Insulin Receptor Isoforms and Insulin Receptor/ Insulin-Like Growth Factor Receptor Hybrids in Physiology and Disease

Antonino Belfiore,* Francesco Frasca,* Giuseppe Pandini, Laura Sciacca, and Riccardo Vigneri

Endocrinology, Department of Clinical and Experimental Medicine (A.B.), University of Catanzaro, 88100 Catanzaro, Italy; and Endocrinology, Department of Internal Medicine (F.F., G.P., L.S., R.V.), University of Catania, Ospedale Garibaldi-Nesima, 95122 Catania, Italy

In mammals, the insulin receptor (IR) gene has acquired an additional exon, exon 11. This exon may be skipped in a developmental and tissue-specific manner. The IR, therefore, occurs in two isoforms (exon 11 minus IR-A and exon 11 plus IR-B). The most relevant functional difference between these two isoforms is the high affinity of IR-A for IGF-II.

IR-A is predominantly expressed during prenatal life. It enhances the effects of IGF-II during embryogenesis and fetal development. It is also significantly expressed in adult tissues, especially in the brain. Conversely, IR-B is predominantly expressed in adult, well-differentiated tissues, including the liver, where it enhances the metabolic effects of insulin.

Dysregulation of IR splicing in insulin target tissues may occur in patients with insulin resistance; however, its role in type 2 diabetes is unclear.

IR-A is often aberrantly expressed in cancer cells, thus increasing their responsiveness to IGF-II and to insulin and explaining the cancer-promoting effect of hyperinsulinemia observed in obese and type 2 diabetic patients. Aberrant IR-A expression may favor cancer resistance to both conventional and targeted therapies by a variety of mechanisms.

Finally, IR isoforms form heterodimers, IR-A/IR-B, and hybrid IR/IGF-IR receptors (HR-A and HR-B). The functional characteristics of such hybrid receptors and their role in physiology, in diabetes, and in malignant cells are not yet fully understood. These receptors seem to enhance cell responsiveness to IGFs. **(*Endocrine Reviews* 30: 586–623, 2009)**

I. Introduction
II. Insulin Receptor Structure and Signaling
  A. Insulin receptor gene
  B. Insulin receptor expression
  C. Insulin receptor structure, ligand binding, and autophosphorylation
  D. Insulin receptor signal transduction pathways
III. The Biology of Insulin Receptor Isoforms
  A. IR isoform generation
  B. IR isoforms and ligand specificity
  C. IR isoforms and signaling specificity
IV. Insulin Receptor Isoforms in Physiology
  A. Role of IR isoforms in development
  B. IR isoforms and cell differentiation
  C. Role of IR isoforms in aging
V. Insulin Receptor Isoforms in Disease
  A. Insulin resistance and altered IR-A/IR-B ratio: the model of myotonic dystrophy
  B. Insulin resistance and type 2 diabetes mellitus: a pathogenic role for differential splicing of IR?
  C. Aberrant expression of IR-A in cancer
  D. IR isoforms and cancer risk in type 2 diabetic patients treated with insulin or insulin analogs

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/er.2008-0047 Received November 3, 2008. Accepted August 6, 2009.
First Published Online September 14, 2009.
* A.B. and F.F. contributed equally to this work.

Abbreviations: APS, Associate protein substrate; AT, ataxia telangiectasia; ATM, AT mutated; AT/RT, atypical teratoid/rhabdoid tumors; BRET, bioluminescence resonance energy transfer; CR, cysteine-rich region; dmpk, dystrophia myotonica protein kinase; E, embryonic day; EGF, epidermal growth factor; ER, estrogen receptor; FAK, focal adhesion kinase; FnIII, fibronectin type III; FoxO, forkhead box 'other'; HR, hybrid receptor; HR-A, HR containing IR-A hemidimers; HR-B, HR containing IR-B hemidimers; ID, insert domain; IGFBP, IGF binding protein; βIGFRKO, *Igf1r* knockout; ILP, insulin-like peptide; IR, insulin receptor; IR-A, IR isoform A; IR-B, IR isoform B; IRS, IR substrate; MBNL, muscleblind; MD1, myotonic dystrophy type 1; MEK1, MAPK kinase 1; MPO, myeloperoxidase; mTOR, mammalian target of rapamycin; PCOS, polycystic ovary syndrome; PI3K, PI3-kinase; PPP, picropodophyllin; raptor, regulatory-associated protein of mTOR; SH2, Src homology 2; T2DM, type 2 diabetes mellitus; TSC2, tuberous sclerosis complex 2.

VI. Possible Implications of Aberrant IR-A Expression for Cancer Therapy
A. Role of the IGF system in mediating resistance to anticancer therapies
B. Role of IR and hyperinsulinemia in resistance to IGF-IR or IGF-targeted therapies
C. Targeting IR-A signaling in cancer
VII. IR Isoforms and Hybrid Receptors
A. IR-A/IR-B hybrids
B. IR/IGF-IR hybrids: functional characteristics and the role of IR isoforms
C. IR/IGF-IR hybrids and cancer
D. IR isoforms in the regulation of the IGF system
VIII. Summary, Conclusion, and Perspectives
A. The physiological role of IR isoforms
B. Implications for type 2 diabetes and associated disorders
C. Implications for oncology
D. Perspectives

I. Introduction

In 1985, two different groups reported cloning of the insulin receptor (IR) cDNA. These cDNAs were of slightly different lengths (1, 2), indicating that the IR exists in two isoforms. These two isoforms were found to be IR isoform A (IR-A), lacking exon 11, and IR isoform B (IR-B), including exon 11. Several subsequent studies were performed to correlate IR isoform expression with insulin resistance/type 2 diabetes mellitus (T2DM) (3–5). The role of IR isoforms in T2DM has remained elusive because the data obtained by different groups conflicted (6–9). Further studies indicated subtle differences between IR-A and IR-B in terms of receptor activation and signaling, suggesting different functions for each IR isoform (10–12). This concept was confirmed by the observation that IR-A, but not IR-B, is a high-affinity receptor for IGF-II (13). These studies placed IR-A in the IGF system, which regulates several biological events, including cancer, aging, and diabetes (14–16). IGF system dysregulation frequently occurs in malignancies and includes IGF-IR overactivation, by both receptor overexpression and autocrine/paracrine overproduction of IGF-I and IGF-II (17, 18). In this system, IR-A overexpression may contribute to modulation of tissue responses to insulin and IGF-I/II (16, 19).

Herein, we review data indicating that IR splicing is an evolutionary conserved mechanism in mammals that is responsible for the specificity of insulin and IGF signaling. Indeed, IR-A up-regulation is associated with decreased metabolic insulin signaling and increased IGF signaling, whereas IR-B up-regulation is associated with predominant metabolic insulin signaling. Predominant IR-A expression may therefore be important in embryonic life for growth and fetal development, whereas predominant IR-B expression has a role in metabolic insulin action in adult life. Dysregulation of IR splicing, *i.e.*, increased IR-A expression in adult life, may play an underestimated role in cancer progression and the aging process.

II. Insulin Receptor Structure and Signaling

A. Insulin receptor gene

1. IR gene structure

The human IR is encoded by a 22-exon gene (*Ir*) spanning 120 kb that is located on chromosome 19. Isolation and characterization of the human IR cDNA (approximately 5 kb) by the groups of Axel Ullrich (2) and Bill Rutter (20) in 1985 revealed predicted human insulin proreceptor sizes of 1343 and 1355 amino acids, respectively. The difference in size was found to be due to exclusion or inclusion of a 12-amino acid segment at the C-terminal end of the IR α-subunit. Studies on the exon-intron organization of *Ir* indicated that the 12-amino acid region is encoded by exon 11, spanning 36 bp, that could be alternatively spliced, thus generating two IR isoforms (21).

Homology between IR and IGF-IR is high, ranging from 45–65% in the ligand binding domains to 60–85% in the tyrosine kinase and substrate recruitment domains (22, 23). Indeed, *Ir* and *Igf1r* have evolved from an ancestral gene and are part of a system that is highly conserved in vertebrates and invertebrates, which coordinates metabolic, growth, and differentiation responses to environmental conditions and nutrient availability (24, 25) (Fig. 1). Although only one receptor protein is present in invertebrates (26, 27), three distinct receptors have been identified in vertebrates: IR (1, 2) IGF-IR (23), and an orphan receptor named the IR-related Receptor (IRR) (28, 29). The observation that the triple *Ir/Igfr/Irr* knockout, at variance with the single and double knockouts, does not develop the male phenotype suggests that the IRR signaling pathway is required for male sexual differentiation (30).

2. Phylogeny of the Ir gene family

The first duplication of the ancestral *Ir-like* gene led to the *Ir* paralog and the common ancestral gene of both *Igf1r* and *Irr*, which were generated in a second round of duplication (23, 29, 31) (Fig. 2). Approximately 20% of all sites in *Ir* transcripts are invariant across vertebrates (31). Such conserved sites are abundant in the tyrosine kinase domain (32), indicating that this domain is under strong selection pressure to maintain the general IR signal transduction functions.

In contrast, only 2% of the overall *Ir* transcript is specific and not shared by *Igf1r* or *Irr*. IR-specific sites are mainly located in the extracellular portion of the receptor, suggesting that differences in the function of paralogs in

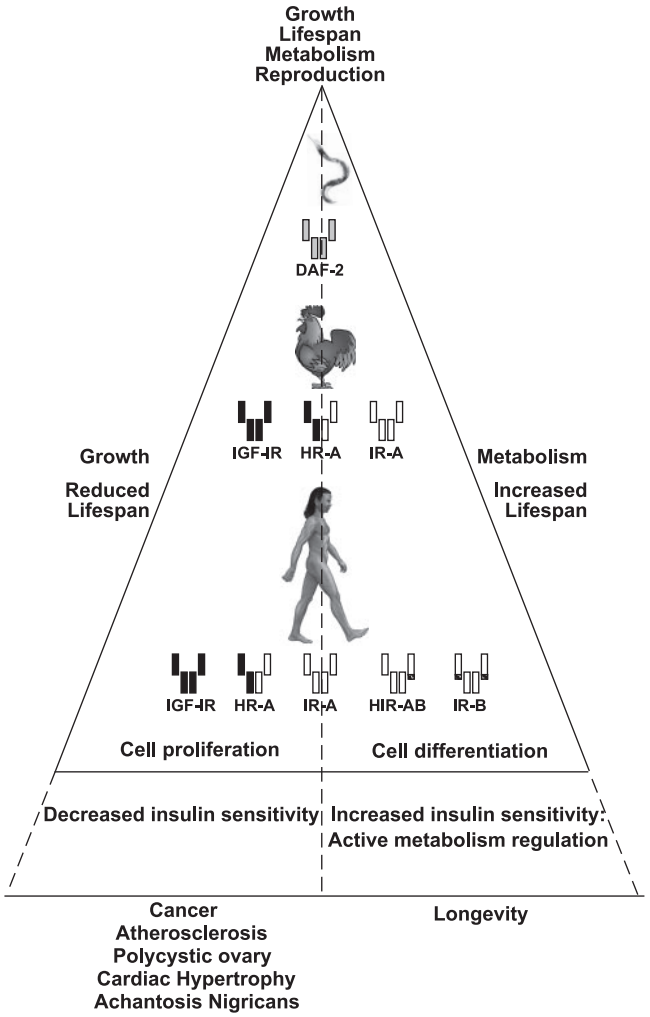

FIG. 1. Signaling diversification in the IGF system, a key regulator of metabolism and life span. In invertebrates (*C. elegans*; *top of the triangle*), only one receptor of the IGF system is present (DAuer Formation-2). Nutrient availability activates DAuer Formation-2 and elicits coincident biological functions, including activation of metabolism and growth, reproduction, and death. In contrast, low nutrient availability causes poor DAuer Formation-2 activation. In this case, *C. elegans* will neither grow nor reproduce, and it becomes a long-lived dauer larva. In vertebrates (chicken; *middle of the triangle*), the presence of two different receptors of the IGF system leads to functional diversification: whereas IR-A mainly regulates glucose metabolism, IGF-IR regulates growth and survival. HRs (HR-A) may have some overlapping functions, including the proliferative effects of insulin and proinsulin. In mammals (*Homo sapiens*; *bottom of the triangle*), the appearance of IR-B, a receptor with exquisite metabolic functions, makes the scenario even more complex. The assumption that IGF system down-regulation is coincident with a prolonged life span becomes less evident. Despite receptor subtype complexity, however, functional studies on receptor subtypes maintain the validity of this paradigm. The key for interpretation of such receptor complexity is IR-A; overactivated/overexpressed IR-A leads to a reduced metabolic effect of insulin and increased activation of IGF-like signaling (*left side of the triangle*). As a consequence, increased cell proliferation, atherosclerosis and cancer may be promoted. In contrast, decreased IR-A signaling is associated with restrained IGF signaling and increased insulin metabolic effects, as observed in healthy centenarians. In light of this view, human longevity is inversely related to insulin/IGF signaling promiscuity.

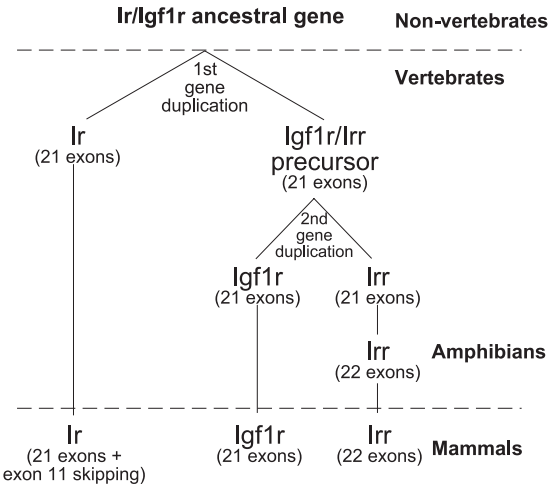

FIG. 2. Schematic representation of IR phylogeny. An ancestral gene exists in nonvertebrates (DAuer Formation-2), which encodes for a common receptor for insulin-like peptides. A gene duplication occurred in vertebrates, resulting in two 21-exon genes, one encoding the *Ir* and the other one a precursor of the *Igf-ir* and *Irr*. A second gene duplication resulted in the formation of two separate genes for the IGF-IR and the IRR, both formed by 21 exons. In amphibians, the *Irr* gene acquired one more exon, becoming a 22-exon gene. Moreover, in mammals, the *Ir* gene also acquired an additional exon (exon 11) together with the ability to skip the exon in a developmental and tissue-specific fashion.

the family have been evolutionarily achieved more through changes in ligand-binding affinity (33) than by modifications in the intracellular tyrosine kinase domain (31).

At variance with *Igf1r*, *Ir* and *Irr* include exon 11. Interestingly, *Ir* exon 11 is found exclusively in mammals (31). This finding implies that functional diversification of the *Ir* family during evolution was achieved through alternative splicing (Fig. 2).

Alternative splicing of *Ir* exon 11 in mammals and its physiological implications will be discussed in the next paragraphs.

3. IR promoter

The *Ir* promoter spans 2000 bp (21). It contains many GCs, no TATA or CAAT boxes, and multiple transcription initiation sites (34). It contains a largely GC-rich region, located between −1818 and −288 bp (35). The region spanning −646 to −489 bp is important for activity of the proximal promoter (36). A positive regulatory element is localized between −1823 and −1311 bp, whereas a negative regulatory element is found between −1311 and −877 bp (37). This organization suggests that IR expression is highly regulated in different tissues. In tissues that are typical targets of insulin action, like hepatocytes, *Ir* promoter activity may be regulated by the HT-FIR transcription factor (38), IRNF-I, and IRNF-II (IR nuclear factors I and II) (39). Also, the nuclear protein HMGA1, which is up-regulated in embryonic tissues and human malignancies (40), may activate *Ir* promoter (41).

Interaction of Sp1 with wild-type p53 reduces *Ir* promoter activity, whereas p53 mutants may directly bind and stimulate the *Ir* promoter. This effect of p53 mutants may be responsible for IR overexpression in human cancer (42). Estrogen receptors (ERs) bound to estrogens may also inhibit *Ir* promoter activity. Indeed, two estrogen response elements were found in the *Ir* promoter (43). The existence of these modulatory mechanisms suggests that IR expression is highly regulated in cells in a development- and tissue-specific manner.

