

THEMATIC REVIEW

40 YEARS OF IGF1

IGF system in sarcomas: a crucial pathway with many unknowns to exploit for therapy**Caterina Mancarella and Katia Scotlandi**

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Abstract

The insulin-like growth factor (IGF) system has gained substantial interest due to its involvement in regulating cell proliferation, differentiation and survival during anoikis and after conventional and targeted therapies. However, results from clinical trials have been largely disappointing, with only a few but notable exceptions, such as trials targeting sarcomas, especially Ewing sarcoma. This review highlights key studies focusing on IGF signaling in sarcomas, specifically studies underscoring the properties that make this system an attractive therapeutic target and identifies new relationships that may be exploited. This review discusses the potential roles of IGF2 mRNA-binding proteins (IGF2BPs), discoidin domain receptors (DDRs) and metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) in regulating the IGF system. Deeper investigation of these novel regulators of the IGF system may help us to further elucidate the spatial and temporal control of the IGF axis, as understanding the control of this axis is essential for future clinical studies.

Key Words

- ▶ sarcomas
- ▶ IGF system
- ▶ targeted therapy
- ▶ clinical trials

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Introduction

The insulin-like growth factor (IGF) system is involved in many physiological and pathological processes throughout the life span. For example, longitudinal growth; metabolism; longevity and cell development and diseases, such as cancer, obesity, eating disorders and neurodegenerative illnesses, are influenced by the IGF pathway. Thus, IGF signaling must be carefully regulated not only in its magnitude but also in its timing and understanding the mechanisms behind the cell-/tissue-specific regulation of IGF system components is critical for efficiently targeting the system. However, much still needs to be understood. This review highlights key examples of the role of the IGF system in sarcoma pathogenesis and tumor progression and identifies new questions that need to

be addressed to provide new fuel for the development of therapeutic strategies.

Key points

- Role of IGF system in sarcomas.
- Lessons learned from clinical studies on sarcomas vs carcinomas.
- Critical issues and novel perspectives.

The IGF system

The IGF system is a very complex system with multiple actors in play. Molecular details of the IGF system have been excellently reviewed by Samani and coworkers

(Samani *et al.* 2007) and Taniguchi and coworkers (Taniguchi *et al.* 2006). It is beyond the scope of this manuscript to provide a detailed description of the complexity and interactions of this system at the molecular level. However, to inform the reader of the peculiarities of this signaling axis, the most relevant and critical issues are briefly described.

The IGF system is classically described as composed of three ligands (IGF1, IGF2 and insulin), their receptors (IGF1 receptor (IGF1R), mannose 6-phosphate/IGF2 receptor (M6P/IGF2R), insulin receptor (IR) and hybrid IR/IGF1R), at least six IGF-binding proteins (IGFBP1–6), acid labile subunit (ALS) and binding protein proteases. Please refer to Fig. 1 for a schematic diagram of the system.

Receptors, ligands and IGFBPs

Insulin and IGFs activate intracellular signaling pathways by binding with high affinity to their cognate receptors (e.g. insulin → IR, IGFs → IGF1R) and with lower affinity to a noncognate receptor (e.g. insulin can also activate IGF1R and IGFs can activate IR). IGF2 also binds IGF2R, which is a mannose 6-phosphate scavenger receptor that does not transmit signals intracellularly. IR has two splice isoforms, IRA, which is highly expressed in fetal tissues and cancer, and IRB, which is mainly present in adult tissues (for a detailed review, please refer to Belfiore *et al.* 2009). While IRB is a specific receptor for insulin and thus primarily mediates metabolic effects, IRA binds IGF2 and IGF1 (at a lower affinity) and may induce biological effects in response to both IGFs with substantial crosstalk with the IGF1R mitogenic signaling pathway. IGF1R shares 70% homology with IR. Because of the close homology between IR and IGF1R, hybrid receptors can be formed via an interaction between an insulin alpha-beta hemireceptor and an IGF1 alpha-beta hemireceptor in cells expressing both. Hybrid receptors appear to bind IGF1 and IGF2 with a high affinity similar to IGF1R. The interaction between the ligands (IGF1 and IGF2) and IGF1R, IR or the hybrid receptors results in trans-autophosphorylation of the intracellular portion of the receptors and the subsequent recruitment of downstream signaling adaptor proteins, IR substrate (IRS) 1–6 and Src homology 2 domain-containing transforming protein (Shc), to the cell membrane (for reviews see Butler *et al.* 1998, Samani & Brodt 2001). The subsequent phosphorylation of these proteins induces the activation of phosphoinositide 3-kinase (PI3K) (Giorgetti *et al.* 1993) and mitogen-activated protein kinases (MAPK) pathways (Grey *et al.* 2003),

