Targeting the Insulin-Like Growth Factor (IGF) System Is Not as Simple as Just Targeting the Type 1 IGF Receptor

By Katia Scotlandi, PhD, and Antonino Belfiore, MD

Overview: **Increased signaling of the insulin-like growth factor (IGF) system via alterations in expression levels of its components has been demonstrated in various tumor types. Numerous experimental studies have supported the involvement of the IGF system signaling axis in tumor initiation and progression. These studies, combined with data that link alterations in the levels of circulating IGFs with cancer risk and prognosis, have focused on the IGF-1 receptor (IGF-1R) as a therapeutic target for patients with cancer. As a consequence, most therapeutic strategies have been designed to specifically inhibit IGF-1R but have for the most part ignored the insulin receptor (IR), based on concerns that targeting IR would lead to unacceptable toxicity both because of its role in physiologic metabolism and because we frequently try to oversimplify biologic complexity whenever we are urged to find practical, friendly solutions for clinical practice. Although this is an understandable and necessary starting point in the complex and long-lasting processes that leads to translational biology, the crude reality of the results obtained from phase I and II studies suggest a need for researchers to be humble and go back to the drawing board. Cancer research has substantially neglected the role of IR, and it remains unclear whether and to what extent avoiding the inhibition of IR has compromised the efficacy of anti–IGF-1R therapy. Clarifying its role might also help us take advantage of older drugs that could offer new perspectives in cancer care.**

THE IGF system comprises a phylo-genetically ancient family of peptides involved in growth, development, and metabolism, as well as in cellular processes such as proliferation, survival, cell migration, and differentiation. The IGF system is composed of three ligands (IGF-1, IGF-2, and insulin), their receptors (the IGF-1 receptor [IGF-1R], the mannose 6-phosphate/IGF-2 receptor [M6P/IGF-2R], the insulin receptor [IR], and the hybrid IR/IGF-1R), at least six high-affinity binding proteins and binding protein proteases. IGF binding proteins modulate the activity of IGFs but also have a life of their own inducing cellular processes in an IGF-independent way. Molecular details of the IGF system have been excellently reviewed by Samani and colleagues.[1]

In this context, it is important to highlight the complexity of the system and the presence of several critical nodes that within the signaling networks control various cellular processes. A simple scheme of divergent pathways is usually sufficient to describe and explain IGF/insulin signaling (Fig. 1). However, when examined in detail, the number of genes and protein isoforms involved in the activation of mitogen-activated protein kinase (MAPK) or AKT signaling pathways, the two main signaling mediators of the IGF system or in genes involved in the generation of proliferative, anti-apoptotic, differentiating, or metabolic effects, it becomes clear that hundreds of molecules are involved in the IGF/insulin-signaling pathway. It is beyond the scope of this manuscript to describe in detail this molecular level of complexity and interactions[2] but, to make the reader more aware of the peculiarities of this signaling axis, some examples of at least the best-defined critical nodes are described.

For instance, the IR has two splice isoforms, IR-A that is highly expressed in fetal tissues and cancer, and IR-B that is mainly found in adult tissue (details to follow). Both are usually coexpressed in cells that also express IGF-1R. Insulin and IGFs bind with high affinity to their cognate receptors (e.g., insulin → IR; IGFs → IGF-1R), whereas IGF-2R serves mainly as a sink for the regulation of IGF2 levels. However, IGF-2 also binds IR-A with high affinity,[3] although at lower affinity, IGF-1R can also be activated by insulin, and IR can be activated by IGFs. This implies that whenever we study the effects of IGF-1R, IR, or both, we should also pay attention to the most prevalent types and expression levels of the ligand(s) in that specific cellular context.

