REVIEW

Signalling through IGF-I and insulin receptors: where is the specificity?

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Summary Receptor tyrosine kinases of the insulin–insulin-like growth factor (IGF) family promote growth and mediate metabolic signals. Despite their extensive structural homology, genetic evidence indicates that their physiological functions are distinct. Nevertheless, there is limited evidence from cell culture systems suggesting that their signalling capabilities differ. Thus, it remains unclear whether the different physiological roles of insulin and IGF-I receptors result from intrinsic differences in their abilities to activate distinct signalling pathways, or arise from extrinsic differences, such as tissue distribution, relative abundance and developmental regulation.

Key words: growth, metabolism, substrate phosphorylation, genetics, mouse models

INTRODUCTION

The insulin receptor (IR) and insulin-like growth factor type I receptor (IGF-IR) (protein products are indicated in upper case, murine genes in lower case italics, e.g. Ir) are members of the receptor tyrosine kinase family. Like their ligands, they are encoded by distinct genetic loci believed to have evolved from a common ancestral gene, probably through a duplication event during the evolutionary transition from protochordates to vertebrates. Reflecting this common heritage, they share a high degree of homology.1,2 Unlike other receptor tyrosine kinases, which are activated by ligand-induced dimerization, IR and IGF-IR exist at the cell surface as \(2^2\) tetramers. Ligand binding is thought to induce a conformational change, resulting in ATP binding and autophosphorylation.3 The two receptors become autophosphorylated on conserved tyrosine residues and activate similar signalling substrates. When studied in cultured cell lines, the two receptors are capable of producing similar cellular responses. This observation raises the question of whether the physiological roles of these two highly conserved receptors are indeed distinct or overlapping.

EVIDENCE THAT IR AND IGF-IR ARE FUNCTIONALLY DISTINCT

IR and IGF-IR have different, but partially overlapping, physiological functions. This is illustrated most clearly from the phenotype of the relevant knockout mice. Mice lacking IR are born with modest growth retardation (\(-10\%\)).4 Their embryonic development is otherwise unimpaired. After birth, they rapidly develop diabetic ketoacidosis and die within few days.5,6 This experiment indicates that Ir is necessary for postnatal, but not for embryonic, metabolism. IGF-IR-deficient mice, on the other hand, are severely growth retarded (\(-45\%\) of normal) and die within minutes of birth, probably as a result of respiratory failure caused by impaired development of the diaphragm and intercostal muscles.3 In addition, they are born with multiple abnormalities, including muscular hypoplasia, delayed ossification and thin epidermis.7 Igf1r null mice have also been reported to develop metabolic abnormalities. These include mild hyperglycaemia (\(\sim 250 \text{ mg/dL}\)) and decreased \(\beta\)-cell mass.8 It is unlikely that...
hyperglycaemia is a contributory cause of death, because IR null mice survive longer with considerably higher glucose levels.5,6

Thus, within the experimental limits of this approach, there appears to be an almost total dichotomy between IR function in fuel metabolism and IGF-IRs in growth. However, a genetic analysis based on mutant mice lacking both receptors reveals a more complex truth. IR null/mice are smaller than mice lacking a single receptor (30% of normal body weight vs. 90% and 45% respectively), suggesting that the two receptors contribute independently of one another to embryonic growth. Genetic evidence indicates that IR mediates embryonic growth in response to IGF-I, whereas IGF-IR mediates the actions of IGF-I.7 This conclusion is borne out from studies of double-mutant Igf1r−/−/Igf2rNull−/− mice. In these mice, the lack of IGF-IR impairs IGF-II clearance, resulting in increased IGF-II levels. Excess IGF-II stimulates IR to promote embryonic growth in the absence of IGF-IR, so that Igf1r−/−/Igf2rNull−/− mutant mice have normal size at birth.9 The conclusion from these experiments is that IR has an intrinsic ability to mediate growth, independent of its metabolic actions.