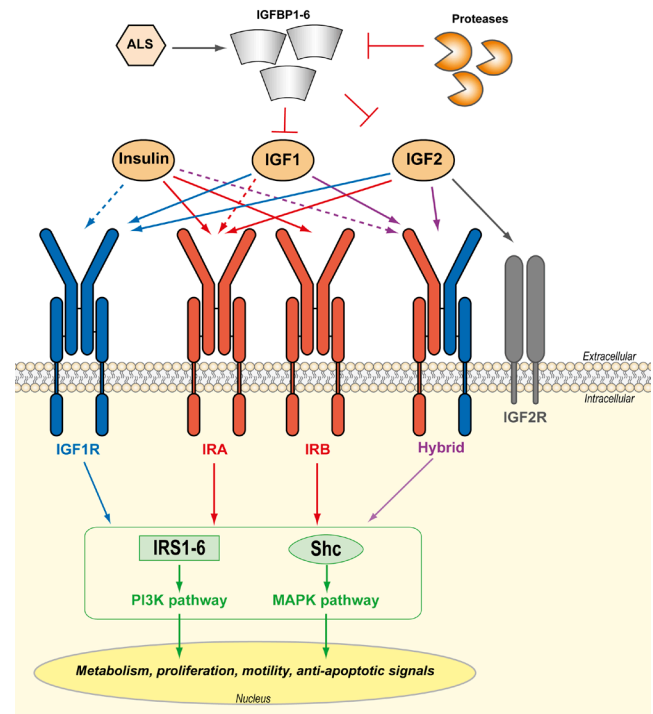


Figure 1

Schematic representation of the IGF system, interactions among its components and signal transduction. In the extracellular compartment, the ligands (IGF1, IGF2 and insulin) and the family of 6 IGFBPs, which are controlled by the activity of proteases and ALS, are depicted. Each ligand displays a specific binding affinity for the receptors: continuous arrows indicate high affinity, while dotted arrows indicate low affinity. IGF1R, IR isoforms (IRA and IRB), hybrid receptors and IGF2R are shown in the cell membrane. In the intracellular compartment, upon binding the ligand, the kinase domains of the receptors are activated and serve as docking sites for downstream substrates including IRS1–6 and Shc. These substrates initiate phosphorylation cascades through the PI3K or MAPK pathways, which transmit the signal to the nucleus. ALS, acid labile subunit; IGF, insulin-like growth factor; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IGF1R, insulin-like growth factor 1 receptor; IGF2R, insulin-like growth factor 2 receptor; IGFBPs, insulin-like growth factor binding proteins; IR, insulin receptor; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; Shc, Src homology 2 domain-containing transforming protein.

resulting in the stimulation of cellular proliferation and cell motility and the inhibition of apoptosis. Although the IGF system receptors have many similarities, the biological response elicited by each IGF system receptor can vary depending on (1) the ligands involved, (2) the expression of a specific IR isoform and (3) the recruitment of certain docking proteins and intracellular mediators, and the dynamics and regulation involved are still not completely understood (Frasca *et al.* 2008).

These complex functional interconnections between receptors and ligands imply that whenever the effects of IGF1R, IR or both are studied, the prevalent types and

expression levels of the ligand(s) in that specific cellular context should also be examined. When exploring IGF1R and IR signaling in cancer, considerable attention was given to abnormal signaling after the interactions between IGFs and their receptor/s. However, the mechanism by which IGFs and/or insulin in plasma are regulated prior to interacting with their receptor/s is as important as the intracellular signaling pathway. Many epidemiological studies reported that individuals with IGF1 levels at the upper end of the normal range have an increased risk of developing certain cancers, e.g. prostate, breast and colon cancers (Chan *et al.* 1998, Endogenous *et al.* 2010, Rinaldi *et al.* 2010). Conversely, individuals with Laron's syndrome, which is characterized by very low IGF1 levels, appear to be protected from the development of cancer (Shevah & Laron 2007); moreover, reducing circulating IGF1 levels by caloric restriction decreases tumor growth (Brandhorst *et al.* 2015).

Relevant checkpoints in regulating the IGF system

The level of free IGFs is affected by the rate of IGF production, clearance and binding to IGFBPs. IGFs circulate in large part bound to one of the six IGFBPs, mainly IGFBP3 and IGFBP5, fractions of which further form ternary complexes with ALS. This results in an increase in IGFs half-life (Baxter 2000). The IGFBPs circulate in molar excess of IGFs and have ligand affinities at the same order of magnitude as the ligands have for the IGF1R (Clemmons 1998). Most IGFBPs compete with IGFs for binding to respective receptors and antagonize IGF function, while some (e.g., IGFBP2) appear to amplify IGF signaling (Grimberg & Cohen 2000). Mohan and Baylink provided a detailed review on IGF-binding proteins that regulate the biological accessibility and activity of IGFs (Mohan & Baylink 2002). In addition to IGF-dependent mechanisms of actions, IGFBPs bind non-IGF ligands in the extracellular space, cell membrane, cytoplasm and nucleus, thereby modulating cell proliferation, survival and migration in an IGF-independent manner (Granata *et al.* 2004, Han *et al.* 2011).

Alterations in IGF-mediated functions also involve modulating IGF2R, which lacks transketolase activity, does not transmit signals, and may serve as a scavenger receptor for IGF2. Loss-of-functional IGF2R, a condition largely observed in tumors, likely facilitates an enhanced interaction between IGF2 and IGF1R (Devi *et al.* 1999, Martin-Kleiner & Gall Troselj 2010). In addition, alterations in phosphatase and tensin homologue (PTEN), which is a tumor suppressor and lipid phosphatase (Zhao *et al.*

2004), and altered expression of adaptor proteins IRSs (Chang *et al.* 2002) and Shc (Ursini-Siegel *et al.* 2008) are also involved in IGF signaling regulation.

Finally, recent discoveries on post-transcriptional and proteolytic regulation of IGF activity have provided novel insights into the intricate control of the IGF system.

To date, no reports have described mutations in any genes encoding IGFs, receptors or the six IGFBPs; however, a notable exception is osteosarcoma, where recurrent mutations in genes mediating IGF signaling via IGF1R (focal amplification of *IGF1R* and *IGF1*; frameshift indels in recessive cancer genes, *IGF2R* and *IGFBP5*) have been recently described in 7% of tumors (Behjati *et al.* 2017). More commonly, reports have described the expression of IGF system players, to a large extent, at the transcriptional and post-transcriptional levels with functions that can be influenced by genetic and epigenetic changes. Interestingly, mutations in metalloproteinase pregnancy-associated plasma protein-A2 (PAPP-A2), one of the proteases believed to increase the local bioavailability of IGFs by cleaving inhibitory IGFBPs (Conover 2012), were recently discovered in patients with growth failure (Dauber *et al.* 2016). The impact of these mutations in cancer, as well as the expression of stanniocalcin 1 and 2 (STC1 and STC2), which are potent proteinase inhibitors of PAPP-A (Jepsen *et al.* 2015, Klooverpris *et al.* 2015), have been poorly explored in relation to the IGF system; however, these alterations may significantly affect the role of PAPP-A in certain malignancies. Targeting the proteolytic activity of PAPP-A is relevant to preventing cancer growth and metastases (Becker *et al.* 2015), thus sustaining further investigations.

