Binding properties of chimeric insulin receptors containing the cysteine-rich domain of either the insulin-like growth factor I receptor or the insulin receptor related receptor. *Biochemistry* 30(21):5113–5117
47. Brown MS, Goldstein JL (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* 232(4746):34–47
48. Backer JM et al (1990) Receptor-mediated internalization of insulin requires a 12-amino acid sequence in the juxtamembrane region of the insulin receptor beta-subunit. *J Biol Chem* 265(27):16450–16454
49. Hsu D et al (1994) NPXY motif in the insulin-like growth factor-I receptor is required for efficient ligand-mediated receptor internalization and biological signaling. *Endocrinology* 134(2):744–750
50. Prager D et al (1994) Human insulin-like growth factor I receptor internalization. Role of the juxtamembrane domain. *J Biol Chem* 269(16):11934–11937
51. Hanks SK, Quinn AM, Hunter T (1988) The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241(4861):42–52
52. O'Connor R, Fennelly C, Krause D (2000) Regulation of survival signals from the insulin-like growth factor-I receptor. *Biochem Soc Trans* 28(2):47–51
53. Patti ME, Kahn CR (1998) The insulin receptor—a critical link in glucose homeostasis and insulin action. *J Basic Clin Physiol Pharmacol* 9(2–4):89–109
54. Blakesley VA et al (1996) Signaling via the insulin-like growth factor-I receptor: does it differ from insulin receptor signaling? *Cytokine Growth Factor Rev* 7(2):153–159
55. Lammers R et al (1989) Differential signaling potential of insulin- and IGF-1-receptor cytoplasmic domains. *EMBO J* 8(5):1369–1375
56. Favelyukis S et al (2001) Structure and autoregulation of the insulin-like growth factor I receptor kinase. *Nat Struct Biol* 8(12):1058–1063
57. Wang LM et al (1993) IRS-1: essential for insulin- and IL-4-stimulated mitogenesis in hematopoietic cells. *Science* 261(5128):1591–1594
58. Goel HL et al (2004) Selective modulation of type I insulin-like growth factor receptor signaling and functions by beta1 integrins. *J Cell Biol* 166(3):407–418
59. Fukushima T et al (2011) Insulin receptor substrates form high-molecular-mass complexes that modulate their availability to insulin/insulin-like growth factor-I receptor tyrosine kinases. *Biochem Biophys Res Commun* 404(3):767–773
60. Yoshihara H et al (2012) Insulin/insulin-like growth factor (IGF) stimulation abrogates an association between a deubiquitinating enzyme USP7 and insulin receptor substrates (IRSs) followed by proteasomal degradation of IRSs. *Biochem Biophys Res Commun* 423(1):122–127
61. Wills MK, Jones N (2012) Teaching an old dogma new tricks: twenty years of Shc adaptor signaling. *Biochem J* 447(1):1–16
62. Werner H, Le Roith D (2000) New concepts in regulation and function of the insulin-like growth factors: implications for understanding normal growth and neoplasia. *Cell Mol Life Sci* 57(6):932–942
63. Fukushima T et al (2012) Phosphatidylinositol 3-kinase (PI3K) activity bound to insulin-like growth factor-I (IGF-I) receptor, which is continuously sustained by IGF-I stimulation, is required for IGF-I-induced cell proliferation. *J Biol Chem* 287(35):29713–29721
64. Alessi DR et al (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 15(23):6541–6551
65. Balendran A et al (1999) Evidence that 3-phosphoinositide-dependent protein kinase-1 mediates phosphorylation of p70 S6 kinase in vivo at Thr-412 as well as Thr-252. *J Biol Chem* 274(52):37400–37406
66. del Peso L et al (1997) Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science* 278(5338):687–689
67. Cardone MH et al (1998) Regulation of cell death protease caspase-9 by phosphorylation. *Science* 282(5392):1318–1321
68. Dupont J et al (2003) The insulin-like growth factor axis in cell cycle progression. *Horm Metab Res* 35(11–12):740–750
69. Blume-Jensen P, Hunter T (2001) Oncogenic kinase signaling. *Nature* 411(6835):355–365
70. Zhang D et al (2004) Dual regulation of MMP-2 expression by the type 1 insulin-like growth factor receptor: the phosphatidylinositol 3-kinase/Akt and Raf/ERK pathways transmit opposing signals. *J Biol Chem* 279(19):19683–19690
71. Mayo LD, Donner DB (2001) A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci USA* 98(20):11598–11603
72. Peeper DS et al (1997) Ras signaling linked to the cell-cycle machinery by the retinoblastoma protein. *Nature* 386(6621):177–181
73. Bates S et al (1998) p14ARF links the tumour suppressors RB and p53. *Nature* 395(6698):124–125
74. Sherr CJ, Weber JD (2000) The ARF/p53 pathway. *Curr Opin Genet Dev* 10(1):94–99
75. Marchetti A et al (2004) p53 can inhibit cell proliferation through caspase-mediated cleavage of ERK2/MAPK. *Cell Death Differ* 11(6):596–607
76. Walsh S, Margolis SS, Kornbluth S (2003) Phosphorylation of the cyclin b1 cytoplasmic retention sequence by mitogen-activated protein kinase and Plx. *Mol Cancer Res* 1(4):280–289
77. Strausfeld U et al (1991) Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein. *Nature* 351(6323):242–245
78. Morrione A et al (1996) Grb10: a new substrate of the insulin-like growth factor I receptor. *Cancer Res* 56(14):3165–3167
79. Wang J et al (1999) Grb10, a positive, stimulatory signaling adapter in platelet-derived growth factor BB-, insulin-like growth factor I-, and insulin-mediated mitogenesis. *Mol Cell Biol* 19(9):6217–6228
80. Uddin S et al (1996) Insulin-like growth factor-I induces rapid tyrosine phosphorylation of the vav proto-oncogene product. *Exp Hematol* 24(5):622–627
81. Zeng L et al (2000) Vav3 mediates receptor protein tyrosine kinase signaling, regulates GTPase activity, modulates cell morphology, and induces cell transformation. *Mol Cell Biol* 20(24):9212–9224