Similar to IR, IGF-1R consists of two extracellular ligand-binding subunits (the alpha subunits) and of two transmembrane beta subunits, which are linked to alpha subunits by disulfide bonds and are composed of a transmembrane domain, an intracellular tyrosine kinase (TK) domain, and a C-terminal tail. IGF-1R has 70% homology to IR, with which it shares some signaling pathways. As a consequence of the close homology of IR and IGF-1R, hybrid receptors can be formed by an insulin alpha-beta hemireceptor and an IGF-1 alpha-beta hemireceptor in cells expressing both. The biologic response elicited by these hybrid receptors can vary, depending on the ligands involved and the specific IR isoforms.[4] These hybrid receptors appear to bind IGF-1 and IGF-2 with high affinity similar to IGF-1R. Ligand binding induces tyrosines within the TK domain to be transphosphorylated by the dimeric subunit partner. Phosphorylated residues serve as docking sites for other signaling molecules, such as IR substrates (IRS) and the adaptor protein Shc, which leads to the activation of the phosphatidylinositol-3 kinase (PI3K)-Akt and MAPK pathways (Fig. 1). However, both IR and IGF-1R can phosphorylate at least six known substrate proteins (IRS1-6) that are capable of interacting with eight known forms of the PI3K regulatory subunit, which leads to the activation of three known isoforms of AKT signaling, besides being able to crosstalk with the MAPK signaling pathway. Moreover, these signaling pathways are shared with most other TK receptors and it is possible that other pathways that have yet to be identified are involved or

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that subtle differences in the recruitment of certain docking proteins and intracellular mediators exist about which we still know little regarding their dynamics and biologic effects. Finally, in addition to the well-established signaling pathway from the cell membrane, recent reports have highlighted how IGF-1R or IR signaling, or both, may also be dependent on cell localization. Differential endocytosis and signaling dynamics have been reported for IGF-1R[5] as well as for IR-A and IR-B in relation to the mitogenic or metabolic activities of the two receptors.[6] Nuclear translocation of IGF-1R has also been reported.[7] The modification of IGF-1R by small ubiquitin-like modifier (SUMO) proteins occurs in a ligand-dependent manner and is necessary for nuclear translocation of the receptor. As expected, nuclear IGF-1R has different biologic functions: it binds to genomic DNA and may act as a transcriptional enhancer.

This level of complexity has been grossly ignored when targeted therapies against IGF-1R were developed. However, despite the difficulties of deeply understanding such complex signaling, steps have been recently taken and indeed some achievements have already been obtained.

Why Was IGF-1R Chosen as a Therapeutic Target?

The first inkling that IGF-1R played a crucial role in malignant transformation was provided by Sell and colleagues (1993), who found that R cells could not be transformed by the SV40 large T antigen. R cells are 3T3 cells originating from mouse embryos with a targeted disruption of the IGF-1R gene. These cells have a tendency to transform spontaneously in culture, and the SV40 T antigen is, by itself, a strong transforming agent in 3T3c cells. The failure of R cells to become transformed by SV40 T antigen indicated a role of the IGF-1R in transformation of cells in culture. This finding has since been confirmed with different viral and cellular oncogenes and in different laboratories.[8] Reintroduction of an IGF-1R into R cells promptly renders these cells susceptible to transformation. Thus, there should be a signal originating from the IGF-1R that facilitates and is quasi-necessary for the transformation by the usual agents (i.e., physical, chemical, and/or genetic). The level of expression of IGF-1R does not need to be high. Even low levels of expression are sufficient to send the permissive signal that allows oncogenes to transform mammalian cells. Accordingly, IGF-1R is overexpressed in some malignant tissues but amplification and overexpression are less common for IGF-1R than for other oncogenetic receptors. Similarly, mutations have not been described as a way to increase receptor activity. Activation of IGF-1R is indeed mainly induced by either circulating or locally synthesized IGFs in an autocrine or paracrine manner.[9] Although some studies report no relationship between IGF-1 levels and cancer risk, many others report that individuals with IGF-1 levels at the upper end of the normal range have an increased risk of developing certain cancers (e.g., colon, breast, and prostate).[10] Conversely, individuals with growth hormone receptor deficiency, also known as Laron syndrome, who have very low IGF-1 levels, appear to be protected from the development of cancer when compared with their relatives without hormonal deficiency.[11,12] Dietary factors and lifestyle have also been shown to have a substantial effect on the activation of the IGF system. In animal models, caloric restriction reduced circulating IGF-1 levels as well as bladder tumor growth, by increasing apoptosis and decreasing cell proliferation.[13]