Beyond their canonical role in digesting extracellular matrix proteins, matrix metalloproteinases (MMPs) have a specific role in cleaving IGFBPs, thus leading to IGF liberation. In epithelial cells, IGFBP5 is cleaved by MMP7, which consequently releases IGF2 and promotes a proliferative cellular response (Hemers *et al.* 2005). In prostate cancer cells, MMP9 was described as having a proteolytic effect on IGFBP3 (Manes *et al.* 1999). In this perspective, targeting MMPs was postulated as a novel therapeutic strategy against cancer (Egeblad & Werb 2002).

Another interesting regulator of the IGF system are the IGF2 mRNA-binding proteins, a family of mRNA-binding proteins (RBPs) consisting of three paralogs (IGF2BP1, 2 and 3) involved in RNA localization, translation and stability (Christiansen *et al.* 2009). These RBPs are oncofetal proteins that are highly expressed during both human and mouse embryogenesis. Although

these RBPS may affect stability and translation of many transcripts, Nielsen and colleagues demonstrated that IGF2BPs specifically drive physiological regulation of *IGF2* translation at both a temporal and spatial level during late mammalian development (Nielsen *et al.* 1999). With the notable exception of IGF2BP2, IGF2BP1 and IGF2BP3 are expressed at low levels in adult tissues and are re-expressed in malignancies. Of note, IGF2BP3 has been described as playing a specific role in regulating *IGF2* and *IGF1R* mRNA expression that leads to increased IGF signaling in different tumor types (Hafner *et al.* 2010, Fawzy *et al.* 2016, Panebianco *et al.* 2017).

Moreover, previous studies described novel relevant interactions between IGF1R or IR and discoidin domain receptor 1 (DDR1). DDR1 belongs to a subfamily of membrane tyrosine kinase receptors, including five DDR1 isoforms and DDR2, and these DDRs bind to and are activated by different types of collagen (for a review, consider (Valiathan *et al.* 2012)). DDRs are characterized by an extracellular discoidin domain, a long juxtamembrane region and 13–15 tyrosine residues in their cytoplasmic domain that serve as binding sites for SH2 and phosphotyrosine-binding (PTB) domain-containing molecules for signal transduction. Interestingly, DDR1 constitutively associates with IGF1R, and this interaction is enhanced after IGF stimulation and leads to an increase in IGF1R expression levels as well as signaling and biological effects. Importantly, silencing DDR1 was found to decrease the IGF1R biological response after IGF stimulation, and the absence of IGF1R was reported to impair collagen-dependent DDR1 activation, thus further indicating the close crosstalk between the two molecules (Malaguarnera *et al.* 2015). Similarly, in response to insulin or IGF2, DDR1 co-localizes with IRA and influences its biological actions. Indeed, DDR1 knockdown inhibits IRA signaling, as well as IRA-elicited proliferation and migration after ligand stimulation. Importantly, DDR1 regulates IR expression levels through both transcriptional and post-transcriptional mechanisms (Morcavallo *et al.* 2011, Vella *et al.* 2017).

Please refer to Fig. 2 for a schematic representation of the IGF system and all relevant regulators controlling this axis.

Despite this level of complexity, IGF1R signaling has been the only focus of previous studies, and selective IGF1R inhibitors have been the first and, unfortunately, the only therapy to progress from bench to bedside. Considering these intricacies of the IGF system, it is now clear that the current understanding of this system is a

gross oversimplification of the real interconnections that may alter IGF-mediated signals.

Historically, IGF1R was chosen as the therapeutic target because of the following: (i) the first evidence by Sell and colleagues demonstrated that IGF1R is quasi-obligatory for cell transformation (Sell *et al.* 1993) and (ii) multiple preclinical studies demonstrated that downregulation or inhibition of IGF1R in malignant cells induces cell death, inhibits tumorigenesis and metastasis and increases chemosensitivity to conventional and targeted drugs.

It was therefore not surprising that targeting IGF1R became popular with pharmaceutical companies. Monoclonal antibodies and tyrosine kinase inhibitors were designed to specifically target IGF1R, and several phase I to III clinical trials were conducted. From these studies, we obtained some important indications: (1) anti-IGF1R drugs have modest toxic effects and (2) anti-IGF1R drugs show limited effectiveness. Because of this disappointing evidence, the development of anti-IGF1R agents was largely abandoned. However, sarcoma studies found a few extraordinary results that deserve deeper analysis and follow-up investigations.

IGF system in sarcomas

The IGF system has been demonstrated to be clearly involved in the pathogenesis and progression of sarcomas that tend to occur in younger patients, such as osteosarcoma, Ewing sarcoma and rhabdomyosarcoma. Although there are no direct data describing a relationship between IGF levels and sarcoma risk, the peak incidence of primary bone sarcoma (osteosarcoma and Ewing sarcoma) correlates with increased levels of IGF ligands in puberty. Growth hormone (GH) and IGFs are important regulators of growth and development in normal bone and contribute to approximately 50% of basal bone cell proliferation (for reviews see Cannata *et al.* 2010, Al-Kharobi *et al.* 2014). IGFs also promote differentiation of myoblastic or osteoblastic tissues into muscle and bone (Schmid *et al.* 1983), and glucose uptake favors osteoblast differentiation by suppressing AMP-activated protein kinase (AMPK)-dependent proteasomal degradation of Runx2 (Wei *et al.* 2015), a master determinant of osteoblast differentiation. Thus, it is conceivable that defects in molecules involved in IGF signaling may play a role in the formation of musculoskeletal tumors.