82. Beitner-Johnson D, LeRoith D (1995) Insulin-like growth factor-I stimulates tyrosine phosphorylation of endogenous c-Crk. *J Biol Chem* 270(10):5187–5190
83. Baron V et al (1998) p125Fak focal adhesion kinase is a substrate for the insulin and insulin-like growth factor-I tyrosine kinase receptors. *J Biol Chem* 273(12):7162–7168
84. Seely BL et al (1995) Localization of the insulin-like growth factor I receptor binding sites for the SH2 domain proteins p85, Syp, and GTPase activating-protein. *J Biol Chem* 270(32):19151–19157
85. Arbet-Engels C, Tartare-Deckert S, Eckhart W (1999) C-terminal Src kinase associates with ligand-stimulated insulin-like growth factor-I receptor. *J Biol Chem* 274(9):5422–5428
86. Dey BR et al (1998) Interaction of human suppressor of cytokine signaling (SOCS)-2 with the insulin-like growth factor-I receptor. *J Biol Chem* 273(37):24095–24101

87. Hermanto U et al (2002) RACK1, an insulin-like growth factor I (IGF-I) receptor-interacting protein, modulates IGF-I-dependent integrin signaling and promotes cell spreading and contact with extracellular matrix. *Mol Cell Biol* 22(7):2345–2365
88. Kiely PA, Sant A, O'Connor R (2002) RACK1 is an insulin-like growth factor I (IGF-I) receptor-interacting protein that can regulate IGF-1-mediated Akt activation and protection from cell death. *J Biol Chem* 277(25):22581–22589
89. O'Connor R et al (1997) Identification of domains of the insulin-like growth factor I receptor that are required for protection from apoptosis. *Mol Cell Biol* 17(1):427–435
90. Baserga R (2012) The decline and fall of the IGF-I receptor. *J Cell Physiol* 228(4):675–679
91. Yee D (2012) Insulin-like growth factor receptor inhibitors: baby or the bathwater? *J Natl Cancer Inst* 104(13):975–981
92. Werner H (2012) Tumor suppressors govern insulin-like growth factor signaling pathways: implications in metabolism and cancer. *Oncogene* 31(22):2703–2714
93. Pollak M (2012) The insulin and insulin-like growth factor receptor family in neoplasia: an update. *Nat Rev Cancer* 12(3):159–169
94. LeRoith D, Roberts CT Jr (2003) The insulin-like growth factor system and cancer. *Cancer Lett* 195(2):127–137
95. Larsson O, Girnita A, Girnita L (2005) Role of insulin-like growth factor I receptor signaling in cancer. *Br J Cancer* 92(12):2097–2101
96. Pearse BM, Robinson MS (1990) Clathrin, adaptors, and sorting. *Annu Rev Cell Biol* 6:151–171
97. Robinson MS (1989) Cloning of cDNAs encoding two related 100-kD coated vesicle proteins (alpha-adaptins). *J Cell Biol* 108(3):833–842
98. Koenig JA, Edwardson JM (1997) Endocytosis and recycling of G-protein-coupled receptors. *Trends Pharmacol Sci* 18(8):276–287
99. Hicke L (1997) Ubiquitin-dependent internalization and down-regulation of plasma membrane proteins. *FASEB J* 11(14):1215–1226
100. Hicke L (1999) Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels. *Trends Cell Biol* 9(3):107–112
101. Hicke L (2001) Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol* 2(3):195–201
102. Haglund K, Dikic I (2012) The role of ubiquitylation in receptor endocytosis and endosomal sorting. *J Cell Sci* 125(Pt 2):265–275
103. Andersson ER (2011) The role of endocytosis in activating and regulating signal transduction. *Cell Mol Life Sci* 69(11):1755–1771
104. Luo Y et al (2011) Endosomal signaling of epidermal growth factor receptors contributes to EGF-stimulated cell cycle progression in primary hepatocytes. *Eur J Pharmacol* 654(2):173–180
105. Huynh J et al (2012) CSF-1 receptor signaling from endosomes mediates the sustained activation of Erk1/2 and Akt in macrophages. *Cell Signal* 24(9):1753–1761
106. Shih SC, Sloper-Mould KE, Hicke L (2000) Monoubiquitin carries a novel internalization signal that is appended to activated receptors. *EMBO J* 19(2):187–198
107. Acconcia F, Sigismund S, Polo S (2009) Ubiquitin in trafficking: the network at work. *Exp Cell Res* 315(9):1610–1618
108. Pickart CM (2001) Mechanisms underlying ubiquitination. *Annu Rev Biochem* 70:503–533
109. Weissman AM (2001) Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol* 2(3):169–178
110. Bremm A, Komander D (2011) Emerging roles for Lys11-linked polyubiquitin in cellular regulation. *Trends Biochem Sci* 36(7):355–363
111. Kulathu Y, Komander D (2012) Atypical ubiquitylation—the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol* 13(8):508–523
112. Trempe JF (2011) Reading the ubiquitin postal code. *Curr Opin Struct Biol* 21(6):792–801
113. Nakasone MA et al (2013) Mixed-linkage ubiquitin chains send mixed messages. *Structure* 21(5):727–740
114. Sepp-Lorenzino L et al (1995) Herbimycin A induces the 20 S proteasome- and ubiquitin-dependent degradation of receptor tyrosine kinases. *J Biol Chem* 270(28):16580–16587
115. Carelli S et al (2006) Degradation of insulin-like growth factor-I receptor occurs via ubiquitin-proteasome pathway in human lung cancer cells. *J Cell Physiol* 208(2):354–362
116. Girnita L, Girnita A, Larsson O (2003) Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor I receptor. *Proc Natl Acad Sci USA* 100(14):8247–8252
117. Sehat B et al (2008) Identification of c-Cbl as a new ligase for insulin-like growth factor-I receptor with distinct roles from Mdm2 in receptor ubiquitination and endocytosis. *Cancer Res* 68(14):5669–5677
118. Glogowska A et al (2012) Epidermal growth factor cytoplasmic domain affects ErbB protein degradation by the lysosomal and ubiquitin-proteasome pathway in human cancer cells. *Neoplasia* 14(5):396–409