In any case, if IGF-1R is quasi-obligatory for cell transformation, downregulation of IGF-1R in malignant cells ought to reverse the transformed phenotype. Downregulation or inhibition of IGF-1R functions, by neutralizing antibodies or small-molecule TK inhibitors (TKIs), by antisense strategies, or by developing agents to modulate IGF binding proteins, causes massive apoptosis of tumor cells in vitro and in vivo and results in the inhibition of tumorigenesis and metastasis formation. Preclinical data suggest that agents used to target the IGF system may be more effective when used in combination with chemotherapy compared with when used as monotherapy. In addition, the induction of IGF-1R signaling has been described to be involved in mediating resistance to both conventional and some targeted drugs.[14-16] It is therefore not surprising that targeting IGF-1R has become popular with pharmaceutical and biotechnology companies. Currently, most therapeutic agents, monoclonal antibodies (MAbs), or TKIs have been designed to specifically target IGF-1R while sparing IR, on the basis of the concern that cotargeting IR would lead to unacceptable toxicity. MAbs targeting IGF-1R were the furthest in development and had the benefit of inhibiting hybrid receptors besides IGF-1R. Recently, several phase I to III clinical trials have been conducted to evaluate the safety and efficacy of drugs targeting the IGF-1R. From these studies, we obtained some important indications: (1) anti–IGF-1R drugs have modest toxic effects, with mild and reversible hyperglycemia as the most common toxicity; and (2) anti-IGF-1R drugs show limited effectiveness. In particular, the best tumor responses have been observed in Ewing's sarcoma, in which IGF/IGF-1R functions have been clearly associated with the pathogenesis of this tumor and in which few, if any, other TK receptors are fundamentally activated.[9] However, despite the presence of the target

KEY POINTS

- The insulin-like growth factor (IGF) system is an important mediator of cancer pathogenesis and progression. Drug resistance to conventional or targeted therapies frequently involves components of the insulin-like growth factor system.
- Researchers and pharmaceutical companies have focused on IGF-1 receptor (IGF-1R), which is clearly able to deliver a proliferative, antiapoptotic, and promigratory signal in cancer cells.
- Several approaches inhibiting IGF-1R functions have shown very encouraging results in preclinical conditions, but only limited evidence of efficacy has been demonstrated in phase I and II clinical studies.
- The IGF system is quite complex, with many players in the field. Insulin receptor function in cancer cells has been underestimated, but also little attention has been paid to the type of ligands that are mainly involved in each tumor type.
- Strategies considering the IGF system in all its complexity are encouraged.