Previous studies described the expression of IGFs and IGF1R in tumor cells from primary high-grade osteosarcoma, which is an aggressive tumor derived from

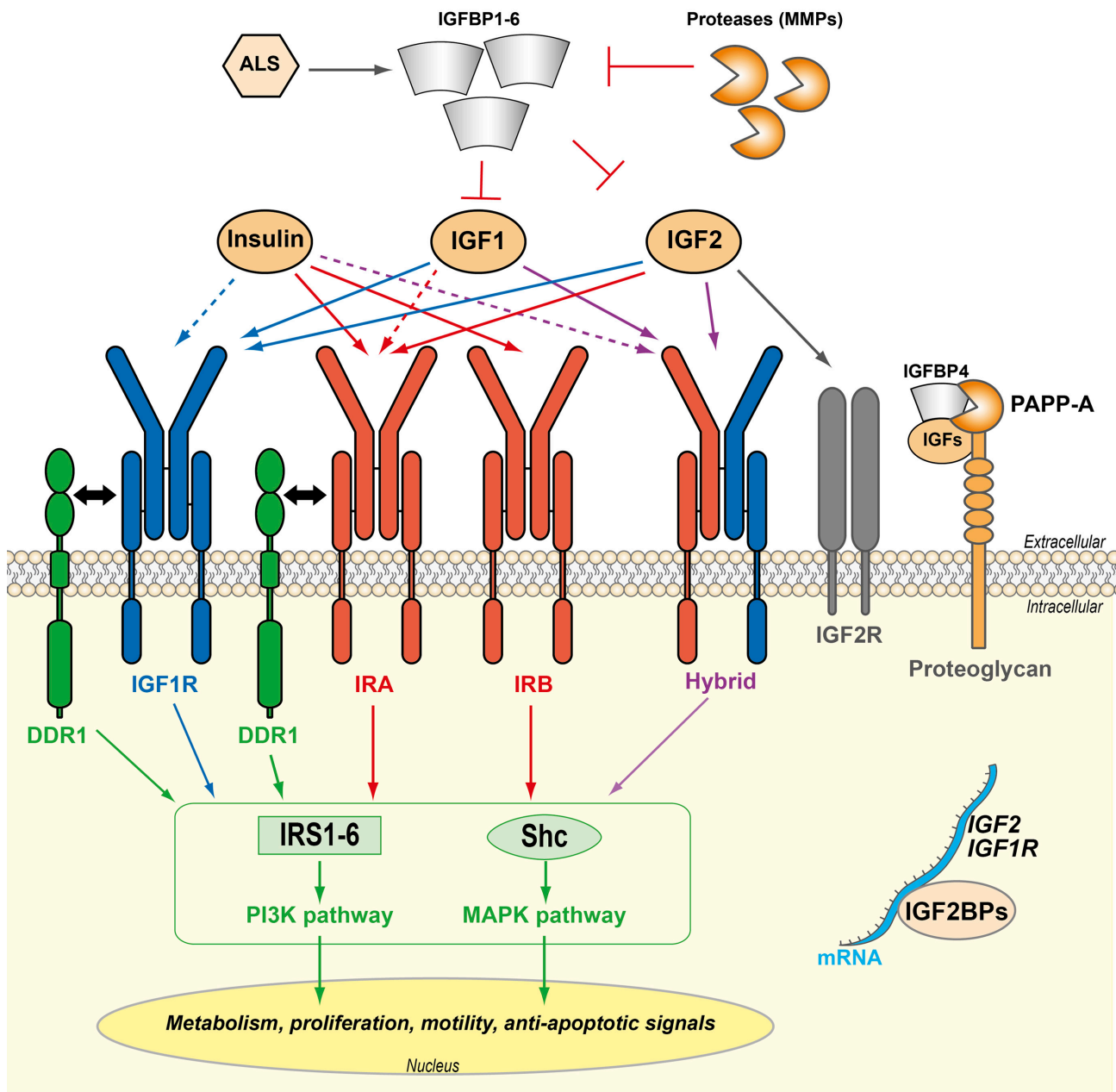


Figure 2

Schematic representation of the complexity surrounding the IGF system. Canonical components and interactions as well as novel regulators and binding partners of the IGF system are presented. The IGFBP family, ligands and their receptor-binding affinities are shown in the extracellular compartment: continuous arrows indicate high affinity, while dotted arrows indicate low affinity. As reported, IGFBP proteases include MMPs and PAPP-A. Specificity of PAPP-A for binding to the IGFBP4/IGF complex is shown. Alongside IRB, hybrid receptors and IGF2R, the direct interaction between DDR1 and IGF1R or IRA and the impact of these interactions on the PI3K and MAPK downstream pathways are shown in both the membrane and intracellular compartment. Cytoplasmic regulation of *IGF2* and *IGF1R* mRNA by IGF2BPs is depicted. ALS, acid labile subunit; DDR1, discoidin domain receptor 1; IGF, insulin-like growth factor; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IGF1R, insulin-like growth factor 1 receptor; IGF2BPs, insulin-like growth factor 2 mRNA-binding proteins; IGF2R, insulin-like growth factor 2 receptor; IGFBPs, insulin-like growth factor binding proteins; IR, insulin receptor; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; PI3K, phosphoinositide 3-kinase; Shc, Src homology 2 domain-containing transforming protein.

osteoblastic precursors and found that IGF and IGF1R expression levels are functionally related to malignant growth and invasion in several preclinical models