119. Ancot F et al (2012) Shedding-generated Met receptor fragments can be routed to either the proteasomal or the lysosomal degradation pathway. *Traffic* 13(9):1261–1272
120. Vecchione A et al (2003) The Grb10/Nedd4 complex regulates ligand-induced ubiquitination and stability of the insulin-like growth factor I receptor. *Mol Cell Biol* 23(9):3363–3372
121. Sehat B et al (2007) Role of ubiquitination in IGF-I receptor signaling and degradation. *PLoS ONE* 2(4):e340
122. Dore S, Kar S, Quirion R (1997) Presence and differential internalization of two distinct insulin-like growth factor receptors in rat hippocampal neurons. *Neuroscience* 78(2):373–383
123. Segretin ME et al (2003) Insulin-like growth factor-I receptor regulation in activated human T lymphocytes. *Horm Res* 59(6):276–280
124. Zheng H et al (2012) Selective recruitment of G-protein-coupled receptor kinases (GRKs) controls signaling of the insulin-like growth factor I receptor. *Proc Natl Acad Sci USA* 109(18):7055–7060
125. Ceresa BP, Schmid SL (2000) Regulation of signal transduction by endocytosis. *Curr Opin Cell Biol* 12(2):204–210
126. Johnson KF, Kornfeld S (1992) The cytoplasmic tail of the mannose 6-phosphate/insulin-like growth factor-II receptor has two signals for lysosomal enzyme sorting in the Golgi. *J Cell Biol* 119(2):249–257
127. Bremnes B et al (1994) An LI and ML motif in the cytoplasmic tail of the MHC-associated invariant chain mediate rapid internalization. *J Cell Sci* 107(Pt 7):2021–2032
128. Miura M, Baserga R (1997) The tyrosine residue at 1250 of the insulin-like growth factor I receptor is required for ligand-mediated internalization. *Biochem Biophys Res Commun* 239(1):182–185
129. Morrione A et al (1997) The role of mGrb10alpha in insulin-like growth factor I-mediated growth. *J Biol Chem* 272(42):26382–26387
130. Morrione A et al (1999) mGrb10 interacts with Nedd4. *J Biol Chem* 274(34):24094–24099
131. Monami G, Emiliozzi V, Morrione A (2008) Grb10/Nedd4-mediated multiubiquitination of the insulin-like growth factor receptor regulates receptor internalization. *J Cell Physiol* 216(2):426–437
132. Werner H et al (1996) Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. *Proc Natl Acad Sci USA* 93(16):8318–8323

133. Girmita L et al (2005) β -Arrestin is crucial for ubiquitination and down-regulation of the insulin-like growth factor-1 receptor by acting as adaptor for the MDM2 E3 ligase. *J Biol Chem* 280(26):24412–24419
134. Girmita L et al (2007) Beta-arrestin and Mdm2 mediate IGF-1 receptor-stimulated ERK activation and cell cycle progression. *J Biol Chem* 282(15):11329–11338
135. Zwang Y, Yarden Y (2009) Systems biology of growth factor-induced receptor endocytosis. *Traffic* 10(4):349–363
136. Cao XR et al (2008) Nedd4 controls animal growth by regulating IGF-1 signaling. *Sci Signal* 1(38):ra5
137. Higashi Y et al (2008) The ubiquitin ligase Nedd4 mediates oxidized low-density lipoprotein-induced downregulation of insulin-like growth factor-1 receptor. *Am J Physiol Heart Circ Physiol* 295(4):H1684–H1689
138. Romanelli RJ et al (2007) Insulin-like growth factor type-I receptor internalization and recycling mediate the sustained phosphorylation of Akt. *J Biol Chem* 282(31):22513–22524
139. Morcavallo A et al (2012) Insulin and insulin-like growth factor II differentially regulate endocytic sorting and stability of insulin receptor isoform A. *J Biol Chem* 287(14):11422–11436
140. Zheng H et al (2012) beta-Arrestin-biased agonism as the central mechanism of action for insulin-like growth factor 1 receptor-targeting antibodies in Ewing's sarcoma. *Proc Natl Acad Sci USA* 109(50):20620–20625
141. Mao Y et al (2011) Polyubiquitination of insulin-like growth factor I receptor (IGF-IR) activation loop promotes antibody-induced receptor internalization and down-regulation. *J Biol Chem* 286(48):41852–41861
142. Broussas M et al (2009) Molecular mechanisms involved in activity of h7C10, a humanized monoclonal antibody, to IGF-1 receptor. *Int J Cancer* 124(10):2281–2293
143. Vasilcanu R et al (2008) Insulin-like growth factor type-I receptor-dependent phosphorylation of extracellular signal-regulated kinase 1/2 but not Akt (protein kinase B) can be induced by picrodopophyllin. *Mol Pharmacol* 73(3):930–939
144. Dalle S et al (2001) Insulin and insulin-like growth factor I receptors utilize different G-protein signaling components. *J Biol Chem* 276(19):15688–15695
145. Rocchi S et al (1996) Interaction of SH2-containing-protein tyrosine phosphatase 2 with the insulin receptor and the insulin-like growth factor-I receptor: studies of the domains involved using the yeast two-hybrid system. *Endocrinology* 137(11):4944–4952
146. Maile LA, Clemmons DR (2002) The alphaVbeta3 integrin regulates insulin-like growth factor I (IGF-I) receptor phosphorylation by altering the rate of recruitment of the Src-homology 2-containing phosphotyrosine phosphatase-2 to the activated IGF-I receptor. *Endocrinology* 143(11):4259–4264
147. Kuemmerle JF (2006) Occupation of alphavbeta3-integrin by endogenous ligands modulates IGF-I receptor activation and proliferation of human intestinal smooth muscle. *Am J Physiol Gastrointest Liver Physiol* 290(6):G1194–G1202
148. Buckley DA et al (2002) Regulation of insulin-like growth factor type I (IGF-I) receptor kinase activity by protein tyrosine phosphatase 1B (PTP-1B) and enhanced IGF-I-mediated suppression of apoptosis and motility in PTP-1B-deficient fibroblasts. *Mol Cell Biol* 22(7):1998–2010
149. Blanquart C et al (2005) Monitoring the activation state of the insulin-like growth factor-1 receptor and its interaction with protein tyrosine phosphatase 1B using bioluminescence resonance energy transfer. *Mol Pharmacol* 68(3):885–894