While IR appears to be intrinsically capable of stimulating growth, evidence for a metabolic role of IGF-IR remains circumstantial. Igf1r−/−/Igf2rNull−/− mice have normal fuel metabolism, indicating that the metabolic role of IGF-IR, if any exists, is ancillary to IR. On the other hand, combined targeted inactivation of IR and IGF-IR in skeletal muscle results in insulin-resistant diabetes,10 while isolated ablation of IR does not.11,12 These data suggest that IGF-IR contributes to metabolic regulation in skeletal muscle in concert with IR. This is consistent with a number of observations indicating that IGF-IR can mediate glucose utilization in skeletal muscle13 and in primary myoblast cultures from Ir−/− mice.14,15 In contrast, IGF-IR fails to mediate insulin-like responses in hepatocytes from Ir−/− mice.16,17 This failure, however, can be partially restored by overexpressing IGF-IR, suggesting that it is not dependent on an intrinsic inability of IGF-IR to mediate insulin-like actions.18

To summarize the findings in genetically engineered mice, it appears that IR and IGF-IR functions are physiologically distinct but overlapping. Thus, IGF-IR is primarily a growth promoter and IR a metabolic mediator. However, each receptor encroaches on the other’s domain, suggesting that they have an intrinsic ability to mediate other functions. Although a discussion of the phenotypes of mice lacking the various IR substrate (IRS) signalling proteins is beyond the scope of this review, it should be mentioned that they are broadly consistent with this interpretation.19

CAN THE DIFFERENCES BETWEEN IR AND IGF-IR BE ASCRIBED TO EXTRINSIC FACTORS?

Despite attempts to elucidate the molecular basis of IR vs. IGF-IR action, it is unclear what determines signalling specificity in vivo. Before embarking on a detailed analysis of signalling differences, it bears emphasizing that signalling along mitogenic vs metabolic pathways is also influenced by properties extrinsic to the receptor’s signalling domain, such as kinetics of ligand binding, number of receptors at the cell surface and their tissue distribution.20 For simplicity, we shall refer to these differences as “extrinsic” to the receptor, although differences in ligand binding kinetics are obviously as intrinsic to the receptor as differences in signalling. What we mean is that these differences are not due to signalling capabilities.) Moreover, the kinetics of insulin and IGF secretion differ substantially, with insulin levels fluctuating vastly during the day, and IGF levels being maintained within a relatively constant range by circulating, high-affinity IGF binding proteins.21 Unlike insulin, IGF-1 is also produced locally in tissues and can therefore act in a paracrine/autocrine manner.22

Undoubtedly, extrinsic factors contribute to the differences between IR and IGF-IR signalling, but they are unlikely to be the sole factor (Fig. 1). For example, insulin-sensitive tissues tend to express larger numbers of IR than IGF-IR: liver and adipose tissue possess few, if any, IGF-IRs, while muscle has sizeable amounts. However, while the liver is virtually devoid of IGF-IR, it is endowed with considerable mitogenic potential, mediated by IR.23 Thus, receptor number alone is not a determinant of the physiological responses that will ensue.

An interesting paradigm for the role of extrinsic factors in mediating the specificity of receptor response is the developmental regulation of IR function. During fetal development in the mouse, IR mediates growth in response to IGF-II.4 After birth, IGF-II expression becomes extinct, and IR becomes mainly, but not exclusively, a metabolic promoter. The switch from high-affinity IGF-II binding to high-affinity insulin binding is associated with splicing of sequences encoded by Ir exon 11, an event that appears to be developmentally regulated.24 The observation that the function of the same receptor can be modulated by different ligands does indeed suggest that ligand–receptor interactions are as important as signalling differences in determining the physiological response.

ARE THERE IR- AND IGF-IR-SPECIFIC SIGNALLING PATHWAYS?

Cloning of cDNAs encoding IR and IGF-IR revealed extensive conservation of their amino acid sequences.1,2

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The extracellular, ligand-binding α subunits share 47–67% overall amino acid homology. The positions of cysteine residues and N-glycosylation sites are highly conserved, but the homology between the cysteinerich domains is relatively low (48%). The potential insulin-binding domain appears to involve the first 500 amino acids of the α subunit, including the cysteinerich region. However, the 12 amino acids encoded by exon 11 at the C-terminal end of the α subunit are also involved in determining insulin-binding affinity. In contrast, high-affinity IGF-I binding, unlike insulin binding to IR, is primarily determined by the cysteinerich region.