(Kappel *et al.* 1994, Benini *et al.* 1999, MacEwen *et al.* 2004, Duan *et al.* 2009, Kuijjer *et al.* 2013, Cao *et al.* 2014). Loss of IGFBPs and IGFBP-related proteins (IGFBP-rPs),

which are generally believed to inhibit the effects of IGFs, has also been found in osteosarcoma but not normal bone cells (Yang *et al.* 2016). Of note, during normal osteoblastic terminal differentiation, the expression of IGFs progressively increased, while the expression of IGF1R progressively decreased (Viereck *et al.* 2007), suggesting that upregulation of the receptor, rather than the ligands, is the aberrant condition in osteosarcoma. Accordingly, using fluorescence in situ hybridization, Behjati and coworkers (Behjati *et al.* 2017) found high-level amplification of *IGF1R* in 14% of cases, while mutations in genes mediating signaling via IGF1R were found in 7% of patients. Polymorphisms of *IGF2R* are also significantly associated with an increased risk for osteosarcoma (Savage *et al.* 2007), likely affecting interactions between ligands and IGF1R and leading to its increased activity. In addition, a multistage genome-wide association study of the incidence of metastasis at diagnosis in 935 osteosarcoma patients identified a SNP rs7034162 in the nuclear factor 1 B-type (*NF1B*) gene that was significantly associated with metastasis in European, African and Brazilian patients (Mirabello *et al.* 2015). This study therefore identified *NF1B*, which encodes a transcription factor that regulates IGF1R expression in human osteoblasts (Perez-Casellas *et al.* 2009), as an osteosarcoma metastasis susceptibility gene. Interestingly, IGF1R, the most abundant IGF1R stored in bone, inhibits tumor growth and metastasis of human osteosarcoma cells (Su *et al.* 2011, Luther *et al.* 2013).

The IGF1R pathway is a major autocrine loop that plays a key role in the pathogenesis and malignant behavior of Ewing sarcoma (Scotlandi *et al.* 1996, 1998, 2002, 2005, Scotlandi & Picci 2008). EWS-FLI1, a fusion gene and genetic hallmark of Ewing sarcoma, was found to have transforming properties only in the presence of IGF1R (Toretzky *et al.* 1997), and more recently, this fusion product was found to directly affect IGF1R signaling by either downregulating *IGF1R3* (Prieur *et al.* 2004) or increasing *IGF1* promoter activation (Herrero-Martin *et al.* 2009, Amaral *et al.* 2015). Robust upregulation of IGF1 was also described in mesenchymal progenitor cells transformed by EWS-FLI1 (Riggi *et al.* 2005).

Data showing that a PAX3/7-FOXO1 fusion protein, which is a genetic hallmark of alveolar rhabdomyosarcoma, activates the *IGF1R* gene promoter and increases expression of IGF1R are compelling (Ayalon *et al.* 2001). In addition, high levels of IGF2 in rhabdomyosarcoma were determined in previous studies describing loss of imprinting (Zhan *et al.* 1994, Wang *et al.* 1998), and IGF2 was found to cooperate with PAX3-FKHR in tumor oncogenesis (Wang

et al. 1998, de Souza *et al.* 2012). Patients with stage III rhabdomyosarcoma can be categorized into patients who will proceed either poorly or well by considering high or low levels of Akt phosphorylation (i.e., higher or lower tonic IGF1R signaling (Crist *et al.* 2001, Petricoin *et al.* 2007)). As such, these findings further support the importance of IGF1R as a potential therapeutic modality in alveolar rhabdomyosarcoma.

Similarly, IGF1R has been found to be highly expressed in desmoplastic small cell tumors due to a direct activation of the *IGF1R* promoter by EWS-WT1, a genetic hallmark of this tumor. Interestingly, a truncated version of the fusion protein EWS-WT1 that has alternative mechanisms of action still stimulated IGF1R expression such that the receptor upregulation remained a consistent consequence of the genetic aberration in the fusion protein (Karnieli *et al.* 1996, Werner *et al.* 2007). In synovial sarcoma, elevated expression of IGF2 is induced by synovial sarcoma oncoprotein SYTSSX and appears to be required for tumor formation *in vivo* (Sun *et al.* 2006).

High expression of IGF2 was also observed in gastrointestinal stromal tumor (GIST) (Steigen *et al.* 2009). In addition, approximately 7% of GISTs without tyrosine-protein kinase *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA*) mutations were found to express IGF1R in excess of that seen in *KIT* or *PDGFRA* mutant GIST, and cell death was observed after treating these cell lines with IGF1R inhibitors (Agaram *et al.* 2008, Tarn *et al.* 2008).

The utility of IGF1R inhibitors (monoclonal antibodies and small-molecule tyrosine kinase inhibitors) was demonstrated in preclinical models against Ewing sarcoma, rhabdomyosarcoma, osteosarcoma, synovial sarcoma cells and desmoplastic round cell tumor, and these inhibitors were convincingly shown to block cancer cell proliferation, survival and anchorage-independent growth *in vitro*; to block tumorigenesis, tumor invasion and metastasis and to sensitize cancer cells to chemotherapy and radiotherapy (Kalebic *et al.* 1994, Scotlandi *et al.* 1998, 2005, Benini *et al.* 2001, Martins *et al.* 2006, Manara *et al.* 2007, Kolb & Gorlick 2009).

Together, these findings strongly support the concept of pursuing IGF1R as a target for therapeutic intervention in these sarcomas.

Results from clinical trials

Numerous therapeutic agents targeting the IGF1R pathway have been developed. These agents include monoclonal

antibodies (mAb) that specifically inhibit IGF1R (dalotuzumab, figitumumab, cixutumumab, ganitumab, R1507 and AVE1642); several IGF1R/IR tyrosine kinase inhibitors (TKIs), including a dual inhibitor of IGF1R and IR OSI-906 (linsitinib) (Mulvihill *et al.* 2009) and, more recently, monoclonal antibodies against IGF1 and IGF2 (MEDI-573 and BI 836845) (Gao *et al.* 2011, Friedbichler *et al.* 2014).

Different anti-IGF1R mAbs have been tested in early clinical trials involving patients with carcinomas and sarcomas. In sharp contrast with the effects of IGF1R inhibitor monotherapy in a subset of patients with sarcoma, largely unimpressive or even pessimistic results were obtained from studies of carcinoma (Qu *et al.* 2017). Table 1 summarizes the main clinical results obtained from sarcoma studies.