150. Deng H et al (2010) Over-accumulation of nuclear IGF-1 receptor in tumor cells requires elevated expression of the receptor and the SUMO-conjugating enzyme Ubc9. *Biochem Biophys Res Commun* 404(2):667–671
151. Sehat B et al (2010) SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor. *Sci Signal* 3(108):ra10
152. Sarfstein R et al (2012) Insulin-like growth factor-I receptor (IGF-IR) translocates to nucleus and autoregulates IGF-IR gene expression in breast cancer cells. *J Biol Chem* 287(4):2766–2776
153. Asmane I et al (2012) Insulin-like growth factor type 1 receptor (IGF-1R) exclusive nuclear staining: a predictive biomarker for IGF-1R monoclonal antibody (Ab) therapy in sarcomas. *Eur J Cancer* 48(16):3027–3035
154. Aleksic T et al (2010) Type 1 insulin-like growth factor receptor translocates to the nucleus of human tumor cells. *Cancer Res* 70(16):6412–6419
155. Sarfstein R, Werner H (2013) Minireview: nuclear insulin and insulin-like growth factor-1 receptors: a novel paradigm in signal transduction. *Endocrinology* 154:1672–1679
156. Pandini G et al (2007) Functional responses and in vivo anti-tumour activity of h7C10: a humanised monoclonal antibody with neutralising activity against the insulin-like growth factor-1 (IGF-1) receptor and insulin/IGF-1 hybrid receptors. *Eur J Cancer* 43(8):1318–1327
157. Belfiore A (2007) The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer. *Curr Pharm Des* 13(7):671–686
158. Belfiore A et al (1999) Insulin/IGF-I hybrid receptors play a major role in IGF-I signaling in thyroid cancer. *Biochimie* 81(4):403–407
159. Riedemann J et al (2007) The EGF receptor interacts with the type 1 IGF receptor and regulates its stability. *Biochem Biophys Res Commun* 355(3):707–714
160. Legate KR, Wickstrom SA, Fassler R (2009) Genetic and cell biological analysis of integrin outside-in signaling. *Genes Dev* 23(4):397–418
161. Ivaska J, Heino J (2010) Interplay between cell adhesion and growth factor receptors: from the plasma membrane to the endosomes. *Cell Tissue Res* 339(1):111–120
162. Maile LA et al (2002) Insulin-like growth factor I increases alpha Vbeta 3 affinity by increasing the amount of integrin-associated protein that is associated with non-raft domains of the cellular membrane. *J Biol Chem* 277(3):1800–1805
163. Manes S et al (1999) Concerted activity of tyrosine phosphatase SHP-2 and focal adhesion kinase in regulation of cell motility. *Mol Cell Biol* 19(4):3125–3135
164. Ling Y et al (2005) DOK1 mediates SHP-2 binding to the alphaVbeta3 integrin and thereby regulates insulin-like growth factor I signaling in cultured vascular smooth muscle cells. *J Biol Chem* 280(5):3151–3158
165. Jones JI et al (1996) Ligand occupancy of the alpha-V-beta3 integrin is necessary for smooth muscle cells to migrate in response to insulin-like growth factor. *Proc Natl Acad Sci USA* 93(6):2482–2487
166. Brooks PC et al (1997) Insulin-like growth factor receptor cooperates with integrin alpha v beta 5 to promote tumor cell dissemination in vivo. *J Clin Invest* 99(6):1390–1398
167. Ling Y, Maile LA, Clemmons DR (2003) Tyrosine phosphorylation of the beta3-subunit of the alphaVbeta3 integrin is required for membrane association of the tyrosine phosphatase SHP-2 and its further recruitment to the insulin-like growth factor I receptor. *Mol Endocrinol* 17(9):1824–1833
168. Maile LA, Badley-Clarke J, Clemmons DR (2003) The association between integrin-associated protein and SHPS-1 regulates insulin-like growth factor-I receptor signaling in vascular smooth muscle cells. *Mol Biol Cell* 14(9):3519–3528
169. Clemmons DR, Maile LA (2005) Interaction between insulin-like growth factor-I receptor and alphaVbeta3 integrin linked

- signaling pathways: cellular responses to changes in multiple signaling inputs. *Mol Endocrinol* 19(1):1–11
170. Clemmons DR, Maile LA (2003) Minireview: integral membrane proteins that function coordinately with the insulin-like growth factor I receptor to regulate intracellular signaling. *Endocrinology* 144(5):1664–1670
 171. Edderkaoui M et al (2007) Insulin-like growth factor-I receptor mediates the prosurvival effect of fibronectin. *J Biol Chem* 282(37):26646–26655
 172. Shakibaei M et al (1999) Signal transduction by beta1 integrin receptors in human chondrocytes in vitro: collaboration with the insulin-like growth factor-I receptor. *Biochem J* 342(Pt 3):615–623
 173. Kiely PA et al (2006) Insulin-like growth factor I controls a mutually exclusive association of RACK1 with protein phosphatase 2A and beta1 integrin to promote cell migration. *Mol Cell Biol* 26(11):4041–4051
 174. O'Donovan HC, Kiely PA, O'Connor R (2007) Effects of RACK1 on cell migration and IGF-I signaling in cardiomyocytes are not dependent on an association with the IGF-IR. *Cell Signal* 19(12):2588–2595
 175. Saegusa J et al (2009) The direct binding of insulin-like growth factor-1 (IGF-1) to integrin alpha5beta3 is involved in IGF-1 signaling. *J Biol Chem* 284(36):24106–24114
 176. Fujita M et al (2013) An integrin-binding-defective mutant of insulin-like growth factor-1 (R36E/R37E IGF1) acts as a dominant-negative antagonist of IGF1R and suppresses tumorigenesis, while the mutant still binds to IGF1R. *J Biol Chem*
 177. Fujita M et al (2012) Cross-talk between integrin alpha6beta4 and insulin-like growth factor-1 receptor (IGF1R) through direct alpha6beta4 binding to IGF1 and subsequent alpha6beta4-IGF1-IGF1R ternary complex formation in anchorage-independent conditions. *J Biol Chem* 287(15):12491–12500