B. Insulin receptor expression

1. IR expression in physiological conditions

Although the major insulin target tissues are liver, adipose tissue, and skeletal muscle, IRs have also been found in the brain, heart, kidney, pulmonary alveoli, pancreatic acini, placenta vascular endothelium, monocytes, granulocytes, erythrocytes, and fibroblasts (44). The observation that IRs are not restricted to insulin target tissues suggests that IRs may be functionally linked to multiple systems, in addition to their known role in the metabolic actions of insulin. The findings of both metabolic and nonmetabolic effects of IRs are supported by the effects of insulin on embryonic development. Briefly, pancreatic islet β-cell secretory granules have been observed in the human fetus at 14 wk gestation, and IRs have been detected in the liver as early as 15 to 18 wk gestation. Furthermore, monocytes and erythrocytes from human cord blood have higher IR content than similar cells harvested from adult individuals (44). Moreover, the fetal IR is not down-regulated by hyperinsulinemia (44); rather, hyperinsulinemia is associated with increased IR content in cord blood monocytes. In accordance with the notion that insulin may regulate fetal growth, fetal hyperinsulinemia leads to macrosomia, whereas insulin deficiency leads to growth impairment (44). After birth, IR expression in monocytes and erythrocytes decreases, remains unchanged until puberty (44), and then increases in adult life (44). In accordance with the inhibitory effect of estrogens on *Ir* promoter activity, monocyte IR content is higher during the luteal phase in adult females (43). This elevation is abolished by use of oral contraceptives (44) or pregnancy (45). Moreover, glucocorticoids and thyroid hormones have been reported to enhance IR expression (46).

Finally, nutrition and exercise have been reported to influence IR expression (47), although the data obtained in experimental animals were not always confirmed in humans (48).

2. IR expression in various tissues

IR expression in mammalian tissues has been determined by both binding assays and selective immunoprecipitation. The highest expression of IR protein was found in adipose tissue. Relative IR expression in liver, heart, and lung tissue was approximately 30% of that in adipose tissue, whereas it was approximately 10% in muscle, brain, spleen, placenta, and kidney tissue (49). Quantification of IR isoforms has been determined by both RT-PCR and Western blot using antibodies specifically recognizing the IR-B protein. IR-B expression accounted for approximately 80% of total IR expression in liver, 60% in muscle and adipose tissue, 40% in placenta, and 0% in lymphocytes (6).

In the brain and pancreas, the IR expression level is much lower than that in typical insulin-responsive tissues. Moreover, the effect of insulin is typically not metabolic in these organs. Recent data support a specific role of IR isoforms in the biology of these tissues.

3. IR expression in brain

Both IR and insulin are present in the brain (50, 51). Cerebral insulin is transported from the plasma into the cerebrospinal fluid (52) through IR-based, saturable transport (53), occurring mainly in the olfactory bulb and hypothalamus (53). Synthesis of insulin has likewise been detected in neuronal tissue, including neonatal rabbit and adult rat brain (52, 54). Cerebral IRs and insulin are present in the olfactory bulb, hypothalamus, cerebral cortex, cerebellum, hippocampus, and pituitary intermediate lobe (55, 56). The increase in circulating insulin is associated with an increase in insulin in the cerebrospinal fluid (57). Persistent hyperinsulinemia down-regulates IR-mediated insulin transport to the brain (57). In mammalian brain, two IR types are present. In addition to the typical IR present in glial cells, a brain-specific IR is present in neurons (52). This brain-specific IR is mainly the IR-A isoform with a lower molecular weight as a result of differential glycosylation. Brain insulin is believed to have a role in neuronal survival, learning, memory, regulation of energy homeostasis, and reproduction (58). It is also involved in the control of energy balance in mammals because delivery of insulin to the brain is anorexigenic and reduces body weight (58). Accordingly, mice with a brain conditional knockout of IR (NIRKO mice) are insulin resistant, glucose intolerant, and overweight (59). The reduction of food intake caused by centrally administered insulin is lower in overweight animals compared with lean and normal-weight animals, suggesting that insulin resistance in the brain may contribute to weight gain (60).

Brain IR also regulates cognitive functions. Patients with type 1 or 2 diabetes have been found to have diminished mental performance (61). Moreover, patients with T2DM are at higher risk for cognitive decline and Alzheimer's disease (62). In Alzheimer's patients, the activity of brain IR is reduced (60) and insulin concentrations in the

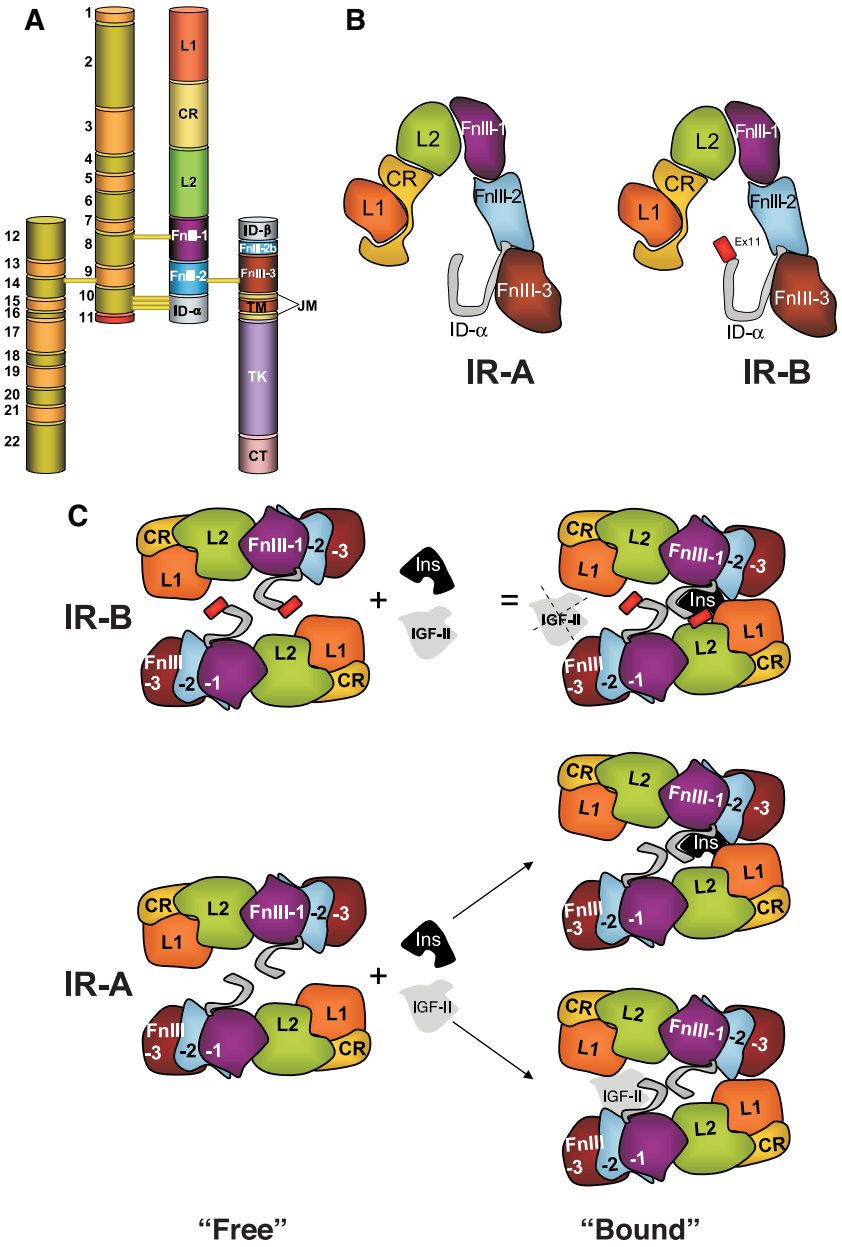

FIG. 3. Modular structure of IR isoforms. A, Diagram of the $\alpha 2\beta 2$ structure of the IR. On the *left half of the figure*, the IR is represented by the 22 exon-encoded sequence. On the *right half*, predicted protein modules are indicated. L1, Large domain 1; CR, cysteine-rich domain; L2, large domain 2; Fn, fibronectin type III domains; TM, transmembrane domain; JM, juxta membrane domain; TK, tyrosine kinase domain; CT, C-terminal domain. B, Schematic side view of the 3-D structure of the IR isoform α-subunit (L1, CR, L2, FnIII-1/2) and part of the extracellular portion of the β-subunit (FnIII-3). The *gray loop* indicates the insert domain (ID-α). The *red fragment* on top of ID-α is encoded by exon 11 and is present in IR-B, but not in IR-A. C, Schematic top view of the 3-D structure of the IR heterotetramer extracellular portion. Insulin (*black molecule*) binds to one site of IR-B. The presence of the red fragment encoded by exon 11 does not allow for binding of IGF-II (*gray molecule*) to IR-B. Both insulin and IGF-II may bind to IR-A, due to the absence of the red fragment.

cerebrospinal fluid are lower than normal, indicating impaired insulin transport to the brain (56). Notably, insulin treatment ameliorates cognitive impairment in diabetic patients (57). These observations are in accordance with data obtained in animals. Additionally, insulin-deficient rats display reduced performance in complex tasks (63). Overall, animal and human studies indicate that insulin may affect cognitive processes (52).

4. IR expression in endocrine pancreas

Both IR and IGF-IR have been shown to be present in β-cells (64, 65). IGF-I acts as a negative regulator of insulin secretion, whereas the role of IR in β-cells remains controversial because IGF-IR activation by insulin may interfere with the evaluation of IR function (66, 67). Recent studies that circumvent the confounding presence of the IGF-IR have concluded that insulin is a positive regulator of its own production (68, 69).

The relative role of IR and IGF-IR in β-cells has been investigated in transgenic models. The β-cell-specific *Ir* knockout (βIRKO) (70) manifests progressive glucose intolerance, as well as reduced islet size and insulin content. *Igf1r* knockout (βIGFRKO) mice (71, 72) display normal β-cells and islets but show defects in glucose-stimulated insulin secretion (71, 72). At 2 months of age, they display hyperinsulinemia, in accordance with the inhibitory effect of IGF-I on insulin secretion (73, 74). Remarkably, both βIRKO and βIGFRKO mice show a loss of acute phase insulin secretion, probably due to common defects in signaling downstream IR and IGF-IR (75, 76). Although IR and IGF-IR are unlikely to play a critical role in islet development, they are important for the islet hyperplastic response to insulin resistance (77) and pancreatic injury (78).

The predominant isoform in human pancreatic β-cells is IR-B (79), suggesting that the IGF-II/IR-A interaction does not play a major role in β-cell physiology and that the effects of IGF-II in β-cells are mediated by IGF-IR, as suggested by the βIGFRKO phenotype.

5. IR expression in malignant cells

An unexpected observation was the ability of mitogens, such as concanavalin A and Epstein-Barr virus, to increase IR expression in lymphocytes (80). Moreover, human lymphocyte-derived malignant cells, such as the IM-9 cells (B-type lymphoblasts derived from a subject with multiple

myeloma), are abundantly endowed with high-affinity IRs (80). IR up-regulation in these cells has no major effect on cell metabolism, but coincides with the development of increased sensitivity to the nonmetabolic effects of insulin. A role for IR in tumor progression was suggested by studies indicating that IR is overexpressed in several tumors, including breast, colon, lung, ovary, and thyroid carcinomas (13, 81, 82). The biological relevance and the putative mechanisms involved in IR overexpression in malignant cells will be discussed below (see *Section V.C*).

C. Insulin receptor structure, ligand binding, and autophosphorylation

1. IR protein: structure and glycosylation

The IR protein is a heterotetramer consisting of two extracellular α-subunits and two transmembrane β-subunits held together by disulfide bonds (83, 84). The α-chain and 194 residues of the β-chain form the extracellular portion of the IR; in the β-chain, there is a single transmembrane sequence and a 403-residue cytoplasmic domain containing the tyrosine kinase activity (Fig. 3). Ligand binding to the IR α-subunit stimulates tyrosine kinase activity intrinsic to the β-subunit of the receptor (84–86). The α- and β-chains are both synthesized from a unique mRNA, which is constituted by 22 exons. *Ir* mRNA encodes for a protein of 1370 amino acids, indicating a predicted molecular mass of 153,917 Da. The protein is cleaved by furin into α- and β-subunits. The α-subunit contains 723 amino acids, with a molecular mass of 130 kDa (83, 84). The β-subunit contains 620 amino acids, with a molecular mass of 95 kDa (83, 84). Both subunits are glycosylated, thus accounting for most or all of the molecular mass difference in the observed *vs.* predicted values in various tissues and cells (87).

Comparative sequence analyses have shown that the IR family members are composed of a number of different, repeated structural units (83, 84). The N-terminal half of each ectodomain monomer consists of two homologous leucine-rich repeat domains (L1 and L2) of approximately 150 amino acids each, separated by a cysteine-rich region (CR), consisting of seven smaller repeats, also containing approximately 150 residues, with one or two disulfide bonds (83, 84) (Fig. 3). The C-terminal half of each ectodomain monomer (approximately 460 residues) consists of three fibronectin type III domains (FnIII-1, FnIII-2, and FnIII-3). They are relatively small (approximately 100 amino acids) and form a seven-stranded β-sandwich. The FnIII-2 domain of IR contains a large, 120-amino acid residue fragment, termed the insert domain (ID) (83, 84), and the furin cleavage site (83, 84) (Fig. 3).

The IR splice variant IR-B differs from IR-A by the presence of a 12-amino acid segment (coded by exon 11) inserted between IR-A residues 716 and 717, three residues before the C-terminus of the α-chain (84).

The intracellular part of each IR monomer contains a tyrosine kinase domain flanked by two regulatory regions (the juxtamembrane region and the C-tail) that contain the phosphotyrosine binding sites for signaling molecules. In particular, the juxtamembrane region is involved in docking IR substrates (IRS) (88) 1–4 and Shc, as well as in receptor internalization (85, 86, 89).

2. IR binding and phosphorylation

Analysis of insulin binding to IR reveals curvilinear Scatchard plots and negative cooperativity (84), thereby implying the existence of both low-affinity and high-affinity insulin binding sites. These two different ligand-binding regions are termed site 1 (low-affinity site) and site 2 (high-affinity site) (83, 84, 86, 89). Photo-affinity labeling and alanine scanning data indicate that insulin binds to the site composed of the L1 domain and α-subunit C-terminal peptide sequence (amino acids 704–715, CT domain) (Fig. 3). A second binding site exists within the C-terminal part of L2 and the first FnIII domain (Fn0) (84). Insulin binds first to the low-affinity site (site 1) on one α-subunit and then to the second site (site 2) of the other IR α-subunit (84, 90). A second insulin molecule bridging both leftover sites 1 and 2 accelerates dissociation of the first bound insulin molecule (84). This behavior explains the bell-shaped curve and negative cooperativity and provides a molecular basis for receptor tyrosine kinase activation, by approximating the two-kinase domains and permitting transphosphorylation. The biological role of IR-negative cooperativity is an interesting issue. The acceleration of the dissociation rate of the IR complex by increased insulin concentration is important for insulin actions. Indeed, the mitogenic effects of insulin are disproportionately enhanced by insulin analogs that have slower dissociation rates relative to native human insulin, suggesting that the negative cooperativity is important for limiting the mitogenic effects of insulin (84). Computational Boolean analyses have demonstrated that ligand residence time on the IR may indeed determine choices in branching signaling pathways (85, 86, 89).

The three-dimensional structure of the IR ectodomain dimer (90) has allowed for better understanding of the localization of insulin binding sites 1 and 2. Each monomer of the IR ectodomain exhibits an inverted-V arrangement relative to the cell membrane, with the L1-CR-L2 domains forming one leg and the three FnIII domains forming the other one (90). In the dimer, the second monomer is related to the first by a 2-fold rotation around the axis of the inverted V, with the L1-CR-L2 domains of one monomer packed against the three FnIII domains of the other (Fig. 3B). At the apex of the inverted V, the L2 do-

main of each monomer contacts the FnIII-1 domain of the other, whereas the base of the C terminus of the FnIII-3 domains is oriented in such a way that it is capable of being extended through the cell membrane to the kinase domains of the intact receptor. The first component of site 1 in both α-subunits is the central β-sheet of L1 (91, 92). The second component of site 1 is formed by the central modules of the CR. The larger size of this module in IR is partly responsible for the lower IR affinity of the bulkier IGF-I and IGF-II ligands compared with its affinity for insulin (Fig. 3C). The third component of site 1 is the CT peptide, as demonstrated by chemical cross-linking. The CT peptide sequence, belonging to the other IR monomer, is closely juxtaposed to the L1 domain of the contralateral IR monomer and contacts amino acid B25 of the insulin molecule (93) (Fig. 3C). It is reasonable to suppose that the exon 11-encoded 12-amino acid peptide present in IR-B may contribute, together with the CR module, to reduce the affinity of IR-B for IGF-II by further restraining the site 1 pocket. Site 2 is formed by the C-terminal loops of FnIII-1 and the N-terminal loops of FnIII-2 that are adjacent to the L1 face (90). The three-dimensional structure of the IR may explain the contribution of each domain to insulin binding and the complexity of the binding characteristics of the IR isoforms and IR/IGF-IR hybrids.