Initial clinical trials in patients with advanced, treatment-refractory sarcoma treated with IGF1R mAbs provided some positive indications (Haluska *et al.* 2007, Tolcher *et al.* 2009, Kurzrock *et al.* 2010) and led to larger trials (for a review, refer to Iams & Lovly 2015). The most remarkable responses were obtained from studies describing Ewing sarcoma, in which a subset of patients showed a unique dependence on IGF1R signaling. To summarize, a complete response (CR) was achieved by a few patients with Ewing sarcoma, a partial response (PR) was achieved by 2–12% of patients, and disease stabilization rates ranged from 16% to 40% (Olmos *et al.* 2010, Juergens *et al.* 2011, Pappo *et al.* 2011, Tap *et al.* 2012). Much less impressive responses were observed in patients with recurrent or refractory rhabdomyosarcoma, synovial sarcoma and osteosarcoma (Pappo *et al.* 2014,

Table 1 Most important clinical studies involving anti-IGF1R agents in sarcomas.

Drug	Phase	Tumor types	Disease control	References
R1507	I	9 pts with multiple Sarcoma subtypes; 9 pts with Ewing sarcoma	Ewing sarcoma: 2 PR; 2 SD Leiomyosarcoma: 1 SD	Kurzrock <i>et al.</i> (2010)
Figitumumab (CP-751,871)	I	13 pts with multiple sarcoma subtypes; 16 pts with Ewing sarcoma	Ewing sarcoma: 1 CR; 1 PR; 6 SD Synovial sarcoma: 1 SD Fibrosarcoma: 1SD	Olmos <i>et al.</i> (2010)
Figitumumab (CP-751,871)	I	16 pts with Ewing sarcoma; 11 with osteosarcoma; 4 with other sarcomas 106 pts with Ewing sarcoma 16 pts with Ewing sarcoma; 11 with osteosarcoma; 4 with other sarcomas		Juergens <i>et al.</i> (2011)
Ganitumab	II	106 pts with Ewing sarcoma	15 PR; 25 SD	
	II	22 pts with Ewing sarcoma 16 with desmoplastic small round cell tumors	Ewing sarcoma: 1 PR; 8 SD DSRCT: 1 PR; 13 SD	Tap <i>et al.</i> (2012)
Cixutumumab (IMC-A12)	II			Schoffski <i>et al.</i> (2013) Pappo <i>et al.</i> (2011)
R1507	II	115 pts with Ewing sarcoma	1 CR; 10 PR; 18 SD	Pappo <i>et al.</i> (2014)
R1507	II	38 pts with osteosarcoma 36 pts with rhabdomyosarcoma 23 pts with synovial sarcoma 66 pts with other sarcomas	Osteosarcoma: 2 PR; 10 SD Rhabdomyosarcoma: 1PR; 6 SD Synovial Sarcoma: 4 SD Other: 1 PR; 22 SD	
Robatumumab	II	144 pts with Osteosarcoma or Ewing sarcoma metastasis	Osteosarcoma: 3/60 CR or PR Ewing sarcoma: 6/84 PR; 23/84 SD	Anderson <i>et al.</i> (2016)
Cixutumumab (IMC-A12)+temsirolimus	I	17 pts with Ewing's sarcoma 3 pts with desmoplastic small round cell tumors	Ewing sarcoma: 2 CR; 5 PR DSRCT: 1 PR, 1 SD	Naing <i>et al.</i> (2012)
Figitumumab (CP-751,871)+everolimus	I	19 pts with multiple Sarcoma subtypes	Solitary fibrous tumor: 1 PR; various: 14 DS	Quek <i>et al.</i> (2011)
Cixutumumab (IMC-A12)+temsirolimus	II	388 pts with bone and soft tissue sarcomas (61 pts with Ewing sarcoma)	No CR Ewing sarcoma: 4/27 PR Osteosarcoma: 3/24 PR Chondrosarcoma: 1/17 Solitary fibrous tumor: 1/8	Schwartz <i>et al.</i> (2013)
Cixutumumab + doxorubicin	I	Pts with multiple soft tissue sarcoma subtypes	Various: 5/26 PR 14/26 SD	Chugh <i>et al.</i> (2015)

CR, complete response; PR, partial response; pts, patients; SD, stable disease.

Anderson *et al.* 2016). In osteosarcoma, the lack of responses may have been due to the redundancy of autocrine loops that characterize this tumor (Benini *et al.* 1999), which likely prevented any efficacy of inhibitors specifically targeting single pathways. Of note, some evidence of efficacy was observed in osteosarcoma when multikinase inhibitor sorafenib was used (Grignani *et al.* 2015) in combination with everolimus, an mTOR inhibitor.

The combination of mTOR and IGF1R inhibition has been tested in patients with several types of advanced sarcomas and has occasionally yielded responses or prolonged disease stability in patients with metastatic solitary fibrous tumor; Ewing sarcoma; high-grade spindle cell sarcoma; osteosarcoma and, among soft tissue sarcoma, leiomyosarcoma (Quek *et al.* 2011, Naing *et al.* 2012, Schoffski *et al.* 2013). Considering the role of IGF1R in mediating resistance to chemotherapy, combinations of anti-IGF1R antibodies with cytotoxic agents, such as docetaxel, gemcitabine, erlotinib and doxorubicin, have also been tested (Macaulay *et al.* 2013, Chugh *et al.* 2015) and have yielded durable responses in a few patients with leiomyosarcoma. Toxicities observed were of a similar character and severity as those reported for each single agent, confirming the lack of serious safety concerns for agents targeting IGF1R even when used in combination. Moreover, the most common side effects, hyperglycemia and hyperlipidemia, are mostly tolerable and manageable. Combining anti-IGF1R mAbs with metformin, an anti-diabetic drug with anticancer efficacy (Martin & Marais 2012), may be a good choice to control hyperglycemia.