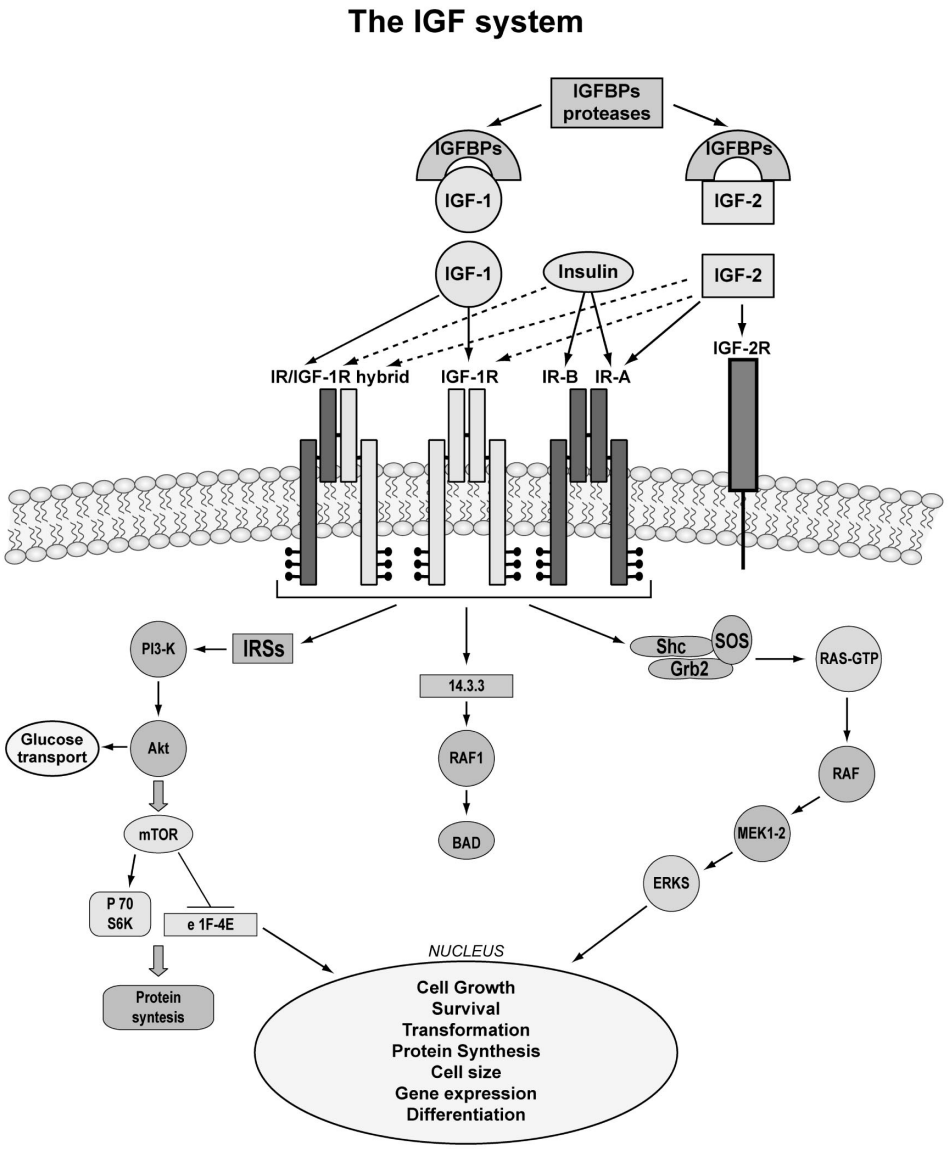

Fig. 1. The IGF system.[5]
Abbreviations: ERK, extracellular regulated kinase; IGF, insulin-like growth factor; IRS, insulin receptor substrate; PI3-K, phosphoinositide-3-kinase; mTOR, mammalian target of rapamycin.

in all tumors and ample preclinical evidence supporting the potential value of anti–IGF-1R agents, less than 10% of patients respond to this therapy with extraordinary results.[17]

On one side, this implies that the presence of the target is not sufficient to benefit from this targeted therapy and that other redundant pathways may be present to render IGF-1R–targeted cells resistant to anti–IGF-1R MAb. Recent studies in cell lines have demonstrated that knocking out, downregulating, or pharmacologically inhibiting IGF-1R can lead to a compensatory increase in IR signaling.[18-21] So, the take-home messages of these studies are: (1) the ratio IGF-1R/IR as well as the type of ligand(s) that are prevalent in the specific cellular context should be considered to identify patients that may benefit from anti–IGF-1R therapy; (2) we need to better identify the mechanisms of action of IR in cancer, viewing this receptor in a new light.

Insulin/IR in Cancer

As mentioned above, IR shares high homology to IGF-1R. However, unlike IGF-1R, the IR is characterized by the ability to alternatively splice a small exon (exon 11) encoding a 12–amino acid stretch contiguous to the CT peptide (encoded by the C-terminal sequence). The exclusion of exon 11 generates isoform A (IR-A); its inclusion generates isoform B (IR-B).[4] Although the current thinking is that IR primarily mediates the metabolic effects of insulin through the activation of the PI3K pathway and IGF-1R mainly mediates the growth effects of IGFs via the activation of MAPK, it is now clearly established that in cancer cells IR, particularly IR-A, is often overexpressed and its signaling pathway deregulated with substantial crosstalk with the IGF-1R pathway. Several factors account for the loss of IR physiologic specificity in cancer. First, cancer cells predominantly overexpress the IR-A isoform.