In the insulin-free state, the inhibitory IR conformation maintains a minimum separation between the two intracellular tyrosine kinases. This distance between the tyrosine kinases prevents the tyrosine kinase activation loop (94) of one tyrosine kinase from reaching the catalytic transphosphorylation site of the other tyrosine kinase (Fig. 3). Binding of a single insulin molecule joins the two ectodomains with a consequent reduction of separation between the associated tyrosine kinase domains, thereby allowing transphosphorylation of the tyrosine kinase activation loops at the catalytic loci of the opposing tyrosine kinase domains. Phosphopeptide mapping techniques (95, 96) have resolved tyrosine kinase residues (in the juxtamembrane region: amino acids 953, 960, and/or 972; in the catalytic domain: 1146, 1151, and 1152; and in the carboxyterminal domain: 1316 and 1322) (1, 2) (Fig. 3). The most important domain for auto- and transphosphorylation is the catalytic domain (amino acids 1146, 1151, and 1152). Phosphorylation of these sites correlates with the acquisition of transphosphorylation activity (97, 98).

Carboxyterminal deletions lacking either 43 amino acids (99) or mutants with tyrosine residues 1316 and 1322 changed to phenylalanines (100, 101) have normal tyrosine kinase activity and diminished autophosphorylation due to loss of the two tyrosine residues. However, these mutants, when transfected into the fibroblast cell line Rat1, show diminished (102, 103) or unchanged (104) glucose uptake and glycogen synthase activity ("metabolic signaling").

In the juxtamembrane region of the β-subunit, Tyr-960 is important for IRS-1 phosphorylation and for metabolic and mitogenic responses to insulin (96), whereas Tyr-953 and Tyr-960 play a role in receptor endocytosis (105, 106) because they exist within consensus endocytosis signals for internalization via clathrin-coated pits (94, 107).

D. Insulin receptor signal transduction pathways

Insulin binding to the IR extracellular α-subunit induces a conformational change in the receptor molecule, which brings the two β-subunits into close opposition. Crystallographic studies of the IR kinase domain in the unphosphorylated and phosphorylated states indicate that autophosphorylation activates the IR tyrosine kinase due to a series of alterations in the β-subunit conformation that facilitate ATP binding, β-subunit phosphorylation, recruitment of membrane and cytosolic protein substrates, and their subsequent phosphorylation.

The activated IR tyrosine kinase phosphorylates several intracellular substrates, including the most extensively characterized IR substrates (IRS-1, -2, -3, and -4), IRS-5/DOK4, IRS-6/DOK5, Shc, Gab1, Cbl, associate protein substrate (APS), and the signal regulatory protein family members (85, 86, 108). Each of these phosphorylated proteins provides specific docking sites for effectors containing Src homology 2 (SH2) domains that specifically recognize different phosphotyrosine residues, including the regulatory subunit p85 of type 1A PI3-kinase (PI3K); the protein tyrosine phosphatase SHP2; the Src family of non-receptor-type tyrosine kinases, including Fyn and Csk; the adaptor proteins Grb2; and the GTPase activating protein (109) of Ras. Some of these molecules contain SH3 domains that bind proline-rich regions with the consensus sequence PXXP and thereby provide additional sites for protein-protein interactions with additional downstream intracellular effectors. In this complex cascade of biochemical signals, two major signaling pathways have been recognized, mediating either prevalent metabolic or mitogenic effects and originating by the activation of PI3K or Ras, respectively.

1. The PI3K pathway

In the PI3K pathway, a regulatory p85 subunit and a catalytic p110 subunit phosphorylate phosphatidylinositol-(4, 5)-bisphosphate (PIP2), thereby generating phosphatidylinositol-(3, 4, 5)-trisphosphate (PIP3). PIP3 recruits and activates pleckstrin homology domain-containing proteins, including enzymes, substrates, adaptors, and cytoskeletal molecules. Among these, phosphoinositide-de-

pendent kinase-1 phosphorylates and activates several downstream enzymes, including the serine/threonine kinase Akt (protein kinase B) and protein kinase C (110). In addition, PIP3 directly facilitates Akt activation by mediating its translocation to the membrane via the pleckstrin homology domain. Akt activation regulates metabolic enzymes, such as glycogen synthase kinase 3 and 6-phosphofructo-2-kinase, and it is involved in glucose metabolism by such activities as induction of glucose transporter (Glut-4) translocation from intracellular storage compartments to the plasma membrane (108). Activated Akt also phosphorylates the Bcl-2 family member BAD, an important proapoptotic protein (111) that, when phosphorylated, is not able to exert its proapoptotic function. Moreover, activated Akt phosphorylates forkhead box 'Other' (FoxO) proteins (FoxO1, FoxO3, and FoxO4), which are signaling molecules that regulate various cell functions (112), including metabolism, apoptosis promotion (*e.g.*, via the proapoptotic member of the Bcl-2 family, Bim), and inhibition of cell growth (113, 114). Upon insulin stimulation, FoxO proteins are phosphorylated by Akt and, after interaction with 14-3-3 proteins and exportins, translocate to the cytoplasm where they undergo ubiquitylation and degradation (113, 114). Therefore, IR activation suppresses the transcriptional program of FoxO proteins.

Another pathway regulated by PI3K-Akt activation is the regulatory-associated protein of mTOR (raptor)–mammalian target of rapamycin (mTOR) pathway, which regulates cell growth and metabolism (86) and integrates signals coming from insulin, as well as other growth factors, with those coming from nutrients. Activated Akt phosphorylates and inactivates TSC2 (tuberous sclerosis complex 2). TSC2 belongs to a heterodimeric complex with TSC1 (tuberous sclerosis complex 1 or hamartin) that functions as the Rheb (Ras homolog enriched in brain) GTPase-activating protein (109). Inactivation of TSC2 by Akt reduces the GAP activity of the TSC1/TSC2 complex, thus increasing the amount of GTP-bound Rheb, a ras-family GTP-binding protein that binds and activates the raptor–mTOR complex.

Two main signaling pathways downstream of the raptor–mTOR complex link IR to the control of mRNA translation: the p70S6 kinase (p70S6K) and eIF4E-binding protein-1 (4E-BP1) pathways. p70S6K is a serine/threonine protein kinase that regulates factors involved in protein synthesis, including ribosomal S6 protein and the translational regulators eukaryotic translation elongation factor 2 kinase and eukaryotic translation initiation factor-4B. When phosphorylated by raptor–mTOR, 4E-BP1 releases eIF-4E, allowing it to interact with eIF-4G, and thus activating cap-dependent mRNA translation (115).

There is also evidence of complex cross-talk between the PI3K-Akt-mTOR pathway and the p53 pathway, which suggests that regulation of cell functions by IR signaling and the p53 pathway may overlap (116).

Activated IR recruits APS (109), an adapter that contains three SH2 domains. Upon binding to the IR β-subunit, APS is tyrosine phosphorylated and acquires the ability to phosphorylate Cbl on three tyrosines (117). Insulin stimulation results in assembly of the Cbl/CrkII/C3G complex and its recruitment to lipid raft domains, where it catalyzes activation of the Rho family small GTP-binding proteins TC10α and TC10β (118).

c-Abl tyrosine kinase is an important component of IR signaling. Insulin stimulates c-Abl tyrosine phosphorylation with consequent focal adhesion kinase (FAK) dephosphorylation. Dephosphorylated FAK mediates the metabolic effects of insulin. Therefore, when c-Abl tyrosine kinase is inhibited, FAK is phosphorylated in response to insulin, which promotes cell proliferation and migration, rather than cell metabolism. Indeed, FAK phosphorylation or dephosphorylation by insulin may mediate alternative mitogenic or metabolic predominance in insulin effects on target cells (119).

2. The Ras pathway

Grb2 is an adaptor that may bind to phosphorylated IRS proteins through its SH2 domain. Via its SH3 domain, Grb2 activates ras guanine nucleotide exchange factor mSos (Son of Sevenless), which, in turn, activates p21ras, a GTP-binding protein with GTPase activity toward active (GTP-bound) or inactive (GDP-bound) p21ras form (120). Phosphorylated IR may also activate p21ras through Shc proteins. In particular, Tyr-972 of IR binds to the N-terminal phosphotyrosine binding domain of Shc. As a consequence, both 52- and 46-kDa isoforms of Shc are phosphorylated at Tyr-317, which recruits the Grb2/Sos/p21ras complex. Therefore, both phosphorylated IRS and Shc bind to Grb2, although the Shc/Grb2/Sos complex is predominantly involved in the activation of p21ras and sustained ERK1/2 activation in response to insulin.

Active p21ras recruits and activates the serine/threonine kinase Raf, which, in turn, phosphorylates the dual specificity kinase MAPK kinase (MEK1), which then phosphorylates ERK1/2, a kinase of the MAPK family. In its inactive form, ERK1/2 is mainly located in the cytoplasm, where it binds to MEK1 to form an MEK/ERK heterodimer (85, 108). When phosphorylated, ERK1/2 dissociates from MEK1 and translocates to the nucleus, where it phosphorylates a number of substrates (SRC-1, Pax6, NF-AT, Elk-1, MEF2, c-Fos, c-Myc, and STAT3) involved in the activation of a complex transcriptional program (120, 121). Moreover, activated ERK1/2 phosphorylates numerous substrates in the cytoplasmic com-

partment. Given that ERK1/2 shuttles between the cytoplasm and the nucleus and has numerous targets in both compartments, its spatial distribution is crucial for determining the biological response.

Other MAPKs, such as Jun N-terminal kinase and p38 kinase, are also activated by insulin and contribute to the complexity of its biological responses (121). Both p70S6K and Jun N-terminal kinase participate in a negative feedback loop involved in the termination of insulin signaling by phosphorylation of serine residues in IRS proteins (85, 108).

III. The Biology of Insulin Receptor Isoforms

A. IR isoform generation

Further insights on IR function were provided by studies on IR isoforms. Indeed, the mature IR exists as two isoforms, IR-A and IR-B, which result from alternative splicing of the primary transcript. IR-B differs from IR-A by the inclusion of exon 11, which encodes 12 amino acid residues (residues 717–728) at the carboxyl terminus of IR α-subunit. Inclusion of this exon is differentially regulated in various tissues. In adult life, IR-A is ubiquitously expressed, whereas IR-B is predominantly expressed in liver and also substantially expressed in muscle, adipose tissue, and kidney, which are all target tissues of the metabolic effects of insulin (10, 122). Moreover, IR-A is predominantly expressed in fetal and cancer tissues. A high IR-A:IR-B ratio has been implicated in the insulin resistance of patients with myotonic dystrophy and possibly in patients with T2DM (123).

The sequences involved in IR exon 11 splicing are only partially known. Transient transfection experiments in human hepatoblastoma cells (HepG2) with minigenes spanning from exon 10 to 12 allowed for identification of a 48-nucleotide purine-rich sequence at the 5′ end of intron 10 that functions as a splicing enhancer and increases exon 11 inclusion (124). Moreover, a 43-nucleotide sequence that favors skipping of exon 11 has been mapped upstream of the break point sequence of intron 10. Mutation studies have also indicated the existence of exon 11 sequences that play a role in determining the degree of exon inclusion in both a positive and negative manner. Further minigene analysis indicated that sequences in exons 10, 11, and 12 regulate the splicing process, perhaps because they are recognized by specific splicing factors including U1 snRNP, SF1, and U2AF65/35. In particular, strengthening of either the 5′ or 3′ splice sites in exon 11 by mutagenesis leads to its constitutive inclusion. In contrast, strengthening of upstream and downstream splice donor and acceptor sites on the neighboring exons (10 and 12) leads to a decreased exon 11 inclusion. Moreover, the interplay of splicing factors involved in the regulation of tissue-specific or developmental-related exon 11 skipping remains unclear (125). Studies of the rat clathrin light chain B and the rat β-tropomyosin genes have shown that overexpression of SF2/ASF proteins induces the use of a proximal splice site, either 5′ or 3′, over a distal site (126, 127). This activity is antagonized by the splicing factor hnRNP-A1, which favors the use of distal splice sites over proximal sites. Hence, the alternative choice of splice sites reflects a balance between SF2/ASF and hnRNP-A1 proteins. These results are in accordance with the observation that cancer cells, which express mostly IR-A, overexpress hnRNP-A1 proteins as well (128).

More recently, a mechanism favoring a high IR-A:IR-B ratio has been described to operate in skeletal muscles of myotonic dystrophy type 1 (MD1) patients. MD1 is caused by an expanded CUG trinucleotide repeat in the 3′ untranslated region of the dystrophia myotonica protein kinase (dmpk) gene transcript (129). As a consequence of CTG expansion, mutant DMPK RNA accumulates in the nucleus, causing dmpk haploinsufficiency (130). Moreover, nuclear accumulation of CUG-containing RNA alters the levels of certain splicing factors and inactivates muscleblind (MBNL) 1 proteins, which are required for inclusion of IR exon 11 in the IR-B transcript (131, 132). In contrast, other splicing factors are abnormally expressed in MD1 myoblasts, including the CUG-binding protein (CUG-BP1), a component of the CELF family of splicing regulators, and hnRNP H proteins (133, 134).

Elevated levels of both hnRNP H and CUG-BP1 result in formation of an RNA-dependent complex that inhibits IR exon 11 inclusion, which results in a predominance of IR-A. This inhibitory effect cannot be blocked by MBNL1 proteins, which normally bind hnRNP and CUG-BP1, because of their nuclear sequestration and inactivation. Studies of a human IR minigene encoding *Ir* exons 10, 11, and 12 have demonstrated that CUG-BP1, hnRNP H and MBNL1 are all able to directly bind RNA sequences encoded by this *Ir-b* minigene (135), but the binding sites remain unknown. These results suggest that the fine-tuning of IR isoform expression may be regulated by a balance between MBLN, CELF, and hnRNP H proteins.

B. IR isoforms and ligand specificity

The alternative splicing of exon 11 in the expression of IR-A and IR-B results in structural and functional differences between the isoforms. Therefore, the two isoforms have unique properties (10–12). Indeed, IR-A displays approximately 1.7-fold higher affinity for insulin than IR-B (10). Moreover, Yamaguchi *et al.* (11) observed that insulin association and dissociation from IR-A is faster than from IR-B. These findings are counterintuitive, given the expectation that receptors with higher binding affinity usually have slower ligand dissociation kinetics. This ap-

parent contradiction might be an artifact of the procedure that was employed in the dissociation studies, given that it was measured in the absence of unlabeled insulin. Negative cooperativity does not occur, and potential changes in receptor site-site interactions are not taken into account under such conditions. Increased IR-A internalization and recycling time (see *Section III.C*) may also contribute to the results obtained in intact cells.

In contrast to the very modest difference in insulin steady-state binding affinity, we found that the two isoforms markedly differ in their IGF-II binding affinity. Bound labeled insulin was displaced from IR-A by low concentrations of IGF-II (ED_{50} = 2.5 nM), whereas high IGF-II concentrations were necessary for displacement from IR-B (ED_{50} >100 nM). These results were obtained in IGF-IR knockout mouse embryo fibroblasts that had been transfected with either human IR-A or IR-B cDNA (R^-/IR-A and R^-/IR-B, respectively) and in transfected NIH-3T3 and CHO cells (13) (Table 1). Moreover, the ability of IGF-I to compete with ^{125}I-insulin binding differs between the two IR isoforms, although much less markedly than IGF-II (Table 1). Half-maximal binding inhibition for IGF-I was about 10-fold higher in cells expressing IR-A than in cells expressing IR-B (41 ± 15 *vs.* 390 ± 50 nM for CHO/IR-A and CHO/IR-B, respectively) (11). Similar differences were found in immunocaptured receptors (136). These data indicate that residues encoded by exon 11 exert a major effect on the conformational state of the IR α-subunit binding site.

As previously described, the insulin binding site on the L1 domain ("classical binding site") of both IR isoforms depends on two functional epitopes. However, the quantitative contribution of single amino acids to insulin affinity differs significantly for each IR isoform. In particular, mutation of Asn^{15} to alanine in IR-A results in a receptor that is completely devoid of insulin binding activity, whereas a similar mutation in IR-B results in a 63-fold decrease in affinity. Mutation of Leu^{37} to alanine in IR-B produces a 40-fold affinity reduction (137). In the C-terminal domain of the α-subunit, alanine mutations of Tyr^{708} or Asn^{711} have a greater effect on the affinity of the B isoform with respect to the A isoform, whereas the reverse occurs for alanine mutations of Leu^{709}, Asp^{707}, Val^{713}, Val^{715}, and Phe^{714} (137).

TABLE 1. IC_{50} values (nM) for insulin, IGF-II, and IGF-I, as measured by either ligand competition assay or BRET, using intact cells or immunopurified receptors

	Insulin	IGF-II	IGF-I	First author, year (Ref.)
IR-A	0.9		41.0	Yamaguchi, 1993 (11)
	0.9	2.5	>30.0	Frasca, 1999 (13)
	0.2	2.2	9.0	Benyoucef, 2007 (136)
IR-B	1.6		390.0	Yamaguchi, 1993 (11)
	1.0	>20.0	>30.0	Frasca, 1999 (13)
	0.5	10.0	90.0	Benyoucef, 2007 (136)
HIR-AB	1.0	10.0	>50.0	Blanquart, 2008 (224)
IGF-IR	>30.0	0.6	0.2	Pandini, 2002 (184)
	>100.0	4.4	0.8	Denley, 2004 (138)
	>1000.0	0.5		Benyoucef, 2007 (136)

HIR-AB, IR-A/IR-B hybrid receptor.