Unlike other receptor tyrosine kinases, IR and IGF-IR exist as covalent dimers of two αβ monomers in the unliganded state. A second difference between the different subtypes of tyrosine kinase receptors relates to the strategies employed to engage downstream signalling molecules. The epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors, for example, form stable complexes between phosphorylated tyrosine residues in the receptor and downstream mediators via src homology (SH-2) domains in the effector proteins. In contrast, the IR and IGF-IR do not form such complexes. Instead, the activated receptor phosphorylates several substrates, of which the members of the IRS family are the best characterized. Specific phosphorylated tyrosines on IRSs engage downstream mediators, analogous to the sites on the PDGF and EGF receptors. Since both IR and IGF-IR have similar subunit structures, it is unlikely that this unique arrangement contributes to the signalling differences between the two receptors.

The IR cytoplasmic domain has 13 tyrosine residues, the IGF-IR has 15. Seven of the 13 tyrosines in the IR are phosphorylated in response to insulin binding, as are eight of the 15 in the IGF-IR following IGF-I binding. These tyrosine residues are clustered in three different domains. The roles of tyrosine residues in the juxta-membrane and catalytic domains appear to be conserved. However, some evidence suggests that intrinsic differences in the catalytic activities of the IR and IGF-IR kinase domains may explain signalling selectivity. When the catalytic domains of the two receptors are expressed as GST (glutathione-S-transferase) fusion proteins in Escherichia coli, they phosphorylate different peptide substrates with different affinities. In addition to regulating the kinase activity, the catalytic domain participates in substrate selection by interacting with the kinase regulatory loop binding domain of IRS2. Thus, it is possible that the different catalytic activities of the two receptors result in altered IRS2 phosphorylation. Studies of hepatocytes from IR knockout mice are consistent with this hypothesis, indicating that IRS2 is a better substrate of IR than IGF-IR.

The role of the C-terminal domain, which extends beyond the tyrosine kinase domain, deserves a closer look. The IR contains two tyrosines that can be phosphorylated and account for ~40% of the insulin-stimulated phosphate incorporation. The IGF-IR contains four tyrosines, only one of which is conserved in the IR sequence (Fig. 2). The conserved tyrosine residue is located within a potential SH2 domain binding site and directly binds the regulatory (p85) subunit of PI3K.
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Fig. 2 Different autophosphorylation sites on IR and IGF-IR. The IR and IGF-IR cytoplasmic domains are highly conserved. Thus, the positions of most autophosphorylation sites are conserved in both receptors. Tyrosine residues in the juxta-membrane and catalytic domains are phosphorylated in both receptors, although there appear to be differences in the ability of the purified kinases to phosphorylate peptide substrates. The autophosphorylation sites in the C-terminal domain are divergent. This domain has been proposed to mediate the transforming and differentiative responses to IGF-IR but not its mitogenic or anti-apoptotic actions. In IR, this domain appears to mediate the mitogenic but not the metabolic response. This subdivision of functions remains controversial. Interestingly, the C-terminal domain is required for specific protein–protein interactions with 14.3.3 and MAD2.

3-kinase. It is unclear whether PI 3-kinase associates with IR and/or IGF-IR physiologically, or only in the test tube. Since it only represents a small fraction of total insulin- or IGF-1-stimulated PI 3-kinase activity, it is unlikely to play a major role in insulin/IGF action. Mutagenesis of the IR C-terminus has shown that this region is not required for IR’s ability to mediate glucose transport but modulates the mitogenic potential of IR. On the other hand, the C-terminus of IGF-IR is believed to be dispensable for mitogenesis and protection from apoptosis but required for cellular transformation and differentiation. These conclusions are supported by domain swapping experiments.

Transfection experiments of chimeric receptors composed of a heterologous ligand binding domain fused to the signalling domains of the IR or IGF-IR tend to support the view that the intracellular domains contain elements required for signalling specificity. These experiments show that the IGF-IR intracellular domain is more effective in stimulating cell proliferation, whereas the IR intracellular domain is more effective in stimulating metabolism.

The main signalling pathways activated by IR and IGF-IR are largely overlapping. Following the discovery of IRS molecules, it was thought possible that, among other things, they might explain signalling differences between IR and IGF-IR. Analysis of IRS1 and IRS3 phosphorylation in response to insulin or IGF-I indicates that the two receptors are able to catalyse identical phosphorylation patterns in these two proteins. The main limitation of these experiments is that overexpression of the various signalling elements may conceal subtle differences in affinity or lead to altered subcellular localization. These differences may be crucial for the in vivo response. For example, when IRS phosphorylation is analysed in hepatocytes from Ir knockout mice, it appears that lack of IR signalling is reflected in a more pronounced loss of IRS2 phosphorylation than IRS1 phosphorylation, leading to the suggestion that IRS2 is more tightly coupled to IR and IRS-1 to IGF-IR. Interestingly, this conclusion is borne out from the phenotypes of the knockout mice, showing that Irs2−/− mice are diabetic and Irs1−/− are growth retarded. Thus, the complexity of the interaction between IR or IGF-IR with the IRSs may not lend itself easily to an in vitro analysis in transfected cells but may require in vivo site-specific mutagenesis in mice.