Although IR-B is a specific receptor for insulin, IR-A also binds IGF-2 and at lower affinity IGF-1, and may induce biologic effects in response to both IGFs. Second, overexpressed IR enhances the effects of IGF-1 and IGF-2 through the formation of IR/IGF-1R hybrid receptors, which bind both IGFs with high affinity. Third, cancers often produce both IGF-2 and IGF-1 in an autocrine/paracrine manner.[1] Finally, in patients with cancer affected by insulin resistance, the elevated levels of circulating insulin induce unbalanced IR activation, with predominant activation in the mitogenic pathway rather than the metabolic pathway.[22,23]

The importance of IR and insulin in tumor development and progression has been demonstrated in both animal models and clinical studies.[10] In humans, high levels of insulin—but not blood glucose or obesity per se—are associated with increased risk for various malignancies.[24] Women with breast cancer that also have insulin resistance show increased cancer-specific mortality.[25,26]

Obesity is a very important determinant for inducing or worsening insulin resistance. Obesity is also a predisposing factor of type 2 diabetes (T2DM), and several studies have now firmly established that both obesity and/or T2DM are associated with an increased risk of cancer.[23,27,28] Patients with T2DM carry an increased risk for almost every cancer histotype, except prostate cancer. Obese patients are at increased risk for a variety of malignancies, including most common cancers and hematologic malignancies. Metabolic syndrome, a disorder characterized by obesity, hypertension, dyslipidemia, and long-term insulin resistance, is also associated with worse cancer prognosis.[29] Conversely, body weight reduction decreases cancer risk.[22] Because of these findings and considering the studies suggesting that insulin analogs may promote tumorigenesis,[10] the effects of insulin/IR on tumor growth have recently received greater attention.

New Opportunities for Cancer Prevention and Therapy Involving the IR pathway

As long-term exposure to hyperinsulinemia is an important risk factor for cancer development and progression in patients with obesity, T2DM, or both, measures and drugs aimed at improving insulin resistance and reducing circulating insulin levels should contribute to prevent cancer in these patients and to ameliorate prognosis in patients with cancer.[25,26] Nonpharmacologic measures, such as lifestyle changes involving caloric restriction and physical exercise, may also be useful.

Among drugs aimed at reducing insulin resistance and circulating insulin levels, biguanides and thiazolidinediones (TZDs) (collectively classified as insulin sensitizers) have received attention as potential anticancer agents. Metformin is the only biguanide used in the clinical setting and is currently recommended as first-line therapy in patients with T2DM for its excellent long-term safety profile. Metformin impairs the production of adenosine 5′-triphosphate (ATP) by targeting complex I in the mitochondrial electron transport chain. This event activates AMP-activated protein kinase (AMPK), a kinase with a key role in the regulation of cellular energy homeostasis and growth. AMPK causes, on one hand, downregulation of gluconeogenesis in the liver with reductions in blood glucose and insulin levels and, on the other hand, direct reduction of cell growth through the inhibition of the mTOR/AKT pathway. Indeed, in vitro studies and animal models strongly suggest that metformin may have anticancer effects.[30,31] In humans, observational clinical studies have actually shown a decrease in cancer risk in patients with T2DM using metformin compared with those following other treatment regimens. In a case-control study, metformin use was associated with a reduced risk for breast cancer.[32] Moreover, the adjunct of metformin to insulin was reported to offset the increased risk for colorectal or pancreatic cancer observed when insulin was used as monotherapy, and patients with T2DM treated with metformin were found to have a reduced cancer-specific mortality compared with those using insulin. From a therapeutic point of view, metformin may improve response rates in women with breast cancer that have T2DM who are receiving adjuvant chemotherapy,[33] as well as progression-free survival for chemotherapy-treated patients with advanced cell lung cancer that have diabetes.[34] Currently, pilot clinical trials are being conducted with women without diabetes to evaluate the possible effect of metformin on the outcome of breast cancer (clinical trials NCT00897884 and NCT01101438).

TZDs, the second class of insulin sensitizers available for clinical use, belong to the group of peroxisome proliferator-activated receptor gamma (PPARgamma) agonists. These drugs have shown substantial antitumoral effects in vitro and in some, but not in all, animal models. However to date, enthusiasm for the anticancer potential of the currently available TZDs has declined because of toxicity and an associated increased risk of tumors.[35] When new TZDs reach the market, more studies are warranted to explore the effects of these drugs for patients with cancer who have insulin resistance.

As mentioned above, IR-A overexpressed by cancer cells may be stimulated not only by circulating insulin, but also by autocrine- or paracrine-produced IGF-2 and IGF-1. In patients with cancers overexpressing IR-A and IGFs, therefore, lowering insulin levels with the use of insulin sensitizers is not sufficient and direct inhibition of this IGFs/IR-A pathway should be pursued.