Recently, Denley *et al.* (138) evaluated regions of IGF-II that may determine its high affinity for IR-A. Studies with chimeras, where the C and D domains of IGF-II were exchanged with the analogous domains of IGF-I, indicated that C and D domains of IGF-II are involved in high-affinity binding to IR-A and low-affinity binding for IR-B. A and B domains of IGF-II and IGF-I are apparently not important for binding specificity (138).

Taken together, these data indicate that the binding site structures of IR-A and IR-B are quite different. Importantly, a direct interaction of either insulin or IGF-II with the peptide encoded by exon 11 has not yet been demonstrated.

C. IR isoforms and signaling specificity

Exon 11 inclusion in the IR transcript determines differences in ligand binding affinity, receptor internalization, and recycling time and intracellular signaling. The faster internalization and recycling time (12, 139) of IR-A has been attributed to the CEACAM1 protein, which selectively increases IR-A internalization despite being equally phosphorylated by both IR isoforms (140).

Differences between IR isoforms regarding signaling activation have been reported in pancreatic β-cells. In these cells, insulin gene transcription is promoted by signaling through IR-A/PI3K class 1A/p70S6K, whereas βGK gene transcription is regulated through IR-B/PI3K class II-like/protein kinase B (141). These signaling differences have been attributed to different localization of IR isoforms at distinct plasma membrane subdomains, thereby allowing for partitioning of adaptor proteins and subsequent activation of selective signaling pathways (142). Although distinct localization of the two isoforms has not been independently confirmed, examples of differences in signaling between IR isoforms have been reported in other models, including neonatal hepatocytes. Indeed, neonatal hepatocytes expressing IR-A, but not IR-B, have increased basal glucose uptake that does not correlate with insulin stimulation (143). In addition, neonatal hepatocytes expressing IR-B alone have increased caspase-8 and caspase-3 activation and increased apoptosis, whereas the mitochondrial branch of apoptosis is activated in neonatal hepatocytes expressing IR-A. Only coexpression of both IR isoforms protected the hepatocytes from apoptosis (144). Data obtained in murine 32D hemopoietic cells indicated that IR-A preferentially induces mitogenic and antiapoptotic signals, whereas IR-B predominantly induces cell differentiation signals (145).

Moreover, in R$^-$/IR-A cells, activation by either insulin or IGF-II induces nuclear translocation of IRS-1, whereas neither insulin nor IGF-II is able to induce IRS-1 nuclear translocation in R$^-$/IR-B cells (146).

Finally, the abnormally high IR-A:IR-B ratio in muscle cells of patients with myotonic dystrophy seems to play an essential role in the insulin resistance of these patients, suggesting a substantial difference in signaling capability between IR-A and IR-B in muscle cells (see *Section V.A*).

Besides functional differences from IR-B, IR-A may differentially elicit biological effects and intracellular signaling upon insulin or IGF-II binding. R$^-$/IR-A cells preferentially undergo proliferation when stimulated with IGF-II, whereas they preferentially activate glucose uptake when stimulated with insulin (13, 147). These different effects are coincident with quantitative and temporal differences in the phosphorylation of intracellular substrates in response to either insulin or IGF-II binding. In particular, IGF-II is less effective than insulin in stimulating the IRS/PI3K pathway, rather than the Shc/ERK pathway (13), and it induces higher p70S6K:Akt and ERK1/2:Akt ratios than insulin (148). In SKUT-1 human leiomyosarcoma cells, which express IR-A and not IR-B and lack functional IGF-IR, IGF-II is a more potent activator of the Shc/ERK pathway and stimulator of cell migration than insulin, whereas insulin is a more potent stimulator of the PI3K/Akt pathway and a better protector from apoptosis than IGF-II (148). This differential IR-A signaling activation elicited by insulin and IGF-II is reflected by differential global gene expression in response to treatment with insulin or IGF-II. Microarray technology performed in R$^-$/IR-A cells stimulated with either insulin or IGF-II indicated that 214 transcripts were similarly regulated by insulin and IGF-II, whereas 45 genes were differentially regulated. Among them, 12 genes were responsive only to insulin, whereas six genes were responsive only to IGF-II. The other 27 transcripts were regulated by both ligands, but with a significant difference in response kinetics, because the effect of IGF-II was generally stronger and more persistent than that of insulin (149).

Recently, we showed that IGF-I may induce remarkable activation of intracellular signaling in R$^-$/IR-A cells characterized by a high p70S6K:Akt ratio, despite eliciting only modest IR-A autophosphorylation (150). These studies await independent confirmation.

IV. Insulin Receptor Isoforms in Physiology

A. Role of IR isoforms in development

Insulin and IGFs are important for development in both vertebrates and invertebrates. Genetic studies in *Drosophila melanogaster* have shown that insulin-like peptides (ILPs) regulate development, longevity, metabolism, and female reproduction by acting through a conserved insulin signaling pathway (151, 152). Indeed, altered expression of genes encoding ILPs in this conserved pathway has phenotypic effects on the growth and organogenesis of *D. melanogaster*. In particular, overexpression of *d-ilp* genes results in bigger flies in a *Drosophila* IR-dependent manner (25). In contrast, genetic ablation of *d-ilp* genes causes a development delay and growth retardation (153). These phenotypes are similar to those observed in flies homozygous for a partial loss-of-function mutation in *Drosophila* IR (25).

In vertebrates, IR and IGF-IR are expressed early in fetal tissues (44). IR-A is the predominant IR isoform in fetal tissues, whereas IR-B appears in postnatal life in insulin-target tissues (13). These data suggest that, in fetal tissues, IR-A may play a role in mediating some of the effects of IGF-II. The contribution of IR-A to embryo development has been mainly studied in mice, where IGF-II acts as an IR ligand in the promotion of embryonic growth (154). Mice lacking IR are born with 10–20% growth retardation (154) without apparent metabolic abnormalities; metabolic abnormalities appear after birth. The growth-promoting role of IR in embryos is indicated by studies in mice with combined alterations in various elements of the IGF system. These experiments document that IGF-II induces signals through IR to stimulate embryonic growth. Single mutations that ablate *Ir* function result in embryos that are approximately 80–90% of normal size, whereas single *Igf1r* mutations result in smaller embryos (45% of normal size). Combined ablation of *Ir* and *Igf1r*, however, results in even smaller embryos (30% of normal size). The same "30% phenotype" (155) is brought about by double mutations inactivating *Igf2* and *Igf1r*, but not by inactivating mutations of *Igf1* and *Igf1r*. The latter double mutants have the same phenotype as single *Igf1r* mutants (156), suggesting that IGF-I signals exclusively through IGF-IR. In contrast, the growth retardation observed in *Igf2/Igf1r* double mutants is greater than that observed in *Igf1r* single mutants, indicating that IGF-II signals through an additional receptor besides IGF-IR. Because *Igf2/Igf1r* and *Ir/Igf1r* double mutants have the same phenotype as *Igf2/Igf1r/Ir* triple mutants, the additional receptor for IGF-II is IR. Biochemical evidence that the IR-A is indeed a high-affinity IGF-II receptor (13) supports this conclusion.

The analysis of growth retardation onset in mouse embryos lacking *Ir*, or *Igf1r*, or both indicates that during midgestation [embryonic day (E) 12.5 to 15.5] IGF-IR is the main IGF-II receptor, whereas, starting at about E15.5, the IGF-II/IR interaction becomes quantitatively more important (154).

Unlike mice, humans lacking IRs show more severe intrauterine growth retardation (157–160). Indirect evidence suggests that growth retardation is present as early as 27 wk gestation (161). This discrepancy with *Ir*-deficient mice is probably due to differences in development timing and to a compensatory increase in IGF-IR expression (154). Indeed, rodents are born at a developmental stage corresponding to approximately 26 wk of human gestation. Therefore, the IR-dependent phase of embryonic growth is temporally limited in mouse embryos, whereas it is more extended in humans. This distinction explains why the growth retardation defect in *Ins1/Ins2*- or *Ir*-deficient mice is not as severe as the growth retardation of children with leprechaunism characterized by IR mutations that impair insulin binding.

Moreover, IGF-II expression declines after birth in mice. Therefore, the growth-promoting effect of the IR is mediated by insulin after birth (162). In contrast, IGF-II secretion persists throughout adult life in humans (163), providing an additional element of differentiation between the two species (164).

The IGF system may also influence placental development. Although IGF-I expression is low in the placenta, IGF-II expression is abundant in most species (165). The role of IGF-II in placental development has been confirmed in mice lacking the type 2 IGF receptor (mannose 6-phosphate receptor), which is responsible for IGF-II degradation. These animals have a placental mass that is 25% greater than normal (162). These data suggest an important role for IR-A in mediating the effect of IGF-II on placental development.

Because reduced placental development may result in reduced fetal growth (166), it has been suggested that IGF-II and IR-A may influence fetal growth by promoting normal placental development. This conclusion is supported by the reduced placental and fetal size of the IGF-II knockout mice (155). Accordingly, placental growth is reduced in mice lacking the placental specific *igf-II* gene transcript (postnatal day 0); as a consequence, fetal size is also reduced (167). In contrast, placental weight is normal in IGF-I-deficient mice (156).

IGF-I and IGF-II likely play different roles in placental development and function. Data indicate that IGF-I regulates the differentiation of cytotrophoblast into syncytiotrophoblast (168) and extravillous cells (169). In contrast, IGF-II appears to have a role in regulating nutrient exchange. More specifically, in mice with specific placental IGF-II knockdown, the placenta has significantly reduced diffusional exchange surface area and nutrient permeability (170). The different effects of IGF-I and IGF-II on placental development are compatible with the possibility that IGF-II acts via a different receptor (IR-A) than IGF-IR.

Interestingly, IR-A may contribute to vertebrate fetal development by mediating the growth promoting effects of proinsulin. In several vertebrate species, proinsulin mRNA is first detected before formation of the endocrine pancreas (171) and later in development, in extrapancreatic tissues (172). Although extrapancreatic proinsulin gene expression is much lower than in the pancreas (171), proinsulin mRNA can be detected in the chick embryo as early as d 0.5 of development, (173), as well as in the retinal neuroepithelium at E3 (174). Although proinsulin protein is detected in the three embryonic layers, it is mainly detected in the neuroepithelium (173). Remarkably, the presence of proinsulin can be detected before IGF-I expression (173). Because functional IRs and IGF-IRs are both expressed at early developmental stages (175, 176), it is possible that proinsulin may indeed act via both IR and IGF-IR. Because the only IR isoform expressed in chicken is IR-A (31), this isoform may also act as a receptor for proinsulin, although proinsulin was found to be a weak competitor of ^{125}I-insulin for binding to IR on IM-9 cells, which exclusively express IR-A (177).

Binding studies in the chick neuroretina revealed that proinsulin may act through IR/IGF-IR hybrids [hybrid receptors (HRs)]. Indeed, in the neuroretina, heterodimeric receptors are found at early stages, when the homodimeric IR was apparently absent. The proportion of HRs decreases and that of homodimeric IRs increases as retinal development proceeds (178). Interestingly, binding-displacement studies in this chick neural tissue revealed that HRs bind not only insulin and IGF-I with similar high affinity but also proinsulin (approximate $EC_{50} = 10^{-8}$ M) (178). These data suggest that IR-A hemidimers may confer to HRs the ability to bind proinsulin and to cross-talk with the IGF-IR signaling pathway in the early development of vertebrate embryos.

B. IR isoforms and cell differentiation

IR isoform switching to predominant IR-B expression has been found to be associated with cell differentiation in several models.

1. Adipocyte differentiation

Differentiation of brown preadipocytes into mature adipocytes is associated with a dramatic increase in total IR content and with a marked IR isoform switch from IR-A to IR-B (179). In addition, IR-deficient brown preadipocytes are unable to undergo differentiation. However, excessive IR expression is also associated with the inability to differentiate, suggesting that a high IR:IGF-IR ratio may also inhibit the ability of cells to differentiate (179). Indeed, high

IR levels appear to impair IGF-IR signaling, probably by competing for intracellular substrates (180).

IR isoform switching to IR-B also occurs in 3T3-L1 cells induced to differentiate into adipocytes by dexamethasone (181). In these cells, isoform switching persists up to 8 d after dexamethasone removal, indicating that it directly correlates with cell differentiation. Inactivation of one *Ir* allele by homologous recombination reduced IR expression level by approximately 50% and inhibited the differentiation process (182).

Differentiation of stromal-vascular preadipocytes isolated from rat epididymal white adipose tissue yielded similar results. During preadipocyte differentiation, the proportion of IR-B expression level increases, whereas that of IR-A decreases significantly (183).

Taken together, these results suggest that an increase in the IR-B:IR-A ratio, as well as a certain IR/IGF-IR ratio, is important for adipocyte differentiation.

2. Hepatocyte differentiation

Human hepatoblastoma (HepG2) cells, when cultured in standard conditions, express approximately 80% IR-A and show a fetal phenotype, including increased synthesis of α-fetoprotein. However, in the presence of low serum and dexamethasone, they differentiate and acquire approximately 80% IR-B expression, a percentage similar to that of adult hepatocytes (181, 184). Whether a high IR-B:IR-A ratio is a prerequisite for hepatocyte differentiation remains to be clarified.

3. Hematopoietic cell differentiation

Studies in 32D cells have provided further insights into the role of IR isoforms in cell differentiation (145). 32D cells are murine hematopoietic cells that have an absolute requirement of IL-3 for cell survival (185). 32D cells differentiate into granulocytes when shifted from IL-3 to IGF-I, a process that can be monitored by evaluating the expression of myeloperoxidase (MPO) (186). Transfection of 32D cells with an IR-B expression plasmid resulted in MPO mRNA up-regulation, regardless of the presence of ligands, suggesting that IR-B can initiate a differentiation program. This phenomenon was not observed in cells transfected with an IR-A expression plasmid (145). Cotransfection with an IRS-1 expression plasmid inhibited MPO mRNA expression in both IR-A- and IR-B-transfected cells, suggesting that IRS-1 interferes with the IR-driven differentiation program.

4. The thyroid cancer model

As mentioned in *Section V.C*, thyroid cancer occurs in different histotypes with a variable degree of differentiation. IR-A predominance is directly related to the degree of thyroid cancer dedifferentiation (81).

Taken together, these studies indicate a positive correlation between predominant IR-B expression and cell differentiation. These data fit well with the high IR-A:IR-B ratio observed in poorly differentiated cells, both in physiology (embryonic and fetal cells) and in disease (cancer cells). The molecular bases underlying IR isoform switching and cell differentiation are, however, largely unknown.

C. Role of IR isoforms in aging

Several pieces of evidence suggest that the IGF system is important in the regulation of life span (108) because a variety of biological models indicate that inhibition of insulin/IGF signaling results in increased life span. In *Caenorhabditis elegans* (187), disruption of *daf-2* (the ortholog of the *Ir/Igf1r* gene family) prolongs life by 50% (188). Similar genetic manipulations in *D. melanogaster* result in increased life span (189). Similarly, ablation of *chico* (*Irs1*-like gene) also increases the life span (190).

The IGF system is involved in the control of vertebrate longevity (15, 191, 192) by modulating the levels of antioxidative defense molecules (193). Mice that overexpress GH/IGF-I exhibit 50% life span reduction, and exogenous administration of GH/IGF-I decreases the levels of antioxidative defense molecules (194). In contrast, hemizygous *Igf1r* knockout mice (191) display decreased growth and body weight and increased life span. It is reasonable to suppose that IGF-I may affect life span extension by regulating glucose metabolism because calorie restriction increases life span (195) and reduces plasma levels of GH, IGF-I, and insulin (196). Calorie-restricted animals, dwarf mice, and IGF-I-deficient mice share increased insulin sensitivity (197).

Studies in vertebrates suggest that reduced IGF-I signaling could extend life span in humans. However, in humans, GH and IGF-I deficiency are associated with growth disorders, insulin resistance, increased risk of cardiovascular disease, and atherosclerosis. On the other hand, phenotyping of human centenarians revealed that insulin sensitivity, which is a key feature of long-lived mutant mice (198), is strictly associated with exceptional longevity in humans (199). However, whereas decreased IGF-I levels are associated with increased morbidity and mortality in advanced age, increased IGF-I levels do not promote longevity due to the greater risk of developing certain cancers, including those of the prostate, breast, lung, and colon (200). Therefore, human longevity may be regulated by an optimal IGF system set point (201) that is permissive for optimal insulin sensitivity. A clear-cut interpretation of the role of the IGF system in human longevity has been provided by studies that have identified new nonsynonymous mutations in *Igf1r* in centenarians. These mu-

tations resulted in reduced IGF-I signaling in transformed lymphocytes (202).