An alternative explanation for signalling differences is that IR and IGF-IR recruit different signalling molecules. There are indeed several IR- or IGF-IR-specific substrates, although none of them can be easily identified with a specific cellular function. CEACAM-2, a cell surface molecule that is involved in receptor trafficking and participates in insulin clearance, is phosphorylated by IR but not by IGF-IR. c-Crk, an adapter protein in the Ras pathway that associates with the guanine nucleotide releasing proteins mSOS and C3G, appears to be an IGF-IR-specific substrate. Several specific protein–protein interactions have been demonstrated in yeast two-hybrid screens. 14-3-3 is a scaffolding protein involved in the regulation of apoptosis through proteasome targeting and has been shown to bind specifically to IGF-IR. MAD2, a cell cycle checkpoint regulatory protein, interacts with the
Fig. 3 Potential mechanisms of signalling specificity. Three possible explanations for intrinsic signalling differences between IR and IGF-IR are suggested. The two receptors may phosphorylate different substrates, a possibility for which there is only limited evidence. The cell adhesion molecule CEACAM-2 is an example. The second possibility is that the same substrate is phosphorylated at different sites or interacts with the two receptors at different domains, as shown for the adapter protein Grb-10. The third possibility is that specific protein–protein interactions occur. This has been shown for 14.3.3, MAD and IIP-2.

Cell lines derived from mice lacking IR or IGF-IR can be used to address issues of signalling specificity in vitro. For example, we have made extensive use of hepatocytes transformed with a temperature-sensitive SV40 to try to address the differences between IR and IGF-IR. The advantages of using these cells are that they possess the intracellular machinery required to carry out both metabolic and mitogenic responses, and the genetic ablation of IR removes potential confounders owing to hybrid receptor formation and spare receptors. Analysis of metabolic responses to insulin and IGF-I in these cells has led us to propose that IR and IGF-IR trigger different signalling events along the pathways leading to glucose production and glycogen synthesis. These differences may stem from different phosphorylation patterns of the forkhead transcription factor FOXO1, although the kinase responsible for these differences has, thus far, eluded identification.

WHAT TO DO?

Genetic evidence is strongly suggestive of different roles for IR and IGF-IR, despite their overall structural homology. Therefore, learning how IR and IGF-IR signalling differ may prove useful not only in the parochial context of insulin/IGF action but as a more general paradigm for how signalling specificity is achieved. The lesson of the last 15 years is that the functionally relevant differences may lie in non-canonical signalling pathways or may not be demonstrable by in vitro systems.

Differences between IR and IGF-IR could reside in alternative signalling pathways. This possibility is just beginning to be explored. It has been shown that IGF-IR associates with the G proteins G\(_i\) and G\(_p\), whereas IR fails to do so. These findings suggest that IR and IGF-IR can function as G-protein-coupled receptors and engage different G proteins. Scant attention has been devoted to the various mechanisms of signal termination as a possible site of specific differences between IR and IGF-IR. There is no information, for example, on whether IR and IGF-IR are dephosphorylated by the same tyrosine phosphatases or whether they are equally susceptible to downregulation via serine/threonine phosphorylation, in response to cytokines such as tumour necrosis factor \(\alpha\). These areas are underexplored and deserve a closer look.

Alternatively, proteomics methods could be employed to identify targets of IR and IGF-IR phosphorylation in various cell types. However, it should be emphasized that it is the genetics, and not the cell biology, that has provided the strongest evidence for a different physiological role of the two receptors. Therefore, the burden of proof should rest on the genetics. With recent advances in techniques for gene targeting and in vivo mutagenesis, the time is ripe to begin to address these questions by introducing hypomorphic alleles in mice. In many ways, the insulin/IGF action field is already lagging behind, since elegant examples...
exist in the area of c-Met,58 fibroblast growth factors,59 c-kit60 and PDGF61 signalling. We should follow suit.

REFERENCES