 178. Fujita M et al (2013) An integrin binding-defective mutant of insulin-like growth factor-1 (R36E/R37E IGF1) acts as a dominant-negative antagonist of the IGF1 receptor (IGF1R) and suppresses tumorigenesis but still binds to IGF1R. *J Biol Chem* 288(27):19593–19603
 179. Waters C, Pyne S, Pyne NJ (2004) The role of G-protein coupled receptors and associated proteins in receptor tyrosine kinase signal transduction. *Semin Cell Dev Biol* 15(3):309–323
 180. Daub H et al (1997) Signal characteristics of G-protein-transactivated EGF receptor. *EMBO J* 16(23):7032–7044
 181. Pyne NJ, Pyne S (2011) Receptor tyrosine kinase-G-protein-coupled receptor signaling platforms: out of the shadow? *Trends Pharmacol Sci* 32(8):443–450
 182. Rao GN, Delafontaine P, Runge MS (1995) Thrombin stimulates phosphorylation of insulin-like growth factor-1 receptor, insulin receptor substrate-1, and phospholipase C-gamma 1 in rat aortic smooth muscle cells. *J Biol Chem* 270(46):27871–27875
 183. Zhao D et al (2011) Insulin-like growth factor-1 receptor transactivation modulates the inflammatory and proliferative responses of neurotensin in human colonic epithelial cells. *J Biol Chem* 286(8):6092–6099
 184. Oligny-Longpre G et al (2012) Engagement of beta-arrestin by transactivated insulin-like growth factor receptor is needed for V2 vasopressin receptor-stimulated ERK1/2 activation. *Proc Natl Acad Sci USA* 109(17):E1028–E1037
 185. Luttrell LM et al (1995) G beta gamma subunits mediate mitogen-activated protein kinase activation by the tyrosine kinase insulin-like growth factor I receptor. *J Biol Chem* 270(28):16495–16498
 186. Luttrell L et al (1990) A pertussis toxin-sensitive G-protein mediates some aspects of insulin action in BC3H-1 murine myocytes. *J Biol Chem* 265(28):16873–16879
 187. Delcourt N, Bockaert J, Marin P (2007) GPCR-jacking: from a new route in RTK signaling to a new concept in GPCR activation. *Trends Pharmacol Sci* 28(12):602–607
 188. Heyworth CM et al (1986) The action of islet activating-protein (pertussis toxin) on insulin's ability to inhibit adenylate cyclase and activate cyclic AMP phosphodiesterases in hepatocytes. *Biochem J* 235(1):145–149
 189. Rothenberg PL, Kahn CR (1988) Insulin inhibits pertussis toxin-catalyzed ADP-ribosylation of G-proteins. Evidence for a novel interaction between insulin receptors and G-proteins. *J Biol Chem* 263(30):15546–15552
 190. Hallak H et al (2000) Association of heterotrimeric G(i) with the insulin-like growth factor-I receptor. Release of G(beta gamma) subunits upon receptor activation. *J Biol Chem* 275(4):2255–2258
 191. Lin FT, Daaka Y, Lefkowitz RJ (1998) beta-Arrestins regulate mitogenic signaling and clathrin-mediated endocytosis of the insulin-like growth factor I receptor. *J Biol Chem* 273(48):31640–31643
 192. Shenoy SK et al (2006) beta-Arrestin-dependent, G-protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *J Biol Chem* 281(2):1261–1273
 193. Lefkowitz RJ, Shenoy SK (2005) Transduction of receptor signals by beta-arrestins. *Science* 308(5721):512–517
 194. Lefkowitz RJ (2004) Historical review: a brief history and personal retrospective of seven-transmembrane receptors. *Trends Pharmacol Sci* 25(8):413–422
 195. Shenoy SK, Lefkowitz RJ (2003) Multifaceted roles of beta-arrestins in the regulation of seven-membrane-spanning receptor trafficking and signaling. *Biochem J* 375(Pt 3):503–515
 196. DeWire SM et al (2007) Beta-arrestins and cell signaling. *Annu Rev Physiol* 69:483–510
 197. Reiter E, Lefkowitz RJ (2006) GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab* 17(4):159–165
 198. Gao J, Li J, Ma L (2005) Regulation of EGF-induced ERK/MAPK activation and EGFR internalization by G-protein-coupled receptor kinase 2. *Acta Biochim Biophys Sin (Shanghai)* 37(8):525–531
 199. Freedman NJ et al (2002) Phosphorylation of the platelet-derived growth factor receptor-beta and epidermal growth factor receptor by G-protein-coupled receptor kinase-2. Mechanisms for selectivity of desensitization. *J Biol Chem* 277(50):48261–48269
 200. Usui I et al (2004) GRK2 is an endogenous protein inhibitor of the insulin signaling pathway for glucose transport stimulation. *EMBO J* 23(14):2821–2829
 201. Pitcher JA, Freedman NJ, Lefkowitz RJ (1998) G-protein-coupled receptor kinases. *Annu Rev Biochem* 67:653–692
 202. Perrault R et al (2011) Tyrosine kinase-independent activation of extracellular-regulated kinase (ERK) 1/2 by the insulin-like growth factor-1 receptor. *Cell Signal* 23(4):739–746
 203. Frattali AL, Treadway JL, Pessin JE (1992) Insulin/IGF-1 hybrid receptors: implications for the dominant-negative phenotype in syndromes of insulin resistance. *J Cell Biochem* 48(1):43–50
 204. Povsic TJ, Kohout TA, Lefkowitz RJ (2003) Beta-arrestin1 mediates insulin-like growth factor I (IGF-1) activation of phosphatidylinositol 3-kinase (PI3K) and anti-apoptosis. *J Biol Chem* 278(51):51334–51339
 205. Arteaga CL (1992) Interference of the IGF system as a strategy to inhibit breast cancer growth. *Breast Cancer Res Treat* 22(1):101–106
 206. Kalebic T, Tsokos M, Helman LJ (1994) In vivo treatment with antibody against IGF-1 receptor suppresses growth of human rhabdomyosarcoma and down-regulates p34cdc2. *Cancer Res* 54(21):5531–5534

207. Trojan J et al (1992) Loss of tumorigenicity of rat glioblastoma directed by episome-based antisense cDNA transcription of insulin-like growth factor I. *Proc Natl Acad Sci USA* 89(11):4874–4878
208. Resnicoff M et al (1994) Rat glioblastoma cells expressing an antisense RNA to the insulin-like growth factor-1 (IGF-1) receptor are nontumorigenic and induce regression of wild-type tumors. *Cancer Res* 54(8):2218–2222