These findings suggest a more complex relationship between IGF signaling and longevity in humans. Because insulin and IGF signaling are more convergent in lower animals, longevity in those animals is more directly related to the reduction of common insulin/IGF signaling, regulating both metabolism and growth. Because insulin and IGF signaling have developed more specific and distinct functions in humans, due also to the expression of specific receptor subtypes (see *Section II.A*), human longevity may result from attenuation of IGF signaling (specific growth signaling) and the enhancement of insulin sensitivity (specific metabolic signaling). In this respect, the relative expression of IR-A might contribute to regulate this set point and the predominance of either IR or IGF-IR signal branching.

Indeed, in vertebrates such as rats, advanced age is associated with isoform switching to IR-A (183) and an increased IR-A:IR-B ratio. These changes may contribute to the insulin resistance observed among aged animals, together with other changes, such as decreased IRS proteins. These observations are in accordance with other studies in aged rats, showing an association between insulin resistance/glucose intolerance (203, 204) and decreased IR-B expression in the liver, heart, adipose tissue, and skeletal muscle. It is reasonable to speculate that the same isoform switch may occur in humans with age, although no experimental data are available.

In conclusion, data obtained using experimental models indicate that an increased relative abundance of IR-A and insulin resistance usually occurs in advanced age. Increased IR-A expression may enhance IGF signaling via IR-A/IGF-IR hybrids (184) (see *Section VII.A–B*). In addition, compensatory hyperinsulinemia increases IGF bioavailability by decreasing IGF binding protein (IGFBP)-1 and IGFPB-2 (205). Moreover, *in vitro* data indicate that IR-A activation by IGF-II results in preferential activation of the IRS-2 pathway (13, 206). Brain IRS-2 (207) plays a crucial role in nutrient homeostasis (108), and reduced IRS-2 signaling may result in increased life span (207). Thus, one might speculate that enhanced IRS-2 signaling due to an increased relative abundance of IR-A and increased IGF-II action may play a role in reducing life span in vertebrates.

V. Insulin Receptor Isoforms in Disease

A. Insulin resistance and altered IR-A/IR-B ratio: the model of myotonic dystrophy

MD1 has been used as a model to study the factors involved in aberrant IR mRNA splicing and its possible role in determining insulin resistance. MD1 is a disorder characterized by skeletal muscle abnormalities, such as myotonia, weakness, and atrophy (208). However, several extramuscular abnormalities, including cardiac conduction defects and neuropsychiatric and endocrine abnormalities, are also observed in MD1 patients (208). In particular, these patients show peripheral insulin resistance with glucose intolerance, hyperinsulinemia, and an increased risk of developing type II diabetes (209). As discussed in *Section III.A*, muscleblind proteins that are necessary for inclusion of IR exon 11 are functionally inactivated in MD1 patients (131, 132), thus impairing IR-B expression. Indeed, Savkur *et al.* (210) observed an increased IR-A:IR-B ratio in the skeletal muscle of MD1 patients and provided evidence suggesting that this abnormality may play a role in peripheral insulin resistance.

Similar abnormalities have been observed in myotonic dystrophy type II (MD2), which is caused by accumulation of transcribed but untranslated CCTG expansion and consequent CUG repeat-containing RNA in ribonuclear inclusions (211, 212). As in MD1, the IR-A:IR-B ratio is significantly increased in the muscle of MD2 patients. In both MD1 and MD2 patients, IR abnormalities appear very early and precede the development of dystrophic changes (210, 213).

Taken together, studies on MD1 and MD2 have identified a mechanism of predominant IR-A expression in muscle and other tissues caused by the defective interplay of at least three families of splicing factors. They have also confirmed that predominant IR-A expression in muscle may cause peripheral insulin resistance.

B. Insulin resistance and type 2 diabetes mellitus: a pathogenic role for differential splicing of IR?

In Western countries, more than 6.5% of the adult population and up to 15% of the elderly population is affected by T2DM. The pathogenesis of T2DM includes a variable degree of insulin resistance in muscle, liver, and fat tissues and increasingly impaired insulin secretion.

Whether an altered balance of IR isoforms is involved in insulin resistance and T2DM is unclear. In contrast with results obtained in MD1 and MD2 patients, Mosthaf *et al.* (3) found reduced IR-A expression in skeletal muscles of T2DM patients compared with control subjects and suggested that the reduced expression of this high-affinity isoform could be pathogenetically associated with insulin resistance and T2DM. Importantly, the reduced IR-A:IR-B ratio found in adipocytes and muscle of T2DM patients was confirmed by some (4, 5, 9), but not all studies (6–8).

To address this discrepancy found in human studies, studies in animal models were performed. In pancreatectomized insulin-resistant diabetic Sprague-Dawley rats, no alterations in *Ir* splicing were observed, compared with

control rats (204, 214), suggesting that diabetes, *per se*, does not alter relative IR isoform abundance.

In contrast, studies performed in spontaneously obese and diabetic rhesus monkeys indicated that hyperinsulinemia is associated with preferential IR-A expression. Indeed, hyperinsulinemic monkeys (either normoglycemic or at early-stage diabetes) were found to express a higher proportion of IR-A in skeletal muscle and liver, compared with the normal controls (normoglycemic and hyperinsulinemic) and late-stage diabetic monkeys (hyperglycemic and hypoinsulinemic) (215, 216). This observation was in accordance with the finding that IR-A expression is necessary for the increased insulin secretion observed in early stages of T2DM because insulin gene transcription by pancreatic β-cells requires IR-A signaling (141).

Increased relative abundance of IR-A has been reported in a T2DM patient with extreme insulin resistance and hyperinsulinemia (217). Whether insulin resistance and hyperinsulinemia *per se*, in the absence of T2DM, are associated with altered IR splicing remains unclear. A study carried out in a small series of obese nondiabetic patients did not find any significant changes in IR isoform expression (7). In contrast with data obtained in monkeys, a study carried out in nondiabetic obese and insulin-resistant Pima Indians found that the relative abundance of IR-A in skeletal muscle was reduced compared with matched non-insulin-resistant controls (218). Moreover, patients with insulinoma, characterized by hyperinsulinemia and non-genetically determined insulin resistance, were also found to have reduced IR-A expression in skeletal muscle compared with controls (219).

It is important to understand IR isoform regulation in nonclassical insulin target tissues of insulin-resistant patients because these tissues may respond with hyperplastic or neoplastic changes (220). Currently available data are not sufficient to resolve this issue. In one study, IR isoforms were measured in granulosa cells of insulin-resistant patients with polycystic ovary syndrome (PCOS) (221). IR-A was predominantly expressed (approximately 80%) in granulosa cells of both normal and PCOS patients. Total IR was significantly higher in granulosa cells of PCOS patients than in controls, despite increased intrafollicular insulin levels in PCOS patients (no down-regulation effect) (221).

A conclusive interpretation of data concerning the role of IR isoform regulation in insulin resistance and insulin secretion in T2DM patients is not possible at the moment. T2DM is a complex and heterogeneous syndrome, and IR splicing can be affected by several variables, including hyperinsulinemia, hyperglycemia, age, disease stage, and genetic alterations underlying T2DM. Interpretation of data is also made difficult by the different models and methodologies used and by the limitation that most studies have not measured total IR content. Although it seems unlikely that altered IR splicing plays a major role in insulin resistance in T2DM, insulin resistance, hyperinsulinemia, and altered glucose tolerance are associated with an increased IR-A:IR-B ratio in certain genetic syndromes, both in animals (spontaneously diabetic monkeys) and in humans (patients with MD1 and MD2). Indeed, when studies in humans were reanalyzed, taking the T2DM stage into account, data appeared to be mostly consistent with those obtained in monkeys (215). However, some data obtained in T2DM, in Pima Indians, and in patients with insulinoma do not fit this model.

With regard to potential explanations for the contribution of an increased IR-A:IR-B to insulin resistance, IR-A has a slightly higher binding affinity for insulin than IR-B, but also a lower signaling potential (222, 223). Moreover, because IR-A and IR-B may heterodimerize, IR-A overexpression can cause a significant reduction in IR-B homodimers and an increase in IR-A/IR-B heterodimers, which have high affinity for IGF-II (215, 224). This shift may result in increased IR availability for IGF-II binding and reduced IR availability for the metabolic effects of insulin.

C. Aberrant expression of IR-A in cancer

In the last few decades, accumulating evidence has established that IRs are usually abnormally expressed in cancer cells, where they mediate both the metabolic and nonmetabolic effects of insulin. Most recently, it was observed that *Ir* splicing is altered in cancer cells, thus increasing IR-A:IR-B ratio, which profoundly affects the cell response to circulating insulin and IGFs. These observations may have important implications in cancer biology and treatment.

1. Aberrant IR expression in cancer cells

IR expression has been evaluated in a variety of human malignancies. Papa and colleagues (225, 226) found that IRs were overexpressed in a large series of human breast cancer specimens. Mean IR content was more than 6-fold higher in cancer specimens compared with normal breast tissue ($P < 0.001$) (225). Approximately 80% of breast cancer samples had an IR content higher than the mean value +2 SD in normal breast, and approximately 20% had IR values over 10-fold higher than mean value in normal breast (225). Functional studies indicated a higher IR responsiveness to insulin in breast cancer (227) than in normal breast cells.

Further insights into the role of IR overexpression in cancer were provided by data indicating that IR-A is also an IGF-II receptor (13). IR-A was found to be the pre-

dominant IR isoform expressed in a variety of carcinomas, including carcinomas of the breast, colon, and lung (13).

In particular, IR-A was found to be the predominant IR isoform in a panel of breast cancer cell lines (ranging from 64–100% of total IR) and in a series of breast cancer tissue specimens (ranging from 40–80%) (228) (Fig. 4). In contrast, IR-A represented 30–50% of total IR content in normal breast cells and tissue specimens (228). Interestingly, in several breast cancer cell lines (especially in ER-negative cells), as well as in approximately 40% of breast cancer specimens, IR expression predominates over IGF-IR expression (228, 229). Breast cancer cells produce IGF-II in an autocrine manner. In cells with a high IR-A:IGF-IR ratio, such as the ER-negative breast cancer cell line MDA-MB-157, autocrine production of IGF-II stimulates cell growth through IR-A stimulation. In MDA-MB-157 cells, blocking either IGF-II or the IR markedly inhibited growth, demonstrating the relevance of this loop in cell growth (228).

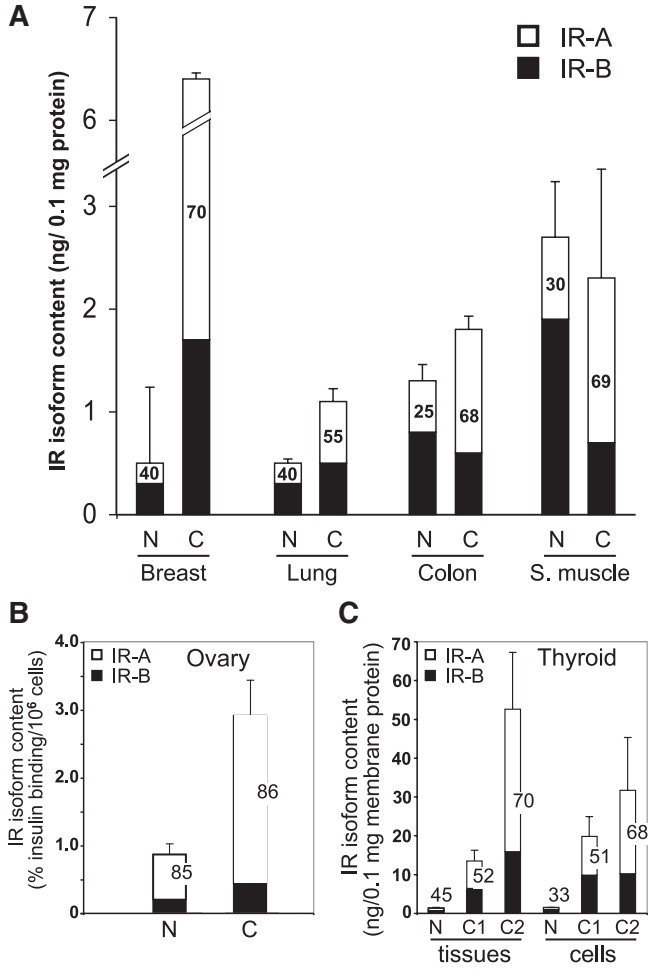

FIG. 4. IR isoform content in various cancer tissues (C), compared with the corresponding normal tissues (N). A, Data pertaining to breast, lung, and colon cancer were acquired by analysis of surgical tissue specimens (13). Data on skeletal muscle (s. muscle) were obtained by analysis of human rhabdomyosarcoma cells (148) and were compared with values obtained by analysis of human muscle specimens (our unpublished data). IR isoform content has been calculated by determining the total IR protein content, as measured by ELISA, and the relative IR isoform transcript abundance, as measured by RT-PCR. B, Data from ovaries have been obtained by analysis of transformed and nontransformed cultured cells. IR isoform content has been calculated by determining the total IR protein content, as measured by a binding assay, and the relative IR isoform transcript abundance, as measured by real time RT-PCR (230). Numbers indicate percentage of IR-A isoform expression. C, IR isoform content in surgical specimens and cultured cells of the normal thyroid compared with differentiated (C1) and undifferentiated (C2) cancer (81). IR isoform content has been calculated by determining the membrane IR protein content, as measured by ELISA, and the relative IR isoform transcript abundance, as measured by RT-PCR.

Both IR and IGF-IR were also found to be overexpressed in well-differentiated thyroid carcinomas (81). In particular, the IR-A:IGF-IR ratio progressively increased in the less-differentiated carcinomas (81). Also, thyroid cancer cell lines, especially when undifferentiated, produce autocrine IGF-II, which can phosphorylate the IR at nanomolar concentrations. The ability of IGF-II to phosphorylate the IR is in close relation with IR-A relative abundance ($r = 0.628$; $P = 0.0001$). Similar data were reported in ovarian cancer cells, in which IR-A is preferentially expressed and mediates mitogenic signaling in response to low doses of insulin and IGF-II (230) (Fig. 4).

The relative contribution of IR-A and IGF-IR to the biological effects of IGF-II was evaluated in thyroid cancer cells, B-CPAP, overexpressing IR-A. In these cells, IGF-II stimulation was similarly inhibited by either an IR-blocking antibody, MA-51, or an IGF-IR-blocking antibody, αIR3 (81, 231). Combination of the two antibodies was the most effective at inhibiting IGF-II stimulation (81). These results indicate that both IR-A and IGF-IR are involved in mediating the effects of IGF-II.

Taken together, these studies indicate that IR-A is aberrantly expressed in breast and thyroid cancer cells. The IR-A:IGF-IR ratio is substantially increased in ER-negative breast cancer cells and in less-differentiated thyroid cancer cells. The ratio correlates with autocrine IGF-II production. Moreover, IR-A significantly contributes to the biological effects of IGF-II. To fully inhibit the biological effects of autocrine IGF-II, both IR-A and IGF-IR need to be blocked (81).

An important role of the IGF-II/IR-A loop has also been observed in gestational trophoblastic neoplasias, including hydatidiform moles, invasive moles, choriocarcinomas, and placental site trophoblastic tumors (232). These findings are in accordance with the notion that IGF-II plays an important role in normal placental development and is expressed in both hydatidiform moles and choriocarcinomas (17). Both IGF-I and IGF-II stimulated choriocarcinoma cell invasion, although they signal through different receptors: IGF-I through IGF-IR, and IGF-II through IR-A. In JEG-3 choriocarcinoma cells, which pre-

dominantly express IR-A, IGF-II stimulated cell invasion more potently than insulin, although both ligands signaled through IR-A (233). These data are reminiscent of those obtained in leiomyosarcoma cells (148). One possible explanation for the stronger effect of IGF-II on cell migration is its ability to bind vitronectin, a ligand of integrin $\alpha V\beta 3$ (234), and therefore to integrate different signaling through this pathway.

IR-A overexpression is not limited to malignancies derived from epithelial cells. Both leiomyosarcoma and rhabdomyosarcoma cells were found to express IR-A and IGF-IR in variable amounts, with IR-A often exceeding IGF-IR. These cells also secrete IGF-II. Moreover, the importance of the IGF-II/IR-A loop is recapitulated in SKUT-1 leiomyosarcoma cells that express high IR-A levels and are almost null for IGF-IR. In these cells, IGF-II was able to elicit its biological effects exclusively via IR-A, whereas IGF-I was totally ineffective due to the absence of functional IGF-IR (148). Similar results were reported for solitary fibrous tumors, which are mesenchymal tumors that are frequently associated with hypoglycemia. More specifically, IR-A was constitutively activated, whereas IGF-IR and IGF-I were not expressed (235).

A peculiar and so far unique role of the insulin/IR/autocrine loop in cancer progression has been reported in atypical teratoid/rhabdoid tumors (AT/RT) (236), a group of highly malignant pediatric tumors of the central nervous system (237). Many AT/RT cell lines not only overexpress IR but also produce insulin, which is able to stimulate an autocrine loop that is biologically relevant for cell proliferation. Insulin activates both the PI3K/Akt/mTOR and the ERK pathways. Furthermore, studies with either pharmacological inhibitors or small interfering RNAs indicated that the IR/PI3K/Akt/mTOR pathway is crucial for AT/RT cell proliferation in response to autocrine insulin (236). Although the IR isoform expressed by AT/RT cells was not investigated, it has been previously reported that central nervous system cells predominantly express IR-A (10). This study, therefore, suggests that a unique insulin/IR-A autocrine loop may contribute to cancer cell proliferation and survival.