These considerations, together with evidence indicating that selective IGF-1R inhibitors can favor the emergence of cell clones with enhanced IGFs/IR-A loops[21,36] and worsen hyperinsulinemia,[37] bring about the concept that cotargeting IGF-1R and IR may be a suitable approach for patients with these malignancies. Small molecules with TK inhibitory activity appear to be the most promising drugs because of their ability to block the ATP-binding site of the kinase domain, which shares a high degree of homology between IR and IGF-1R. These drugs can be given orally and administered in combination with standard chemotherapy. Two currently available TKIs (BMS-754807 and OSI-906) share the ability to inhibit both IR and IGF-1R. BMS-754807 inhibits both IGF-1R and IR with very similar activity (the half maximal inhibitory concentrations [IC_{50}] were 1.8 nmol/L and 1.7 nmol/L, respectively),[38] but also elicits substantial inhibition toward other TK receptors (e.g., Met, recepteur d'origine nantais [RON], TrkA, TrkB) and Aurora A and B. BMS-754807 is currently being evaluated in several clinical trials as a single agent and in combination with other drugs in patients with advanced or metastatic

malignancies (clinical trials NCT00898716, NCT00569036, NCT00908024, NCT00788333, NCT01225172, and NCT-00793897). OSI-906 is a selective dual-inhibitor of IR and IGF-1R (IC_{50} of 19 nmol/L to 35 nmol/L).[39] Preliminary studies with OSI-906 have yielded encouraging results and this drug is now being evaluated in phase I escalation studies as a single agent (clinical trials NCT00514306 and NCT00514007) and in phase I to III trials (clinical trials NCT01101906, NCT00924989, NCT01387386, and others) for various malignancies generally characterized by an activated IGF-2/1R-A loop.

These studies will hopefully provide proof-of-concept that IR inhibition, in addition to IGF-1R inhibition, may be clinically relevant for patients with cancer.

Other Controversial Issues

In addition, IGF-1R was shown to mediate differentiation in some cancers. Specific experimental models indicated that the balance between mitogenesis and differentiation is strongly influenced by the relative level of expression of the two main IGF-1R mediators, Shc and IRS1.[40] Unfortunately, this is not a general rule and the exact mechanism that shifts the message from IGF-1R is still unknown. In any case, evidence indicates that the IGF system is an important mediator of mesenchymal or neural differentiation, an aspect that we need to consider for sarcomas and brain tumors. We need to be aware that IGF-1R and its substrates can also send contradictory signals, signals that can actually lead to growth inhibition or to the inhibition of metastatic spread. These contradictions ought to become fertile areas of investigation for both basic and applied research.

There is no doubt that anti–IGF-1R therapy should be combined with conventional or other targeted drugs. Each tumor requires a unique cocktail of drugs and dedicated studies. This concept is true for most targeted therapies and further effort, time, and resources to be translated into effective treatments are needed. The results achieved to date are not satisfactory to justify routine clinical use of IGF-1R–targeting agents. Nevertheless, for some heavily pretreated patients with refractory rare tumors, responses and clinical benefit in combination with chemotherapy have been observed. Unfortunately, rare tumors seem to be most sensitive to these targeted therapies, which does not inspire pharmaceutical companies. However, we strongly believe that joint efforts between academia and industry are in the interests of both. We have a good level of knowledge in the field and several drugs already developed. Just put them together and take another step toward light.

Conclusion

Recent phase I to II clinical studies with selective anti–IGF-1R MAbs together with epidemiologic data have shifted attention from IGF-1R to IR, and to a more comprehensive view of the IGF system. To date, small molecules acting as dual inhibitors of IGF-1R and IR appear to be the most promising approaches to deprive cancer cells of this important signaling axis. Unfortunately, hyperglycemia and hyperinsulinemia are important adverse effects of dual IGF-1R and IR inhibitors, although hyperglycemia seems to be reversible after the cessation of treatment. It can be hypothesized, therefore, that insulin sensitizers (e.g., metformin) should be given together with these inhibitors to limit these adverse effects.