209. Resnicoff M et al (1994) Growth inhibition of human melanoma cells in nude mice by antisense strategies to the type I insulin-like growth factor receptor. *Cancer Res* 54(18):4848–4850
210. Shapiro DN et al (1994) Antisense-mediated reduction in insulin-like growth factor-I receptor expression suppresses the malignant phenotype of a human alveolar rhabdomyosarcoma. *J Clin Invest* 94(3):1235–1242
211. Reiss K et al (1998) Inhibition of tumor growth by a dominant negative mutant of the insulin-like growth factor I receptor with a bystander effect. *Clin Cancer Res* 4(11):2647–2655
212. Long L et al (1995) Loss of the metastatic phenotype in murine carcinoma cells expressing an antisense RNA to the insulin-like growth factor receptor. *Cancer Res* 55(5):1006–1009
213. Dunn SE et al (1998) A dominant negative mutant of the insulin-like growth factor-I receptor inhibits the adhesion, invasion, and metastasis of breast cancer. *Cancer Res* 58(15):3353–3361
214. Kanter-Lewensohn L et al (2000) Tamoxifen-induced cell death in malignant melanoma cells: possible involvement of the insulin-like growth factor-1 (IGF-1) pathway. *Mol Cell Endocrinol* 165(1–2):131–137
215. Scartozzi M et al (2011) State of the art and future perspectives for the use of insulin-like growth factor receptor 1 (IGF-1R) targeted treatment strategies in solid tumors. *Discov Med* 11(57):144–153
216. Baserga R (2000) The contradictions of the insulin-like growth factor I receptor. *Oncogene* 19(49):5574–5581
217. Kanter-Lewensohn L et al (2000) Expression of insulin-like growth factor-1 receptor (IGF-1R) and p27Kip1 in melanocytic tumors: a potential regulatory role of IGF-1 pathway in distribution of p27Kip1 between different cyclins. *Growth Factors* 17(3):193–202
218. Navab R et al (2001) Inhibition of endosomal insulin-like growth factor-I processing by cysteine proteinase inhibitors blocks receptor-mediated functions. *J Biol Chem* 276(17):13644–13649
219. Ren SG et al (1992) Somatostatin analog induces insulin-like growth factor binding-protein-1 (IGFBP-1) expression in human hepatoma cells. *Endocrinology* 131(5):2479–2481
220. Pietrzowski Z et al (1993) Inhibition of growth of prostatic cancer cell lines by peptide analogues of insulin-like growth factor I. *Cancer Res* 53(5):1102–1106
221. Rohlik QT et al (1987) An antibody to the receptor for insulin-like growth factor I inhibits the growth of MCF-7 cells in tissue culture. *Biochem Biophys Res Commun* 149(1):276–281
222. Rosenzweig SA, Atreya HS (2010) Defining the pathway to insulin-like growth factor system targeting in cancer. *Biochem Pharmacol* 80(8):1115–1124
223. Firth SM, Baxter RC (2002) Cellular actions of the insulin-like growth factor binding-proteins. *Endocr Rev* 23(6):824–854
224. Dransfield DT et al (2010) A human monoclonal antibody against insulin-like growth factor-II blocks the growth of human hepatocellular carcinoma cell lines in vitro and in vivo. *Mol Cancer Ther* 9(6):1809–1819
225. Gao J et al (2011) Dual IGF-I/II-neutralizing antibody MEDI-573 potently inhibits IGF signaling and tumor growth. *Cancer Res* 71(3):1029–1040
226. Zhao Q et al (2011) Human monoclonal antibody fragments binding to insulin-like growth factors I and II with picomolar affinity. *Mol Cancer Ther* 10(9):1677–1685
227. Feng Y et al (2006) Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potently inhibit the IGF receptor type I signal transduction function. *Mol Cancer Ther* 5(1):114–120
228. Olmos D et al (2011) Targeting the insulin-like growth factor I receptor in Ewing's sarcoma: reality and expectations. *Sarcoma* 2011(402508):402508
229. Olmos D et al (2009) Safety, pharmacokinetics, and preliminary activity of the anti-IGF-1R antibody figitumumab (CP-751,871) in patients with sarcoma and Ewing's sarcoma: a phase I expansion cohort study. *Lancet Oncol* 11(2):129–135
230. Juergens H et al (2011) Preliminary efficacy of the anti-insulin-like growth factor type 1 receptor antibody figitumumab in patients with refractory Ewing sarcoma. *J Clin Oncol* 29(34):4534–4540
231. Gualberto A et al (2010) Molecular analysis of non-small cell lung cancer identifies subsets with different sensitivity to insulin-like growth factor I receptor inhibition. *Clin Cancer Res* 16(18):4654–4665
232. Corral J et al (2011) Pathologic complete responses after chemotherapy plus figitumumab in stage IV non-small-cell lung cancer. *J Clin Oncol* 29(7):e163–e165
233. Burgaud JL, Resnicoff M, Baserga R (1995) Mutant IGF-I receptors as dominant negatives for growth and transformation. *Biochem Biophys Res Commun* 214(2):475–481
234. Li S et al (1994) Mitogenicity and transforming activity of the insulin-like growth factor-I receptor with mutations in the tyrosine kinase domain. *J Biol Chem* 269(51):32558–32564
235. Kalebic T et al (1998) Expression of a kinase-deficient IGF-1R suppresses tumorigenicity of rhabdomyosarcoma cells constitutively expressing a wild type IGF-1R. *Int J Cancer* 76(2):223–227
236. Scotlandi K et al (2002) Expression of an IGF-I receptor dominant negative mutant induces apoptosis, inhibits tumorigenesis and enhances chemosensitivity in Ewing's sarcoma cells. *Int J Cancer* 101(1):11–16
237. Brodt P, Samani A, Navab R (2000) Inhibition of the type I insulin-like growth factor receptor expression and signaling: novel strategies for antimetastatic therapy. *Biochem Pharmacol* 60(8):1101–1107
238. Buck E, Mulvihill M (2011) Small molecule inhibitors of the IGF-1R/IR axis for the treatment of cancer. *Expert Opin Investig Drugs* 20(5):605–621