2. Mechanisms of aberrant IR-A expression in cancer

The mechanisms responsible for aberrant IR-A expression in human cancer are largely unknown and may share similarities with the mechanisms operating during embryonic and fetal development, which are also characterized by increased IR expression and increased IR-A:IR-B ratio. As mentioned above (see *Section III.A*), we do not know the molecular mechanisms of tissue-specific and development-driven IR isoform regulation. Similarly, we do not know the mechanisms involved in IR exon 11 skipping in cancer. Alternative IR splicing is only one of the several examples of alternative splicing that may occur both in human development (238) and in cancer cell "retro-differentiation" (239) due to either mutations or cell stressors, such as hypoxia or altered pH (239). As far as IR is concerned, exon 11 skipping may be regulated by hormonal factors, such as dexamethasone and insulin (181). Increased cancer incidence has been observed in individuals with insulin resistance and hyperinsulinemia, consequent to obesity and/or T2DM (240). However, because contradictory data exist on the role of hyperinsulinemia and IR splicing, it seems unlikely that hyperinsulinemia plays a role in IR exon 11 skipping in cancer.

As far as IR overexpression in cancer is concerned, IR gene amplification seldom occurs, and IR gene rearrangement has not been reported. In 96 primary breast carcinomas with high IR expression, only 8% showed IR gene amplification, and none showed IR gene rearrangement (241).

An important mechanism for IR overexpression in cancer may be p53 protein inactivation. Wild-type p53 specifically acts as a repressor of the IR promoter, as indicated by studies in p53 knockout mice that have a higher liver IR content in respect to controls (42). Inactivating mutations of the p53 gene occur in approximately 50% of all human malignancies (242) and may account for decreased p53 inhibition at the IR promoter. In agreement with this hypothesis, undifferentiated thyroid carcinomas, which frequently carry a mutant p53, have higher IR levels than well-differentiated carcinomas carrying a wild-type p53 gene (81). p53 may be functionally inactivated by nonmutational mechanisms. For instance, high levels of HMGA1 proteins may reduce p53 DNA-binding activity by interacting with the p53 oligomerization domain (243). Actually, HMGA1 levels are low in normal differentiated cells, whereas they are high in embryonic and malignant cells (244). In addition, HMGA1 proteins may directly regulate IR gene transcription (see *Section II.B*). Indeed, HMGA1 proteins interact with two Adenine and Thymine-rich sequences of the IR promoter in a multiprotein complex involving Sp1 transcription factor, CCAAT/enhancer binding protein, and AP-2 transcription factor. All of these factors are required for full transactivation of the IR gene (245), and they may be dysregulated in cancer.

Finally, IR up-regulation is present in breast cancers that have been initiated in transgenic mice by various oncogenes, including Wnt, Neu, and Ret (246).

In conclusion, aberrant IR-A expression is strictly bound to cancer development and progression, both as a cause and an effect.

3. Aberrant IR-A expression in cancer: mechanisms of altered signaling and implications for cancer progression

Aberrant IR-A expression may contribute to the dysregulated response of cancer cells to insulin and IGFs in many ways. First, IR overexpression sensitizes cancer cells

to the pleiotropic effects of circulating insulin, especially in hyperinsulinemic patients. Tissue resistance to insulin associated with obesity or T2DM is essentially restricted to metabolic effects, whereas nonmetabolic effects are relatively unimpaired. For instance, hyperinsulinemia in insulin-resistant patients is often associated with increased proliferation of ovary theca cells and keratinocytes, causing PCOS and acanthosis nigricans. Circulating insulin levels in these patients are particularly high (0.2–0.6 nmol/liter), reaching a concentration that is similar to that required for half maximal IR autophosphorylation in breast cancer tissue (227). As already mentioned, the IR is not restricted to mediating metabolic activity; when overexpressed in NIH 3T3 fibroblasts or immortalized human breast epithelial cells at a level similar to that found in some breast carcinomas, IR-A induced a ligand-dependent transformed phenotype, an effect that could be inhibited by an IR-blocking antibody (247, 248). These cells, however, were not able to form tumors in nude mice, suggesting that other factors are required for full malignant transformation. The potential transforming effect of IR is also suggested by the observation that a 24-month treatment of female rats with a AspB10, an insulin analog with enhanced affinity for the IR, induced the formation of both benign and malignant mammary gland tumors (249).

As a second mechanism, IR-A overexpression expands the pool of IGF-I binding sites by forming HRs with the homolog IGF-IR. HRs containing IR-A (HR-A) may bind insulin, although with relatively low affinity (184) (see *Section VII*).

As previously mentioned, a third mechanism favoring IR-driven tumor promotion is the high affinity of IR-A for IGF-II, which is frequently produced by cancer cells.

Moreover, aberrant IR-A expression induces high IR-A:IR-B and IR-A:IGF-IR ratios, causing subtle but biologically significant differences in ligand-stimulated signaling.

As discussed in Section III.C, signaling differences between IR-A and IR-B in response to insulin have been previously described (11, 139, 141). IR-A binding to IGF-II is associated with stimulation of growth and cell invasion (13), whereas IR-B, which does not bind IGF-II, is associated with differentiation and metabolic signals (145). On the other hand, a high IR-A:IGF-IR ratio may favor the effects of IGF-II over the effects of IGF-I. Studies in brown adipocytes suggest that alteration of the IR-A:IGF-IR ratio in favor of IR-A may impair the ability of the cell to undergo a differentiation program (179).

Recent epidemiological studies have demonstrated that both T2DM and obesity are associated with an increased risk for many forms of cancer, including cancer of the breast, colon, liver, pancreas, kidney, and others (250, 251). These studies, together with the finding that IR-A is often aberrantly expressed in cancer cells, have strengthened the hypothesis that insulin resistance and compensatory hyperinsulinemia are a major link between diabetes and cancer (240).

Hyperinsulinemia may indirectly contribute to cancer cell proliferation by a variety of mechanisms, including reduced IGFBP-1 and -2 production (with increased availability of IGF-I and IGF-II) and reduced SHBG. The reduction in SHBG increases the sex steroid-free fraction, which, in turn, increases IGF-IR expression in cancer cells, thus providing an additional indirect mechanism for the mitogenic effect of hyperinsulinemia (252–254).

D. IR isoforms and cancer risk in type 2 diabetes patients treated with insulin or insulin analogs

It is well known that insulin treatment of diabetic patients does not closely mimic endogenous insulin secretion and leads to peripheral tissue hyperinsulinization and liver hypoinsulinization. Although liver cells predominantly express IR-B, most peripheral tissues also express IR-A at a variable degree and may be abnormally stimulated by exogenous hyperinsulinemia. An additional matter of concern is the use of insulin analogs that have been recently developed to improve glycemic control and favor compliance in diabetic patients (255). Today, three short-acting (insulin lispro, insulin aspart, and glulisine) and two long-acting insulin analogs (insulin detemir and insulin glargine) are available for the treatment of diabetic patients (255). Although most of these analogs have been used for over a decade, their long-term safety profile has not been firmly established. Even subtle modifications of the insulin structure may affect receptor specificity and association/dissociation rate, thereby resulting in abnormal signaling and an abnormal mitogenic:metabolic ratio. Both of these mechanisms received experimental support by preclinical studies carried out with the insulin analog AspB10, which was not released for use in humans (256–258). In a variety of cell lines, AspB10 has an increased mitogenic effect (257, 259), a consequence of both concomitant IR and IGF-IR activation (257) and AspB10 slow dissociation from the IR (257, 260). When injected *in vivo*, AspB10 induced mammary tumors in female rodents (261). Studies on commercially available insulin analogs are limited (258, 262, 263). As far as short-acting analogs are concerned, most data indicate that their mitogenic potency is not different from that of insulin, although some divergent data have been reported. For instance, lispro was found to have a slightly reduced affinity for IR-A (264), but an increased (+50%) affinity for IGF-IR, as compared with native insulin (258). This characteristic, however, did not result in an increased mitogenic effect (264). Insulin aspart was reported to give a slight growth

advantage over native insulin to cancer cells (265), whereas insulin glulisine was less potent than native insulin in stimulating growth in nonmalignant MCF-10 mammary cells (266). Moreover, the limited available data do not indicate an increased carcinogenic effect of insulin aspart (data available on http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/020986s047lbl.pdf or glulisine (266) in mice compared with native insulin.

More controversial data have been published on the long-acting insulin analogs. A higher mitogenic:metabolic ratio of glargine compared with native insulin has been reported (258, 267) and attributed to increased affinity for IGF-IR. Other studies have found only minor differences in the mitogenic effect between glargine and native insulin (268). The other long-acting analog, insulin detemir, which does not have an altered amino acid sequence but is acylated at B29Lys, has been reported to have a reduced mitogenic:metabolic ratio (258) (data also available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021-536_Levemir_pharmr.pdf).

While this review was in preparation, four large retrospective studies in humans have addressed the issue of cancer risk in diabetic patients being treated with the long-acting insulin analog glargine (269–272). The effect on cancer risk of insulin detemir, which was introduced only recently, was not addressed by these studies. Three of these studies indicated that treatment with insulin glargine might be associated with a higher risk of cancer than native insulin. However, because of the retrospective, observational characteristics of all three studies, the possible effect of allocation bias and other confounders cannot be ruled out, and they should be regarded as inconclusive. A further small randomized study comparing insulin glargine and neutral protamine Hagedorn (NPH) insulin found no increase in cancer risk associated with the use of glargine (273).

Only limited data are available regarding analog interaction with IR isoforms. Studies have been carried out in cells expressing IR-B (263) or IR-A (258) or variable amounts of the two isoforms (262, 264). In general, only small differences in terms of binding affinity, association, and dissociation were found among native insulin, short-acting insulin analogs, and insulin glargine. We have preliminary data, obtained by analysis of an engineered mouse cell model expressing either human IR-A or IR-B, indicating that long-acting insulin analogs preferentially activate the mitogenic ERK pathway, an effect that is more evident with the IR-A isoform (Sciacca L, Cassarino M, Genua M, Pandini G, Le Moli R, Squatrito S, Vigneri R unpublished data). The issue of the specific insulin analog effect on IR isoform activation requires further study.

VI. Possible Implications of the Aberrant IR-A Expression for Cancer Therapy

A. Role of the IGF system in mediating resistance to anticancer therapies

The crucial role of IGF-IR in promoting resistance to cancer cell killing by conventional anticancer treatments, such as radiation and chemotherapy, is well documented. IGF-IR down-regulation by antisense nucleotides or its inhibition by tyrosine kinase inhibitors increases cancer cell sensitivity to both radiation and chemotherapy in a variety of malignancies (18, 274, 275). Activation of the MEK/ERK pathway or, alternatively, of the PI3K pathway is involved in various cell models of radioresistance (276).

IGF-IR also plays a role in radiosensitivity of patients affected by ataxia telangiectasia (AT) (277), a rare disease resulting from inactivating mutations of the AT mutated (ATM) gene. ATM protein is a sensor of DNA damage and stimulates repair of double-stranded DNA breaks, such as those caused by ionizing radiations (278). Therefore, cells from AT patients are characterized by extreme radiosensitivity. Interestingly, AT cells express low levels of IGF-IR compared with control cells, and IGF-IR forced expression restores radioresistance in AT cells at near normal levels (277). The *Igf-Ir* gene, therefore, is a downstream target of the response pathway of ATM-mediated DNA damage (279). These studies indicate an important role of IGF-IR in several pathways involved in the induction of radioresistance.

So far, studies aiming to evaluate the possible role of IR-A in the regulation of radioresistance and/or resistance to chemotherapy have not been carried out. Given the similarities between IR and IGF-IR regarding both signaling pathways and biological effects, we hypothesize that aberrant IR-A expression in cancer cells may contribute to resistance to both radiation and chemotherapy, especially if the cancer cells have a high IR-A:IGF-IR ratio.

In recent years, a number of approaches have been established to block various growth factor receptors and growth-related signaling pathways in cancer cells. For some of these targeted therapies, a role of the IGF system in mediating cancer resistance has been documented.

1. Therapies targeted to mTOR

The mTOR is a serine/threonine kinase that regulates cell growth and metabolism by affecting multiple pathways involved in ribosome biogenesis, translation initiation, and nutrient uptake (280). When associated with raptor, mTOR forms a complex (TORC1) that induces phosphorylation of p70S6 kinase (p70S6K) and the translation inhibitory factor 4EBP1 (eukaryotic translation initiation factor 4E binding protein 1), thus regulating the

translation of critical mRNAs involved in cell cycle progression and cell proliferation. mTOR is regulated by insulin/IGFs and other growth factors through the PI3K–Akt pathway (281) and is abnormally activated in most cancer cells. TORC1 inhibitors, such as rapamycin and its derivative analogs, have been tested for their anticancer activity. Furthermore, select rapamycin analogs are currently under evaluation in phase I-II clinical trials. Currently available clinical trial results indicate that cancer responsiveness to mTOR inhibitors is variable and somewhat disappointing (282). At least one mechanism of cancer cell resistance to mTOR inhibitors involves Akt activation. Cell pretreatment with an IGF-IR blocking antibody (h7C10) (283) resulted in prevention of Akt activation and improved inhibition of rhabdomyosarcoma cell growth and survival. Treating acute myeloid leukemia (AML) cells with the rapamycin analog everolimus (RAD001) caused IGF-IR-dependent Akt activation in addition to autocrine production of IGF-I (284). Also, in AML cells, coinhibition of the IGF-IR/PI3K/Akt pathway enhanced the antineoplastic effects of the mTOR inhibitor. Whether aberrant IR-A expression in cancer cells may mimic the IGF-IR effect and provide resistance to mTOR inhibitors deserves further study.

2. Therapies targeted to the epidermal growth factor (EGF) receptor family

The receptors of the EGF family and their ligands are frequently overexpressed in advanced malignancies and have been implicated in poor prognosis and resistance to chemotherapy. A variety of approaches have been developed for targeting EGF receptor (EGFR) and human EGFR 2/erythroblastic leukemia viral oncogene homolog 2 (HER2/ErbB2), such as blocking antibodies and small molecules with tyrosine kinase-inhibiting activity. However, only a small proportion of cancer patients receive significant benefit from these therapies, because of either primary or acquired resistance to these agents (274, 285). Mechanisms of resistance are various and not fully elucidated. One mechanism may involve increased IGF-IR signaling, as described for breast cancer cells resistant to the anti-HER2/ErbB2 monoclonal antibody, trastuzumab (286), and for colorectal cancer cells resistant to anti-EGFR monoclonal antibody 225 (287). Moreover, in a variety of cell models, IGF-IR also mediates resistance to EGFR tyrosine kinase inhibitors, such as AG1478 (288) and gefitinib (Iressa) (289, 290). In tamoxifen-resistant breast cancer cells, IGF-IR may modulate EGFR phosphorylation via a mechanism that involves c-Src activation (291).

Recently, Jones *et al.* (292) analyzed a human cancer cell line (LoVo) that expresses IR-A and IGF-II but lacks mature IGF-IR and found that IR-A is involved in *de novo* resistance to gefitinib. Cell exposure to insulin or IGF-II caused EGFR phosphorylation at Tyr845, Tyr1068, and Tyr1173. Treatment with gefitinib had a very small effect on LoVo cell proliferation and caused phosphorylation of both IR-A and Akt. Inhibition of IR-A with a specific inhibitor (ABDP) reduced EGFR phosphorylation and markedly potentiated the antiproliferative effect of gefitinib.

These studies clearly indicate that IGF-IR overactivation is an important mediator of resistance to therapies targeted to receptors of the EGFR family and that IR-A may have a similar role, especially in cells with a high IR-A:IGF-IR ratio.

B. Role of IR and hyperinsulinemia in resistance to IGF-IR or IGF-targeted therapies

1. Therapies targeted to IGF-IR

The most promising approaches to block the effects of IGF-IR in cancer cells include the use of blocking antibodies and small molecules with tyrosine kinase-inhibiting activity. Accumulating *in vitro* and *in vivo* evidence indicate that these therapeutic strategies may indeed inhibit tumor growth and are especially effective at sensitizing cancer cells to conventional anticancer therapies, such as radiation and chemotherapy (293).