The major problems of IGF1R inhibition are not side effects but, rather, the rapid development of resistance with short-lived, clinical responses. Resistance to agents specifically targeting IGF1R is likely intrinsically associated with the complex, redundant interactions that characterize the IGF system. Garofalo and coworkers (Garofalo *et al.* 2011, 2012) and Beltran and coworkers (Beltran *et al.* 2011) proposed a compensatory signaling mediated by IGF2 through the IR that was not downregulated by anti-IGF1R antibodies. Alterations in downstream pathways, such as PI3K/AKT axis or RAS/MAPK, modulation in IGF1R expression or activation of alternative signaling have also been considered as potential mechanisms associated with resistance to anti-IGF1R agents (Cao *et al.* 2008, Haluska *et al.* 2008, Huang *et al.* 2010, Kang *et al.* 2014). Overexpression of epidermal growth factor receptor (EGFR) signaling was particularly suggested in adaptive resistance to IGF1R inhibition and vice versa (for a review see Jones *et al.*

2006), supporting the rationale for combining IGF1R and EGFR inhibitors, even in sarcomas. Particularly, the study performed by Huang *et al.* demonstrated the synergistic or additive effects given by the IGF1R/EGFR combined treatment in rhabdomyosarcoma cells (Huang *et al.* 2009). However, clinical trials have been disappointing. A phase I study in patients with solid tumors, including sarcomas, indicated that the human anti-IGF1R antibody AVE1642 was tolerable when combined with gemcitabine and erlotinib and achieved durable disease control in 44% of patients (Macaulay *et al.* 2013). However, a randomized phase 2 trial of erlotinib with or without the anti-IGF1R mAb R1507 failed to show difference in non-small-cell lung cancer and adding the dual IGF1R/IR linsitinib to erlotinib resulted in inferior outcomes compared with erlotinib alone (Leighl *et al.* 2017). Negative results were also obtained for colorectal cancer (Van Cutsem *et al.* 2014) and pancreatic cancer (Philip *et al.* 2014).

The appreciation of IR-mediated signaling as a potential mechanism of resistance to agents specifically directed against IGF1R has led to studies testing linsitinib, which simultaneously inhibits IGF1R and IR (Mulvihill *et al.* 2009), as well as antibodies directed against the IGF1 and IGF2 ligands (Gao *et al.* 2011) in patients with advanced solid tumors. Linsitinib has been evaluated as a single agent (Fassnacht *et al.* 2015, Jones *et al.* 2015, Puzanov *et al.* 2015) or, more recently, in combinations with paclitaxel or erlotinib in patients with metastatic carcinomas (Bendell *et al.* 2015, Ciuleanu *et al.* 2017, Leighl *et al.* 2017). Only very limited effects were reported. No data are available for sarcomas, although recent preclinical studies have indicated combined inhibition of IR and KIT as a potential therapeutic strategy in imatinib-resistant GISTs (Chen *et al.* 2017).

MEDI-573 has also been tested in patients with advanced, heavily pretreated solid tumors, and these patients exhibited a disease stabilization rate of 30% with no observed PRs or CRs (Haluska *et al.* 2014). A second phase I study of a Japanese population confirmed that MEDI-573 was well tolerated at the doses investigated but with limited efficacy (Iguchi *et al.* 2015).

Overall, clinical trials indicated that some treatment combinations induce disease stabilization in 20–40% of patients and clinical responses in a small percentage of patients with sarcoma, especially Ewing sarcoma.

Most studies noted the need of predictive biomarkers for identifying patients who would likely respond to this therapeutic strategy. To date, tumor expression of IGF1R (Schwartz *et al.* 2013) and its pathway components (Naing *et al.* 2011), serum IGF levels (Juergens *et al.* 2011,

Pappo *et al.* 2011, Macaulay *et al.* 2013), assessment of alternate pathway activation and attempts at identifying specific molecular signatures associated with IGF1R pathway dependence have been analyzed with varying levels of success. Exclusive nuclear localization of IGF1R was associated with responses to various IGF1R Abs in patients with sarcoma, suggesting that IGF1R nuclear translocation could also be a biomarker of IGF pathway activation (Asmane *et al.* 2012). However, despite these findings, the interpretation of the heterogeneous responses in terms of efficacy and tolerability to therapies targeting IGF1R inhibitors is still far from being fully elucidated.

Critical issues

1. Scheduling and identification of predictive biomarkers remain to be resolved.
2. The sample size of clinical studies was too small. Phase 3 clinical trials were only a small proportion of the enrolled studies, and the overall population was not large enough to obtain relevant data.
3. An oversimplified vision of IGF signaling has very likely limited a full appreciation of the pathway, leading to a dismal scenario for the development of therapeutic agents in cancer.

New perspectives

1. Several lines of evidence provide a rationale for combining IGF signaling inhibitors and immune therapies. The contribution of the IGF axis in regulating immune function was first highlighted in a series of studies reporting that downregulating IGF1 or IGF1R enhanced the immunogenicity of glioblastoma models in rats (Resnicoff *et al.* 1994) as well as in a small clinical study on astrocytoma (Andrews *et al.* 2001). IGF1 has also been implicated in the expression of immunosuppressive cytokines, including interleukin-10 (Kooijman & Coppens 2004), and the induction of the M2 macrophage phenotype (Barrett *et al.* 2015). A defect in IGF1/Akt signaling has been associated with a decreased capacity to induce the M2 state and an increased responsiveness to interferon gamma (IFN γ). This observation may be particularly relevant for osteosarcoma, in which tumor-associated macrophages (TAMs) were found to correlate with reduced metastasis and improved survival (Buddingh *et al.* 2011). Liposome-encapsulated muramyl tripeptide (L-MTP-PE), which enhances the

potential antitumor activity of macrophages, has been introduced in the treatment of osteosarcoma patients (Meyers & Chou 2014), and IL-10-polarized M2-like macrophages were able to reduce osteosarcoma cell growth in the presence of the anti-EGFR cetuximab in a mechanism involving antibody-dependent tumor cell phagocytosis (Pahl *et al.* 2014). In addition, inhibition of IGF1R promotes the expansion of activated human NK cells, which maintain antitumor responses against Ewing sarcoma (Jamitzky *et al.* 2015), indicating that combining adoptive NK cell transfer with IGF1R targeting may be an efficient strategy to eliminate minimal residual disease after conventional therapy.