239. Jin M et al (2012) Small-molecule ATP-competitive dual IGF-1R and insulin receptor inhibitors: structural insights, chemical diversity and molecular evolution. *Future Med Chem* 4(3):315–328
240. Buck E et al (2010) Compensatory insulin receptor (IR) activation on inhibition of insulin-like growth factor-1 receptor (IGF-1R): rationale for cotargeting IGF-1R and IR in cancer. *Mol Cancer Ther* 9(10):2652–2664
241. Ulanet DB et al (2010) Insulin receptor functionally enhances multistage tumor progression and conveys intrinsic resistance to IGF-1R targeted therapy. *Proc Natl Acad Sci USA* 107(24):10791–10798
242. Mitsiades CS et al (2004) Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. *Cancer Cell* 5(3):221–230
243. Scotlandi K et al (2005) Antitumor activity of the insulin-like growth factor-I receptor kinase inhibitor NVP-AEW541 in musculoskeletal tumors. *Cancer Res* 65(9):3868–3876
244. Mulvihill MJ et al (2009) Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor. *Future Med Chem* 1(6):1153–1171
245. Vasilcanu D et al (2004) The cyclolignan PPP induces activation loop-specific inhibition of tyrosine phosphorylation of

- the insulin-like growth factor-1 receptor. Link to the phosphatidyl inositol-3 kinase/Akt apoptotic pathway. *Oncogene* 23(47):7854–7862
246. Ahlen J et al (2005) Insulin-like growth factor type 1 receptor expression correlates to good prognosis in highly malignant soft tissue sarcoma. *Clin Cancer Res* 11(1):206–216
 247. Economou MA et al (2005) Receptors for the liver synthesized growth factors IGF-1 and HGF/SF in uveal melanoma: intercorrelation and prognostic implications. *Invest Ophthalmol Vis Sci* 46(12):4372–4375
 248. Economou MA et al (2008) Oral picropodophyllin (PPP) is well tolerated in vivo and inhibits IGF-1R expression and growth of uveal melanoma. *Invest Ophthalmol Vis Sci* 49(6):2337–2342
 249. Menu E et al (2006) Inhibiting the IGF-1 receptor tyrosine kinase with the cyclolignan PPP: an in vitro and in vivo study in the 5T33MM mouse model. *Blood* 107(2):655–660
 250. Natalishvili N et al (2009) Aberrant intracellular IGF-1R beta-subunit makes receptor knockout cells (IGF1R^{-/-}) susceptible to oncogenic transformation. *Exp Cell Res* 315(8):1458–1467
 251. Razuvaev A et al (2007) The cyclolignan picropodophyllin attenuates intimal hyperplasia after rat carotid balloon injury by blocking insulin-like growth factor-1 receptor signaling. *J Vasc Surg* 46(1):108–115
 252. Stromberg T et al (2006) IGF-1 receptor tyrosine kinase inhibition by the cyclolignan PPP induces G2/M-phase accumulation and apoptosis in multiple myeloma cells. *Blood* 107(2):669–678
 253. Vitale M et al (2008) New picropodophyllin analogs via palladium-catalyzed allylic alkylation-Hiyama cross-coupling sequences. *J Org Chem* 73(15):5795–5805
 254. Conti L et al (2007) In the absence of IGF-1 signaling, IFN-gamma suppresses human malignant T-cell growth. *Blood* 109(6):2496–2504
 255. Jernberg-Wiklund H, Nilsson K (2007) Control of apoptosis in human multiple myeloma by insulin-like growth factor I (IGF-I). *Adv Cancer Res* 97:139–165
 256. Menu E et al (2007) Targeting the IGF-1R using picropodophyllin in the therapeutical 5T2MM mouse model of multiple myeloma: beneficial effects on tumor growth, angiogenesis, bone disease and survival. *Int J Cancer* 121(8):1857–1861
 257. Nussbaum T et al (2008) Autocrine insulin-like growth factor-II stimulation of tumor cell migration is a progression step in human hepatocarcinogenesis. *Hepatology* 48(1):146–156
 258. Klinakis A et al (2009) Igf1r as a therapeutic target in a mouse model of basal-like breast cancer. *Proc Natl Acad Sci USA* 106(7):2359–2364
 259. Hashemi J et al (2011) Molecular characterization of acquired tolerance of tumor cells to picropodophyllin (PPP). *PLoS ONE* 6(3):e14757
 260. Rosengren L et al (2006) IGF-1R tyrosine kinase expression and dependency in clones of IGF-1R knockout cells (R⁻). *Biochem Biophys Res Commun* 347(4):1059–1066
 261. Ekman S et al (2011) Clinical phase I study with an insulin-like growth factor-1 receptor inhibitor: experiences in patients with squamous non-small cell lung carcinoma. *Acta Oncol* 50(3):441–447
 262. Dong JY et al (2011) A stable IgG-like bispecific antibody targeting the epidermal growth factor receptor and the type I insulin-like growth factor receptor demonstrates superior anti-tumor activity. *MAbs* 3(3):273–288
 263. Rowinsky EK et al (2007) IMC-A12, a human IgG, monoclonal antibody to the insulin-like growth factor I receptor. *Clin Cancer Res* 13(18):5549S–5555S
 264. Runnels HA et al (2010) Human monoclonal antibodies to the insulin-like growth factor I receptor inhibit receptor activation and tumor growth in preclinical studies. *Adv Ther* 27(7):458–475
 265. Wang Y et al (2005) Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. *Mol Cancer Ther* 4(8):1214–1221
 266. Garofalo C et al (2012) Identification of common and distinctive mechanisms of resistance to different anti-IGF-IR agents in Ewing's sarcoma. *Mol Endocrinol* 26(9):1603–1616
 267. Takahashi K, Yonezawa K, Nishimoto I (1995) Insulin-like growth factor I receptor activated by a transmembrane mutation. *J Biol Chem* 270(32):19041–19045
 268. Craparo A, O'Neill TJ, Gustafson TA (1995) Non-SH2 domains within insulin receptor substrate-1 and SHC mediate their phosphotyrosine-dependent interaction with the NPEY motif of the insulin-like growth factor I receptor. *J Biol Chem* 270(26):15639–15643
 269. Tartare-Deckert S et al (1995) Evidence for a differential interaction of SHC and the insulin receptor substrate-1 (IRS-1) with the insulin-like growth factor-I (IGF-I) receptor in the yeast two-hybrid system. *J Biol Chem* 270(40):23456–23460