Recently, some studies raised the possibility that therapies targeted at IGF-IR may increase IR sensitivity, at least *in vitro* (Table 2). Also, selective IGF-IR down-regulation with small interfering RNAs resulted in increased

TABLE 2. Putative mechanisms involving IR, namely IR-A, which may account for cancer resistance to therapies specifically targeting IGF-IR signaling

Mechanism	Effect	Refs.
Typical cancer features		
Aberrant IR-A expression and activated IGF-II/IR-A loop	Enhanced response to IGF-II and insulin	13, 81, 148, 228
Increased HR-A	Enhanced IGF signaling	184, 231, 301
Possible consequences induced by selective IGF-IR signaling blockade		
Increased IR-mediated IRS-1/Grb-2/ERK activity and/or increased IR-mediated Akt activity	Increased IR-A responsiveness	295, 337
Increased IR-A homodimer formation	Increased IR-A responsiveness	294
Increased circulating GH	Compensatory hyperinsulinemia	302

sensitivity to insulin in cultured MDA-435 breast cancer cells (294). In this case, however, IGF-IR down-regulation caused a decrease of IR/IGF-IR hybrids and, as a consequence, reduced sequestration of IR moieties in hybrids and enhanced IR homodimer formation. Similar data were obtained in primary cultures of osteoblasts subjected to conditional disruption of IGF-IR (295). In these IGF-IR knockdown osteoblasts, increased IRS-1 phosphorylation, ERK/Akt activation, proliferation, and glucose uptake were observed in response to insulin. Insulin also induced the expression of several genes downstream the ERK pathway, such as *Glut-1* and *c-fos*, and genes related to angiogenesis, such as *Vegf* and *iNos*. Also, in this case, the hypothesized mechanism was reduced engagement of IR moieties in IR/IGF-IR hybrids and a consequent increase of IR homodimers. These *in vitro* studies suggest that anticancer therapies targeting IGF-IR may have the unwanted consequence of enhancing IR signaling, which may provide an important mechanism of resistance to IGF-IR-targeted therapies and may favor the selection of cancer cell clones with activated IR-A (Table 2).

Phase II clinical trials with anti-IGF-IR antibodies in patients with various malignancies are under way. It is too early to establish whether these treatments induce enhanced IR-A signaling and clonal selection of IR-A overexpressing cells. Preliminary results indicate that the use of anti-IGF-IR antibodies is associated with insulin resistance and hyperinsulinemia. The mechanism(s) involved may be related to increased circulating GH, a consequence of reduced IGF-I feedback at the pituitary level.

Taken together, *in vitro* and *in vivo* studies strongly suggest that a major mechanism of resistance to IGF-IR-targeted therapies in cancer may involve enhanced IR-A homodimer formation and GH-mediated development of hyperinsulinemia, both of which contribute to IR-A activation (Table 2).

Small molecules with tyrosine kinase inhibitory activity selective for IGF-IR have been studied. These therapeutics are intended to avoid inhibition of the metabolic effects of IR. One such therapeutic is picropodophyllin (PPP), a nontoxic cyclolignan found to be a potent inhibitor of IGF-IR, but ineffective on homologous IRs (296). In mice, PPP selectively inhibited and killed transformed cells overexpressing IGF-IR, as well as a variety of cancer cells (296). Mice treated with PPP did not develop hyperglycemia. Rather, serum glucose slightly decreased in PPP-treated animals. This finding is not fully understood. PPP is thought to interfere with phosphorylation at the substrate level, and its selective effect toward the IGF-IR is probably explained by differences of amino acid sequences outside of the activation loop region, which is identical for IGF-IR and IR. To our knowledge, PPP has never been tested in humans, and therefore its anticancer efficacy, metabolic effects, and adverse effects are unknown. Moreover, selective small molecule inhibitors have the limitation that they leave the IGF-II/IR-A loop intact, which is the principal mechanism of IGF system activation in certain malignancies.

2. Therapies targeted to IGFs

As already mentioned, another strategy for blocking the effects of IGF system dysregulation in cancer involves blockade of the IGF-I and IGF-II ligands using antibodies.

Limited data exist pertaining to antibodies that block both IGF-I and IGF-II. Such antibodies have been shown to reduce cancer progression and metastatic spread of prostate and liver cancer in an animal model (297, 298). Using the mouse monoclonal antibody KM1468, which blocks both IGF-I and IGF-II, a dose-dependent inhibition of human prostate cancer metastasis growth was observed in bone, and a marked reduction of liver foci development was observed in animals injected with human colorectal cancer cells. However, this antibody did not block colorectal cancer cell metastases in the lung, which contains fewer IGFs than bone and liver, suggesting that paracrine IGFs may play a relevant role in metastatic cancer spread. Because KM1468 neutralizes only free/bioactive IGFs, it has been speculated that this antibody is active only in the limited area in which free/bioactive IGFs are released by IGFBP (*e.g.*, in the tumor microenvironment). Although this point was not addressed in the animal model, it is expected that reduced free/bioactive IGF-I stimulates feedback activation of pituitary GH secretion, which, in turn, may adversely affect tumor growth and progression by direct stimulation of cancer cells by GH (299) and by causing insulin resistance with compensatory hyperinsulinemia. Stimulation of cancer-expressed IR-A by hyperinsulinemia may induce partial resistance to this treatment (Table 2).

In contrast, selective inhibition of IGF-II should not affect GH secretion, which is under negative feedback by IGF-I. Feng *et al.* (300) have developed three human monoclonal antibodies that specifically recognize IGF-II and do not cross-react with IGF-I or insulin. Treatment with the most potent of these antibodies (m610) inhibits IGF-IR phosphorylation and downstream kinases, Akt and MAPK, with an IC_{50} on the order of 1 nmol/liter and inhibits growth of prostate cancer cells and migration of breast cancer cells. The same antibody inhibits IR and IGF-IR phosphorylation induced by IGF-II with approximately the same efficiency. These IGF-II specific antibodies have not been tested in humans and, therefore, their anticancer efficacy is unknown. This strategy seems to be promising in view of the fact that the serum IGF-II concentration in humans is approximately 3-fold higher than

that of IGF-I (700 *vs.* 200 ng/ml, respectively), and given that several malignancies overexpress IGF-II and IR-A.

However, it is expected that antibody efficacy will be variably affected by the presence or absence of autocrine IGF-II and by IGF-IR and IR-A expression levels in malignant cells.

A combined approach using a blocking antibody for IGF-IR and HRs together with a blocking antibody for IGF-II may be the most effective approach. This combined therapy would have the advantage of blocking the activities of HR-A and IR-A in response to IGF-II. It is not expected to block the possible development of insulin resistance and hyperinsulinemia caused by the increased GH associated with the use of anti-IGF-IR antibodies.

C. Targeting IR-A signaling in cancer

As previously mentioned, aberrant IR-A signaling may represent an important mechanism of resistance to various anticancer therapies and should be considered an important target in cancer therapy. However, generalized IR inhibition would induce insulin resistance, compensatory hyperinsulinemia, and possibly T2DM. One strategy would be to target only IR-A, leaving the metabolic insulin effects in insulin target tissues, which predominantly express the IR-B isoform, relatively unaffected. However, selective immunological blockade of IR signaling is unlikely to be achieved because no isoform-specific antibody is currently available. Furthermore, it is expected that specific antibodies to IR-A will be difficult to obtain.

A possible targeted strategy would be to block aberrant IR-A expression in cancer by inhibiting the splicing process involved in abnormal IR exon 11 skipping. However, the factors involved in this process in cancer are probably multiple and not yet characterized.

An alternative immunological strategy may take into account the abundance of HR-A in cancer cells. Anti-IGF-IR antibodies, recognizing not only IGF-IR homodimers but also HRs, are expected to reduce HR-A signaling. In fact, in cancer cells with a low IR:IGF-IR ratio, most IR moieties are assembled in HRs. In contrast, in cancer cells with a high IR:IGF-IR ratio, the majority of IRs occur as homodimers and not as HRs. In these cells, IR-A will not be substantially blocked by anti-HR antibodies. Double blockade of IGF-IR and HRs is, in any case, advantageous over simple IGF-IR blockade because most IGF-IR occur as HRs. We have previously obtained evidence that the IR:IGF-IR ratio is an important determinant of the cancer cell response to blocking antibodies. In fact, IR blockade was able to inhibit cancer cell proliferation in response to exogenous and/or autocrine IGF-II in cancer cells with a high IR:IGF-IR ratio (81, 228). In these cells, IR and/or HR blockade is often more useful than IGF-IR blockade (301).

Another possible immunological strategy that will inhibit, at least partially, IR-A signaling is the use of IGF-II blocking antibodies. In cancer cells with a high IR-A:IGF-IR ratio, IR-A will be the most important receptor for IGF-II signaling. Importantly, IR-A and IGF-IR have similar affinity for IGF-II (see *previous paragraph* and *Section III.B*).

A different approach to IR-A blocking is based on the use of small molecules with nonselective tyrosine kinase-inhibiting activity for both IGF-IR and IR, such as BMS-554417 (302). At submicromolar concentrations, BMS-554417 inhibits both IR and IGF-IR with similar potency and with 5-fold selectivity over other kinases. However, variable results were obtained with this compound in various cancer cell lines. In mice, BMS-554417 administration induced a small but statistically significant increase in serum glucose, especially after a glucose load. Moreover, serum insulin markedly increased at 2 h after a glucose load in BMS-554417-treated animals compared with control animals (67.7 ± 10.3 *vs.* 0.152 ± 0.08 ng/ml, respectively; $P = 0.0001$) (302). It is possible that this striking serum insulin increase may overcome IR inhibition in cancer cells overexpressing IR-A, causing tumor resistance to the drug. Moreover, no data on serum GH are available after chronic use of BMS-554417, which is expected to increase serum GH by inhibiting negative feedback of IGF-I on the pituitary.

In summary, the role of IR-A as an important factor for resistance to anti-cancer therapies is just becoming recognized. So far, the attention of researchers has focused on targeting the homolog IGF-IR or IGFs. In light of the recent literature, cancers with a high IR:IGF-IR ratio are unlikely to greatly benefit from this approach. Rather, some evidence suggests that these treatments may increase IR-A signaling and possibly lead to the development of resistant clones. An additional adverse effect of these treatments is the rise of GH with consequent development of insulin resistance and compensatory hyperinsulinemia, which, in turn, can stimulate cancer cell growth. The anti-tyrosine kinase small molecule approach faces a similar problem. If the inhibitors have an elevated selectivity for the IGF-IR, they leave intact or enhance IR-A signaling. In contrast, if they have low selectivity and inhibit IR, they may cause insulin resistance with compensatory hyperinsulinemia.

In any case, future clinical trials using these targeted therapies should be designed to evaluate the effects on IR-A signaling in cancer cells and on circulating levels of GH and insulin. Finally, the efficacy of these therapies should be evaluated in relationship to the IR-A:IGF-IR ratio in cancer cells.

TABLE 3. Receptor subtypes of the IGF system in mammals

	Homotetramers			Heterotetramers (hybrids)		
Receptor	**IR-B/IR-B**	**IR-A/IR-A**	**IGF-IR/IGF-IR**	**IR-B/IR-A**	**IR-B/IGF-IR**	**IR-A/IGF-IR**
Name	IR-B	IR-A	IGF-IR	HIR-AB	HR-B	HR-A
Ligand(s)	Insulin	Insulin IGF-II	IGF-I IGF-II	Insulin IGF-II	IGF-I IGF-II	IGF-I IGF-II Insulin

Variable tetrameric assembly of IR isoforms and IGF-IR moieties confers different ligand binding preferences and different signaling capabilities.

VII. IR Isoforms and Hybrid Receptors

A. IR-A/IR-B hybrids

Both IR isoforms are coexpressed in most cells; therefore, the isoforms are expected to homodimerize (forming IR-A/IR-A and IR-B/IR-B homodimers) and heterodimerize (forming IR-A/IR-B hybrids). It would be important, therefore, to assess the functional characteristics of IR-A/IR-B hybrids (Table 3); however, these studies have been hampered by the lack of adequate methodologies.

Recently, Uhles *et al.* (142) suggested that *Ir* exon 11 may act as a sorting signal that sorts the two IR isoforms into different lipid raft microdomains of the plasma membrane. This differential localization provides a molecular basis for differential signaling by the two isoforms and should prevent heterodimerization of IR-A and IR-B moieties. It is unclear at which stage this targeting process would operate, but it should occur in the endoplasmic reticulum at a very early posttranslational stage, before dimerization of proreceptors and disulfide bond formation between α-subunits (303).

Two recent studies using bioluminescence resonance energy transfer (BRET) (304) technology have independently shown that IR-A and IR-B moieties heterodimerize and that IR-A/IR-B hybrids are randomly formed in cells (136, 224). This finding indicates that the previous negative data obtained in a pancreatic β-cell line is not of general relevance.

One of the two studies compared BRET signals from IR-B homodimers and IR-A/IR-B heterodimers and concluded that their EC_{50} values for insulin stimulation are not significantly different (136).

The other study (224) showed that IR-A/IR-B hybrids are capable of recruiting intracellular partners upon insulin and IGF-II stimulation with the same affinity as IR-A homodimers. IGF-I also activated IR-A/IR-B hybrids, but with a lower affinity. These data suggest that, in the presence of predominant IR-A expression, IR-B moieties are mostly incorporated into IR-A/IR-B hybrids with the consequence that most insulin binding sites will behave as IGF-II binding sites.

B. IR/IGF-IR hybrids: functional characteristics and the role of IR isoforms

As mentioned above, in cells expressing both IR and IGF-IR, IR hemireceptors may heterodimerize with IGF-IR hemireceptors, leading to the formation of HRs (11, 305–308) (Fig. 5). The existence of HRs was first hypothesized by Kasuga *et al.* in 1983 (309). Six years later, Soos and Siddle (306) identified HRs from the human placenta, thus confirming their existence. In accordance with their high affinity for IGF-I, HRs have been measured as the proportion of total ^{125}I-labeled IGF-I that may be immunoprecipitated with an anti-IR antibody. Heterodimerization of the two receptors is due to the high degree of homology between IR and IGF-IR, which ranges from 27 to 84% depending on the region that is compared (310, 311). Heterodimerization is believed to occur with a similar efficiency as homodimerization. Therefore, the proportion of hybrids is a function of the mole fractions of each receptor (49) and the expected proportion of HRs can be calculated as follows: HRs = $2\sqrt{\text{IR}}\sqrt{\text{IGF-IR}}$ (49, 229). According to this model, in a study carried out in differentiated 3T3-L1 adipocytes, a marked IR increase caused most IGF-IR hemidimers to form HR rather than IGF-IR homodimers. In these cells, IGF-I fully stimulated glucose uptake through IR β-subunit activation (312). However, in some cancer tissues, an HR content higher than predicted has been observed (229), suggesting that unknown

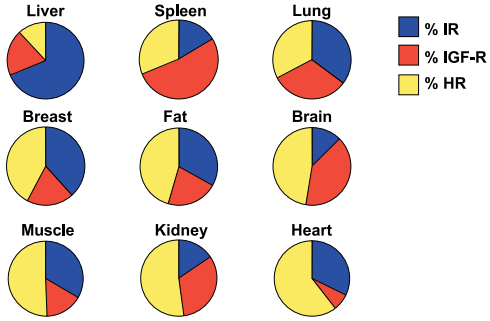

FIG. 5. Expression of IR (*blue*), IGF-IR (*red*), and HRs (*yellow*) in rabbit tissues. Hybrid receptor content was determined by polyethylene glycol assay, as the aliquot of ^{125}I-IGF-I radioactivity that was immunoprecipitated by an anti-IR antibody (49). In most tissues, HRs represent approximately one half of the total amount of IGF system receptors present in the cell.

factors may modulate the assembly of either homodimers or heterodimers.

The physiological role of HRs is still unclear. Studies in cells are complicated by the concomitant variable expression of IRs and IGF-IR with HRs and by the unavailability of specific antibodies recognizing only HRs (11, 305, 308, 313, 314). Moreover, affinity chromatography-purified HRs may have altered functional characteristics and are often contaminated with variable amounts of both homodimeric IR and IGF-IR. Early studies carried out with purified HRs indicate that these receptors mostly bind IGF-I and that they bind insulin with a much lower affinity (305) (Table 3).

When hybrids composed of intact IGF-IR or IR hemidimers plus kinase-inactive IR hemidimers were prepared, both insulin and IGF-I stimulated phosphate incorporation into both β-subunits to a similar extent (315), providing evidence that HR autophosphorylation occurs by intramolecular transreaction independent of which hemireceptor α-subunit is ligand-occupied. Others have confirmed these findings, although IGF-I was found to be more efficient than insulin (307, 316–318).

At variance with homodimeric receptors, HRs have peculiar binding properties. When labeled IGF-I was used as a tracer, binding was displaced by low IGF-I concentrations, but only by high insulin concentrations. In contrast, labeled insulin bound to HRs was displaced by low concentrations of both insulin and IGF-I, with IGF-I being more effective. These data are consistent with a model in which IGF-I allosterically inhibits insulin binding to HR via interaction with the IGF-I hemidimer α-subunit. Similar data were obtained by Soos *et al.* (305), who found high affinity binding to HRs by both insulin and IGF-I, but a limited ability of insulin to displace labeled IGF-I. Labeled insulin, in contrast, was displaced by both insulin and IGF-I (305).