Moreover, we need new biomarkers to select patients suitable for IR and IGF-1R dual inhibition and to monitor therapeutic efficacy. Recently, it has been reported that response to a dual anti–IR/IGF-1R inhibitor may be correlated with an IGF expression signature[41] or with lack of epithelial-mesenchymal transition,[42] but we clearly need more extensive studies and validated biomarkers.

Another possible approach involves the use of antibodies recognizing both IGF-2 and IGF-1. Such antibodies have been described[43] and, in animal models, show promising results in IGFs-driven malignancies.[44] Also in this case, more studies are needed regarding the applicability of this approach in humans.

Overall, recent experimental evidence has shed light on to some new players, like IR, which has been substantially neglected in the field of cancer research as a mediator of tumor progression. We are now well aware of the complexity of the pathway and have some new potentially promising drugs in our hands. Further efforts are needed to learn how to maximize their efficacy in patients with cancer.

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REFERENCES

1. Samani AA, Yakar S, LeRoith D, et al. The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocr Rev.* 2007;28:20-47.

2. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol.* 2006;7:85-96.

3. Frasca F, Pandini G, Scalia P, et al. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol Cell Biol.* 1999;19:3278-3288.

4. Belfiore A, Frasca F, Pandini G, et al. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev.* 2009;30:586-623.

5. Martins AS, Ordonez JL, Amaral AT, et al. IGF1R signaling in Ewing

sarcoma is shaped by clathrin-/caveolin-dependent endocytosis. *PLoS One*. 2011;6:e19846.

6. Giudice J, Leskow FC, Arndt-Jovin DJ, et al. Differential endocytosis and signaling dynamics of insulin receptor variants IR-A and IR-B. *J Cell Sci*. 2011;124:801-811.

7. Sehat B, Tofigh A, Lin Y, et al. SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor. *Sci Signal*. 2010;3:ra10

8. Baserga R. The insulin-like growth factor-I receptor as a target for cancer therapy. *Expert Opin Ther Targets.* 2005;9:753-768.

9. Scotlandi K, Picci P. Targeting insulin-like growth factor 1 receptor in sarcomas. *Curr Opin Oncol.* 2008;20:419-427.

10. Gallagher EJ, LeRoith D. Minireview: IGF, insulin, and cancer. *Endocrinology*. 2011;152:2546-2451.

11. Steuerman R, Shevah O, Laron Z. Congenital IGF1 deficiency tends to confer protection against post-natal development of malignancies. *Eur J Endocrinol*. 2011;164:485-489.

12. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, et al. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med*. 2011; 3:70ra13.

13. Dunn SE, Kari FW, French J, et al. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res.* 1997;57:4667-4672.

14. Benini S, Manara MC, Baldini N, et al. Inhibition of insulin-like growth factor I receptor increases the antitumor activity of doxorubicin and vincristine against Ewing's sarcoma cells. *Clin Cancer Res.* 2001;7:1790-1797.

15. Bodzin AS, Wei Z, Hurtt R, et al. Gefitinib resistance in HCC Mahlavu cells: upregulation of CD133 expression, activation of IGF-1R signaling pathway, and enhancement of IGF-1R nuclear translocation. *J Cell Physiol*. Epub 2011 Sep 29.

16. Rosenzweig SA. Acquired resistance to drugs targeting receptor tyrosine kinases. *Biochem Pharmacol*. Epub 2011 Dec 26.

17. Olmos D, Postel-Vinay S, Molife LR, et al. 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 1 expansion cohort study. *Lancet Oncol.* 2010;11:129-135.

18. Buck E, Gokhale PC, Koujak S, et al. 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*. 2010;9: 2652-2664.

19. Ulanet DB, Ludwig DL, Kahn CR, et al. Insulin receptor functionally enhances multistage tumor progression and conveys intrinsic resistance to IGF-1R targeted therapy. *Proc Natl Acad Sci U S A*. 2010;107:10791-10798.

20. Dinchuk JE, Cao C, Huang F, et al. Insulin receptor (IR) pathway hyperactivity in IGF-IR null cells and suppression of downstream growth signaling using the dual IGF-IR/IR inhibitor, BMS-754807. *Endocrinology*. 2010;151:4123-4132.