 270. He W et al (1996) Interaction of insulin receptor substrate-2 (IRS-2) with the insulin and insulin-like growth factor I receptors. Evidence for two distinct phosphotyrosine-dependent interaction domains within IRS-2. *J Biol Chem* 271(20):11641–11645
 271. Xu P, Jacobs AR, Taylor SI (1999) Interaction of insulin receptor substrate 3 with insulin receptor, insulin receptor-related receptor, insulin-like growth factor-1 receptor, and downstream signaling-proteins. *J Biol Chem* 274(21):15262–15270
 272. Dey BR et al (1996) Evidence for the direct interaction of the insulin-like growth factor I receptor with IRS-1, Shc, and Grb10. *Mol Endocrinol* 10(6):631–641
 273. Beitner-Johnson D et al (1995) Regulation of insulin-like growth factor I receptor gene expression by Sp1: physical and functional interactions of Sp1 at GC boxes and at a CT element. *Mol Endocrinol* 9(9):1147–1156
 274. Koval AP et al (1998) Interaction in vitro of the product of the c-Crk-II proto-oncogene with the insulin-like growth factor I receptor. *Biochem J* 330(Pt 2):923–932
 275. Miura M, Li S, Baserga R (1995) Effect of a mutation at tyrosine 950 of the insulin-like growth factor I receptor on the growth and transformation of cells. *Cancer Res* 55(3):663–667
 276. Jiang Y et al (1996) Effect of tyrosine mutations on the kinase activity and transforming potential of an oncogenic human insulin-like growth factor I receptor. *J Biol Chem* 271(1):160–167
 277. Esposito DL et al (1997) Tyrosine residues in the C-terminal domain of the insulin-like growth factor-I receptor mediate mitogenic and tumorigenic signals. *Endocrinology* 138(7):2979–2988
 278. Yamasaki H et al (1992) Human insulin-like growth factor I receptor 950tyrosine is required for somatotroph growth factor signal transduction. *J Biol Chem* 267(29):20953–20958
 279. Kato H et al (1993) Role of tyrosine kinase activity in signal transduction by the insulin-like growth factor-I (IGF-I) receptor. Characterization of kinase-deficient IGF-I receptors and the action of an IGF-I-mimetic antibody (alpha IR-3). *J Biol Chem* 268(4):2655–2661
 280. Kelly GM et al (2012) Serine phosphorylation of the insulin-like growth factor I (IGF-1) receptor C-terminal tail restrains kinase activity and cell growth. *J Biol Chem* 287(33):28180–28194
 281. Kruis T et al (2010) Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. *J Clin Endocrinol Metab* 95(3):1137–1142
 282. Stannard B et al (1995) Single tyrosine substitution in the insulin-like growth factor I receptor inhibits ligand-induced receptor autophosphorylation and internalization, but not mitogenesis. *Endocrinology* 136(11):4918–4924

283. Hernandez-Sanchez C et al (1995) The role of the tyrosine kinase domain of the insulin-like growth factor-I receptor in intracellular signaling, cellular proliferation, and tumorigenesis. *J Biol Chem* 270(49):29176–29181
284. Blakesley VA et al (1995) Mutation of a conserved amino acid residue (tryptophan 1173) in the tyrosine kinase domain of the IGF-I receptor abolishes autophosphorylation but does not eliminate biologic function. *J Biol Chem* 270(6):2764–2769
285. Brodt P et al (2001) Cooperative regulation of the invasive and metastatic phenotypes by different domains of the type I insulin-like growth factor receptor beta subunit. *J Biol Chem* 276(36):33608–33615
286. Miura M et al (1995) Different effects on mitogenesis and transformation of a mutation at tyrosine 1251 of the insulin-like growth factor I receptor. *J Biol Chem* 270(38):22639–22644
287. Craparo A, Freund R, Gustafson TA (1997) 14-3-3 (epsilon) interacts with the insulin-like growth factor I receptor and insulin receptor substrate I in a phosphoserine-dependent manner. *J Biol Chem* 272(17):11663–11669
288. Furlanetto RW et al (1997) 14-3-3 proteins interact with the insulin-like growth factor receptor but not the insulin receptor. *Biochem J* 327(Pt 3):765–771
289. Peruzzi F et al (2001) Anti-apoptotic signaling of the insulin-like growth factor-I receptor through mitochondrial translocation of c-Raf and Nedd4. *J Biol Chem* 276(28):25990–25996
290. Li S, Resnicoff M, Baserga R (1996) Effect of mutations at serines 1280–1283 on the mitogenic and transforming activities of the insulin-like growth factor I receptor. *J Biol Chem* 271(21):12254–12260
291. Dews M et al (2000) Domains of the insulin-like growth factor I receptor required for the activation of extracellular signal-regulated kinases. *Endocrinology* 141(4):1289–1300
292. Hongo A et al (1996) Mutational analysis of the mitogenic and transforming activities of the insulin-like growth factor I receptor. *Oncogene* 12(6):1231–1238
293. Lamothe B et al (1995) Interaction of p85 subunit of PI 3-kinase with insulin and IGF-1 receptors analysed by using the two-hybrid system. *FEBS Lett* 373(1):51–55
294. O'Connor R (2003) Regulation of IGF-I receptor signaling in tumor cells. *Horm Metab Res* 35(11–12):771–777
295. Fernandez CA et al (2010) The anti-angiogenic peptide, loop 6, binds insulin-like growth factor-1 receptor. *J Biol Chem* 285(53):41886–41895
296. Dey BR, Furlanetto RW, Nissley P (2000) Suppressor of cytokine signaling (SOCS)-3 protein interacts with the insulin-like growth factor-I receptor. *Biochem Biophys Res Commun* 278(1):38–43
297. Dey BR, Furlanetto RW, Nissley SP (1998) Cloning of human p55 gamma, a regulatory subunit of phosphatidylinositol 3-kinase, by a yeast two-hybrid library screen with the insulin-like growth factor-I receptor. *Gene* 209(1–2):175–183
298. Gual P et al (1998) Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. *Endocrinology* 139(3):884–893