2. Future clinical trial should select patients by specific biomarkers to increase efficacy. IGF1R antibodies were well tolerated in some trials, whereas, in others, they caused more severe side effects. The diverse effects and tolerance of IGF1R mAbs in different trials refer to many possible mechanisms, most of which were unclear. Understanding the mechanisms of these mAbs requires a better evaluation of the crosstalk between IGF1R and other signaling components (Lamhamedi-Cherradi *et al.* 2016) as well as consideration of players that have thus far been poorly considered in terms of IGF system regulation. These players may include the following:

- a. Evaluation of IGF2BP family members. Correlative and functional studies have extensively described the elevated expression of IGF2BPs in human cancer but not in adult normal tissues, indicating an oncogenic role in different tumor types, including leukemia, carcinoma and bone and soft tissue tumors. In particular, high IGF2BP3 expression was reported in leiomyosarcoma (Cornejo *et al.* 2012), osteosarcoma (Do *et al.* 2008), chondrosarcoma (Shooshtarizadeh *et al.* 2016) and Ewing sarcoma (Mancarella *et al.* 2016). Due to its ability to sustain *IGF2* expression with a consequent activation of IGF1R signaling pathway, IGF2BP3 may represent an intriguing biomarker to predict the therapeutic response to IGF1R inhibition. Accordingly, *in vitro* and *in vivo* evidence showed that sensitivity to the dual inhibitor linsitinib is higher in cancer cells overexpressing IGF2BP3 than in cells with low IGF2BP3 expression (Panebianco *et al.* 2017). In addition, IGF2BPs are envisioned as potential novel therapeutic targets, but so far, limited data are available regarding the direct inhibition of these proteins. *In vitro* evidence showed that treatment

with an isocorydine derivative (d-ICD) inhibited IGF2BP3 expression and reduced the growth of hepatocellular carcinoma cells (Li *et al.* 2015). Beyond the possibility to directly block IGF2BP3 expression, the role of IGF3BP3 as a vaccine target was postulated. The study from Tomita and coworkers indicated that immunogenic peptides derived from IGF2BP3 can induce tumor-reactive and human leukocyte antigen (HLA)-A2 (A*02:01)-restricted cytotoxic T lymphocytes (CTL) in lung cancer patients (Tomita *et al.* 2011), thus opening potential novel therapeutic avenues.

- b. Evaluation of the metalloprotease PAPP-A. For tumors expressing high levels of PAPP-A, benefits of inhibiting its proteolytic activity have been described. In Mikkelsen *et al.* (2014) developed an inhibitory monoclonal antibody targeting a unique substrate-binding exosite of PAPP-A (mAb-PA). This antibody was shown to inhibit the proteolytic cleavage of IGFBP4, which blocked the intracellular signaling of IGF1R *in vitro* and *in vivo* (Mikkelsen *et al.* 2014). Afterwards, the efficacy of the mAb-PA was tested in primary patient ovarian xenografts showing that the efficacy of mAb-PA was strongly dependent on PAPP-A expression levels. Addition of mAb-PA to standard chemotherapy improved tumor regression (Becker *et al.* 2015). A very interesting aspect of this therapeutic approach is that the mAb-PA reduces the bioavailability of IGF1 and IGF2 but not of insulin, sparing any side effects due to metabolic dysregulation. PAPP-A has been described to be overexpressed in Ewing sarcoma, and TCR transgenic T cells directed against PAPP-A were found to reduce tumor growth in mice (Kirschner *et al.* 2017), thus providing novel possibilities in therapeutic approaches. Considering that even minor modifications in the balance of active and inhibited PAPP-A may have a large effect on the local or systemic generation of bioactive IGF1, more detailed studies on how the expression of PAPP-A and its regulators stanniocalcin 1 and 2 (STC1 and STC2) are controlled are strongly encouraged. STCs are involved in diverse physiological processes, including osteoblast differentiation, adipogenesis and chondrogenesis, indicating a possible diverse, more complex role in sarcomas than those in carcinomas. Deeper investigation of these factors, together with IGFBPs in sarcomas, may help in elucidating the differences observed in clinical trials.
- c. Evaluation of DDRs. DDR1 was reported to act as an oncogene in different tumor types. However, in mesenchymal tumors, the functions of these receptors may be more complex, considering the role that DDRs play in regulating the interactions of mesenchymal cells with collagens and the processes of neural, osteogenic, chondrogenic and myoblast differentiation (Valiathan *et al.* 2012). A complex functional crosstalk involving IGF1R, IRA, DDR1 and other important signaling molecules like G protein-coupled receptors (GPCRs) has been described to affect gene expression and biological effects in response to IGF1 or IGF2 (Malaguarnera *et al.* 2015, Avino *et al.* 2016, Mata *et al.* 2016, Vella *et al.* 2017). This multifaceted signaling network has been poorly considered in both the search of predictive biomarkers and the design of new therapeutic strategies in cancers with a dysregulated IGF system; however, a better understanding of these interactions may be clearly relevant. A recently developed compound 7rh is a selective and orally available specific inhibitor of DDR1 enzymatic activity and was found to potently inhibit proliferation, migration and tumorigenicity of cancer cells expressing high levels of DDR1 (Gao *et al.* 2013) and to improve the chemoresponse when combined with paclitaxel (Aguilera *et al.* 2017). Therefore, based on the biological affinity between the receptors, combined inhibition of IGF1R or IRA and DDR1 may be considered and investigated as a potential therapeutic strategy, especially in the treatment of sarcomas.

Concluding remarks

The IGF system is clearly involved in regulating tumor growth and, even more importantly, in developing resistance to conventional and targeted drugs. Unfortunately, clinical trials have been dismal, and the field has been largely abandoned. However, an exciting new area of research could help us develop new diagnoses and novel therapeutic approaches. The IGF system is complex, and a more profound level of understanding is required to achieve full exploitation of the therapeutic potentialities of the system. Therefore, clinical and basic researchers should continue to work together to provide new treatments for sarcoma patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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