21. Garofalo C, Manara MC, Nicoletti G, et al. Efficacy of and resistance to anti-IGF-1R therapies in Ewing's sarcoma is dependent on insulin receptor signaling. *Oncogene*. 2011;30:2730-2740.

22. Kaaks R, Lukanova A. Effects of weight control and physical activity in cancer prevention: role of endogenous hormone metabolism. *Ann N Y Acad Sci.* 2002;963:268-281.

23. Vigneri P, Frasca F, Sciacca L, et al. Diabetes and cancer. *Endocr Relat Cancer.* 2009;16:1103-1123.

24. Pisani P. Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. *Arch Physiol Biochem.* 2008;114:63-70.

25. Duggan C, Irwin ML, Xiao L, et al. Associations of insulin resistance and adiponectin with mortality in women with breast cancer. *J Clin Oncol*. 2011;29:32-39.

26. Irwin ML, Duggan C, Wang CY, et al. Fasting C-peptide levels and death resulting from all causes and breast cancer: The health, eating, activity, and lifestyle study. *J Clin Oncol*. 2011;29:47-53.

27. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer*. 2004;4:579-591.

28. Rousseau MC, Parent ME, Pollak MN, et al. Diabetes mellitus and cancer risk in a population-based case-control study among men from Montreal, Canada. *Int J Cancer*. 2006;118:2105-2109.

29. Stocks T, Borena W, Strohmaier S, et al. Cohort Profile: The Metabolic syndrome and Cancer project (Me-Can). *Int J Epidemiol*. 2010;39:660-667.

30. Jalving M, Gietema JA, Lefrandt JD, et al. Metformin: taking away the candy for cancer? *Eur J Cancer*. 2010;46:2369-2380.

31. Bost F, Sahra IB, Le Marchand-Brustel Y, et al. Metformin and cancer therapy. *Curr Opin Oncol*. 2012;24:103-108.

32. Bosco JL, Antonsen S, Sorensen HT, et al. Metformin and incident breast cancer among diabetic women: a population-based case-control study in Denmark. *Cancer Epidemiol Biomarkers Prev*. 2011;20:101-111.

33. Jiralerspong S, Palla SL, Giordano SH, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol.* 2009;27:3297-3302.

34. Tan BX, Yao WX, Ge J, et al. Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. *Cancer*. 2011;117:5103-5111.

35. Belfiore A, Genua M, Malaguarnera R. PPAR-gamma agonists and their effects on IGF-I receptor signaling: implications for cancer. *PPAR Res.* 2009. Epub Jul 7.

36. Zhang H, Pelzer AM, Kiang DT, et al. Down-regulation of type I insulin-like growth factor receptor increases sensitivity of breast cancer cells to insulin. *Cancer Res.* 2007;67:391-397.

37. Pollak M. Targeting insulin and insulin-like growth factor signalling in oncology. *Curr Opin Pharmacol.* 2008;8:384-392.

38. Carboni JM, Wittman M, Yang Z, et al. BMS-754807, a small molecule inhibitor of insulin-like growth factor-1R/IR. *Mol Cancer Ther.* 2009;8:3341-3349.

39. Mulvihill MJ, Cooke A, Rosenfeld-Franklin M, et al. Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor. *Future Med Chem.* 2009;1:1153-1171.

40. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. *Mol Pathol.* 2001;54:133-137.

41. Litzenburger BC, Creighton CJ, Tsimelzon A, et al. High IGF-IR activity in triple-negative breast cancer cell lines and tumorgrafts correlates with sensitivity to anti-IGF-IR therapy. *Clin Cancer Res.* 2011;17:2314-2327.

42. Gualberto A, Dolled-Filhart M, Gustavson M, et al. Molecular analysis of non-small cell lung cancer identifies subsets with different sensitivity to insulin-like growth factor I receptor inhibition. *Clin Cancer Res*. 2010;16: 4654-4665.

43. Feng Y, Zhu Z, Xiao X, et al. 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.* 2006;5:114-120.

44. Gao J, Chesebrough JW, Cartlidge SA, et al. Dual IGF-I/II-neutralizing antibody MEDI-573 potently inhibits IGF signaling and tumor growth. *Cancer Res.* 2011;71:1029-1040.