Further studies were designed to evaluate HR characteristics according to whether they incorporate IR-A or IR-B hemidimers. First, experiments carried out in transfected cells indicated that both IR-A and IR-B are able to form hybrids with IGF-IR with the same efficiency (136, 184, 319) and in close agreement with the random assembly model (49, 229). Therefore, the proportion of HR-A (HR containing IR-A hemidimers) or HR-B (HR containing IR-B hemidimers) depends on the relative abundance of the two IR isoforms (Table 3).

To address the functional characteristics of HR-A and HR-B, we studied transfected cells expressing similar amounts of either HR-A or HR-B (184) and evaluated ligand-stimulated receptor autophosphorylation. Experiments were carried out in the presence of a molar excess of an IR-blocking antibody to avoid interference by the homodimeric IR. We found that IGF-I, IGF-II, and insulin were able to stimulate HR-A phosphorylation, although insulin was less effective. In regard to HR-B phosphorylation, IGF-II and insulin were much less effective than IGF-I. We also found that all three ligands, including insulin, were able to induce *in vitro* phosphorylation of specific IGF-IR substrates, such as Crk-II, when binding to HR-A (184). In contrast, in cells expressing HR-B, only IGF-I and IGF-II, but not insulin, were able to phosphorylate Crk-II. Indeed, available studies indicate that Crk-II, a SH2-SH3 adapter protein, is phosphorylated *in vitro* only by IGF-IR tyrosine kinase and not by IR (320). When the binding abilities of HR-A and HR-B were measured in competition-inhibition experiments using HRs that had been immunocaptured with 83-7, an anti-IR antibody recognizing HRs, both IGF-I and IGF-II displaced I^{125}-IGF-I binding. For HR-A, the ED_{50} values were 0.5 and 0.7 nM for IGF-I and IGF-II, respectively, whereas for HR-B, they were somewhat lower, especially for IGF-II (ED_{50} = 2.5 and 15.0 nM for IGF-I and IGF-II, respectively). Insulin was able to displace I^{125}-IGF-I from HR-A (ED_{50} = 3.7 nM), but not from HR-B (ED_{50} >100 nM insulin) (184). These studies suggest that HR-A could be considered a low specificity receptor, which may be activated by high-affinity ligands, IGF-I and IGF-II and, under certain conditions (*e.g.*, hyperinsulinemia), by the low-affinity ligand, insulin (184).

Two recent studies addressed HR-A and HR-B ligand specificity (136, 319) and found that both HR subtypes have a very low affinity for insulin. Relevant differences in the calculation of ED_{50} values and in the displacement efficiency of either IGF-I or IGF-II toward the ^{125}I-IGF-I tracer in competition-inhibition experiments in the two studies were reported. The reasons for these discrepancies are unclear and may reflect differences both in the assay procedure and in the cell models that were used (136, 184, 319). The most important difference may be our use of transfected R^- cells, which are devoid of endogenous IGF-IR, whereas the other two studies used transfected CHO (hamster) cells, in which endogenous hamster IGF-IR homodimers may be captured by the anti-IR antibody that is used to immunopurify HRs (321). Moreover, subtle changes in posttranslational processing may occur in different cell types after transfection with the human IR and IGF-IR cDNAs. In this regard, an interesting model is presented by the developing chick neuroretina (178, 322), where both IR and IGF-IR coexist, but IGF-IR is the predominant receptor. Therefore, in this model, most IRs occur as HRs. Because the chick only expresses the IR-A isoform, only HR-A is present. In this model, whereas IGF-I bound mostly to IGF-IR homodimers, insulin bound

preferentially to HR, which had similar binding affinity for insulin and IGF-I (178).

In summary, available data indicate that both HR-A and HR-B bind IGF-I with high affinity. Some, but not all reports indicate that, by binding to HRs, both IGF-I and insulin may activate autophosphorylation of IR and IGF-IR β-subunits, suggesting that the signaling pathway activated by HR is unique. Whether the IGF-II binding affinity for HRs is similar or lower than that of IGF-I is unclear. IGF-I is able to displace insulin, whereas the opposite does not hold true for HR-B, and it is a matter of discussion for HR-A. As a consequence, whether HR-A functions as a receptor with broader binding specificity than HR-B awaits further study.

C. IR/IGF-IR hybrids and cancer

Most human cancers overexpress both IGF-IR and IR (82, 229, 323). Therefore, it is reasonable to expect that they also overexpress HRs. In the majority of breast cancer specimens (>75%), HRs exceeded IGF-IR in terms of content (229). Upon IGF-I stimulation of breast cancer cells overexpressing HRs, HR autophosphorylation exceeded IGF-IR autophosphorylation, suggesting that most of the IGF-I effect was mediated by HRs. In breast cancer, the measured content of HRs was directly related to the molar ratio of both IRs and IGF-IRs, suggesting that HR formation occurred by random assembly of IR and IGF-IR moieties.

Cell growth studies with specific monoclonal antibodies blocking either HRs or IGF-IR homodimers indicated that, in a given cell type, the IGF-I mitogenic effect occurred predominantly via the receptor type that was more abundant. Thus, an antibody to IGF-IR was a stronger growth inhibitor in breast cancer cells with a low HR:IGF-IR ratio (229) (Fig. 6A). In contrast, an HR blocking antibody markedly inhibited growth in thyroid and breast cancer cells with high HR:IGF-IR ratios (Fig. 6, B and C). When MDA-MB-231 breast cancer cells, which are characterized by a high HR:IGF-IR, were xenografted in mice, a more potent antitumoral response was obtained with h7C10, a monoclonal antibody with neutralizing activity against both IGF-IR and HRs, as compared with antibodies targeting solely IGF-IR or HRs (301) (Fig. 6D).

Because both IR-A and IR-B can form hybrids with IGF-IR with the same efficiency (184), cancer cells overexpressing IR-A are expected to overexpress HR-A.

In thyroid carcinomas, IR-A and, consequently, HR-A are expressed in poorly differentiated histotypes (81, 231). As mentioned above, in these cancer cells, an antibody with blocking activity against both HRs and IGF-IR is a more potent antiproliferative agent than a selective anti-IGF-IR antibody (231) (Fig. 6B). In thyroid cancer specimens, the measured HR content often exceeded HR content calculated on the basis of the random assembly model, suggesting the presence of mechanisms favoring HR over homodimer assembly (231).

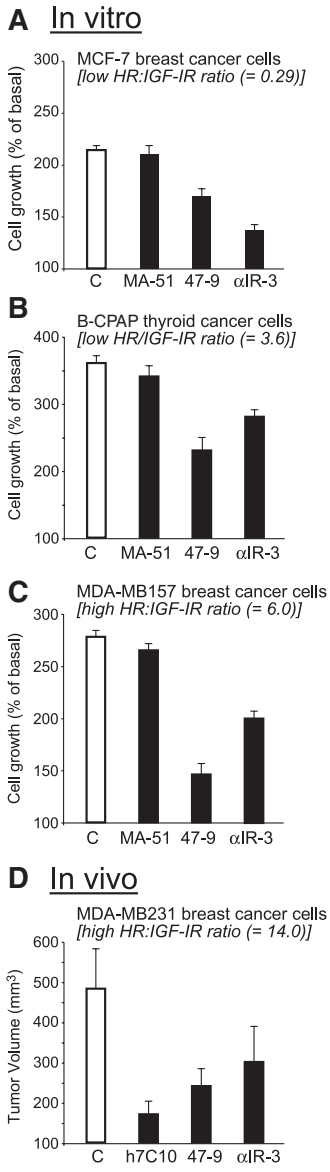

FIG. 6. Effect of IGF-IR or HR targeting in cancer cells with different IGF-IR:HRs ratios. Human breast cancer cells, MCF-7 (A) or MDA-MB157 (C), and papillary thyroid cancer cells, B-CPAP (B), were incubated with IGF-I (10 nM) in the presence of an unrelated antibody (control) or in the presence of antibodies inhibiting IGF-IR (αIR-3), IR (Ab MA-51), or both IR and HRs (Ab 47-9). Cell growth was most greatly inhibited by blocking IGF-IR in MCF-7 cells (high IGF-IR:HRs ratio), whereas it was most greatly inhibited by blocking HRs in MDA-MB157 and B-CPAP cells (both with low IGF-IR:HRs ratios) (229, 231). D, Antitumor activity of antibodies that inhibit IGF-IR with poor (Ab αIR3) or high affinity (Ab h7C10) for HRs as compared with an antibody targeting both IR and HRs (Ab 47-9) using an established xenograft tumor model. Athymic nude mice engrafted with MDA-MB-231 cells were treated for 26 d with a loading dose of 1 mg of antibody. The control group received PBS. Ab h7C10 significantly inhibited tumor growth, whereas Ab αIR3 showed significant minor activity. Ab 47-9 had intermediate activity on tumor growth (301).

In prostate cancer cells, HR formation is favored by nongenomic activity of both androgen and estrogen re-

ceptors, both of which selectively up-regulate IGF-IR but not IR (252, 253). Although IGF-IRs were similarly expressed in benign prostate hyperplasia and cancer tissues, IRs and HRs were more intensely expressed in prostate cancer tissues. In addition, IR and HR expression increased with Gleason score (324), suggesting that the HR:IGF-IR ratio is increased in advanced prostate carcinomas.

High IR-A and HR-A expression levels were also found in human myosarcomas (148) and osteosarcomas (325), which are malignancies that produce autocrine IGF-II. In patients with osteosarcoma, tumor-derived circulating IGF-II was significantly associated with decreased disease-free survival. Simultaneous inhibition of IR-A, HRs, and IGF-IR proved to be a more efficient strategy than selective IGF-IR inhibition (325).

Recently IR-A, IR-B, and HRs were found to coexist with IGF-IR in human papillomavirus-positive cervical cancer cells (326). Although these receptors were found to mediate an IGF-I antiapoptotic effect, full understanding of the biological significance of HRs in these cells requires further study.

In conclusion, a well-defined finding of these studies is that HRs, especially HR-A, should be taken into account for analyses of therapies designed to target the effects of IGFs in cancer.

D. IR isoforms in the regulation of the IGF system

Available data on the functional characteristics of IR isoforms and *Ir* splicing regulation in physiology and disease suggest the hypothesis that a major physiological role of *Ir* splicing is to provide an additional step for IGF system regulation. The insulin/IGF-I signaling pathway is a conserved pathway from yeast to mammals. However, noticeable differences exist between the invertebrate and vertebrate due to an increased number of ligands and receptors (192). Coevolutionary analysis of receptor and ligand transcripts across different species indicates that the IR has a higher coevolutionary relationship with insulin than with IGF-I or IGF-II. Likewise, IGF-IR has a higher coevolutionary relationship with IGF-I than with insulin (327). Interestingly, both IR and IGF-IR share a similar and relatively lower evolutionary relationship with IGF-II (327).

These studies suggest that evolution has separated signaling pathways devoted either to metabolism regulation (insulin/IR) or growth regulation (IGF-I/IGF-IR) but has conserved some cross-talk between the two pathways (IGF-II/IR-A, IGF-II/IGF-IR). By increasing IR-A abundance, the growth pathway is enhanced by various mechanisms. The first mechanism is represented by IGF-II binding to IR-A. The second mechanism is activated by IGF-II binding to IR-A/IR-B hybrids, which may have similar binding characteristics as IR-A homodimers (328). In this case, IGF-II would induce simultaneous phosphorylation of IR-A and IR-B β-subunits. The third mechanism, by which IR-A may enhance the growth pathway, is through formation of HR-A, which may have increased ligand affinity compared with HR-B. Although the increased affinity of HR-A for insulin has been questioned (136), this putative characteristic of IR-A may have considerable relevance because it provides an explanation for activation of the IGF-IR pathway by insulin (184) in hyperinsulinemic patients.

Taken together, these data indicate that *Ir* exon 11 skipping and increased IR-A relative abundance may provide a method by which to enhance the biological effects of IGF-II and favor cross-talk between the IR and IGF-IR signaling pathways, mechanisms that appear to play a role in embryogenesis and fetal development. Conversely, *Ir* exon 11 inclusion and consequent increased relative abundance of IR-B leads to reduced exposure to IGF-II and favors the specific metabolic effect of insulin in differentiated target tissues.

VIII. Summary, Conclusion, and Perspectives

A. The physiological role of IR isoforms

The physiological role of the two IR isoforms is not completely established. Their physiological roles are likely based on their different binding affinities for IGF-II, rather than on their slightly different binding affinities for insulin. Low-specificity receptors, such as IR-A and perhaps HR-A, that are able to bind insulin and IGFs are important for mediating growth and cell metabolism during embryogenesis and fetal life, resembling the promiscuous ancestral receptor of invertebrates. It is reasonable to assume that IR-B, which is predominantly expressed at high levels in differentiated insulin target tissues and selectively binds insulin, mediates metabolic effects in a tissue-specific manner, in relation to the enzymatic machinery for nutrient utilization. On the other hand, it is relatively unclear why IR-A is expressed at variable levels in adult differentiated tissues. It may maintain a "trophic" effect by mediating the effects of both circulating and locally produced IGF-II.

In any case, the fact that mammals have acquired a new *Ir* exon and the ability to skip it by alternative splicing in a developmental and tissue-specific manner suggests that alternative splicing is employed to regulate the IGF system by fine-tuning of ligand specificity.

B. Implications for type 2 diabetes and associated disorders

Studies that have aimed to evaluate whether IR isoform regulation could be associated with insulin resistance and T2DM have yielded inconsistent results, possibly because

T2DM is a heterogeneous and complex disease with variable levels of insulin resistance. Patient age and body mass index, as well as drug therapies, might also influence IR isoform expression. Given this level of complexity, we believe that available data are insufficient to draw a firm conclusion on the role, if any, of IR isoforms in diabetes and insulin resistance. Studies in pancreatic β-cells showing that IR-A promotes insulin gene transcription, whereas IR-B promotes βGK gene transcription, would support a possible role of IR isoforms in type II diabetes development.

Whether IR-A and HR-A may be aberrantly expressed in micro- and macrovessels of diabetic patients, contributing to micro- and macrovascular complications, remains an experimental hypothesis that may be worth testing in future studies.

C. Implications for oncology

Strong evidence suggests that most common malignancies are associated with subtle derangements of the IGF system that are caused by drastic changes in nutrition habits and lifestyle. Systemic disorders, such as obesity, diabetes, and metabolic syndrome, are associated with an increased risk for cancer (240, 250, 251, 329), whereas weight control is associated with a decreased cancer risk (330, 331). Hyperinsulinemia, which is a common characteristic of all of these conditions, seems to be the predominant pathogenetic factor associated with cancer (59, 332–334). The recognition that IR-A and HR-A are aberrantly expressed in cancer cells has provided a framework for better understanding of the cancer-promoting effects of hyperinsulinemia. Moreover, both insulin and IGFs synergize with sex steroids in the promotion of sex steroid-sensitive tumors (254, 335, 336).

Taken together, these studies underscore the utility of preventing the development of obesity and T2DM and consequent hyperinsulinemia as an effective cancer prevention strategy.

As far as cancer therapy is concerned, available evidence indicates that normalization of insulin levels through weight control or insulin sensitizers may improve a cancer prognosis.

It is known that IRs are overexpressed in many cancers and that IR-A is often the prevalent IR isoform in tumors. Aberrant IR-A expression is more pronounced in dedifferentiated cancer and, therefore, IR-A and HR-A overexpression in cancer may be an important factor for tumor resistance to anticancer therapies, including therapies targeting the IGF-IR.

D. Perspectives

Many questions pertaining to IR isoforms remain unanswered, especially with regard to their physiological role and the role of IR-A expression in adult differentiated cells. Whether IR isoforms are located in different membrane subdomains and whether they elicit different signaling pathways in response to insulin remains to be better established. In addition, the possible role of IR isoforms in the regulation of tissue-specific insulin sensitivity remains to be clarified. Clearly, more work is needed to understand the binding affinity of IR isoforms and HRs for synthetic insulin analogs (especially long-acting analogs) used in the treatment of diabetic patients. It is now clear that modifications of the insulin structure may differentially affect both the binding and intracellular signaling of insulin analogs.

There is also an urgent need to fully understand the mechanisms that lead to aberrant IR-A overexpression in cancer cells and the possible ways to block it to overcome IR-A-dependent tumor progression and resistance to anticancer drugs.

Acknowledgments

The authors thank all their co-workers and collaborators who contributed to the work cited in this article.

Address all correspondence and requests for reprints to: Antonino Belfiore, M.D., Endocrinology, Department of Clinical and Experimental Medicine, Campus Universitario, viale Europa, località Germaneto, University of Catanzaro, 88100 Catanzaro, Italy. E-mail: belfiore@unicz.it.

This work was supported by grants from the Associazione Italiana per la Ricerca sul Cancro (to A.B. and R.V.) and PRIN-MIUR 2005 (Ministero Italiano Università e Ricerca; to A.B.).

Disclosure Summary: R.V. has received research grants from Sanofi-Aventis. A.B. has served in advisory councils for AstraZeneca and Pfizer. F.F., L.S., and G.P. have nothing to declare.

References

1. **Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou JH, Masiarz F, Kan YW, Goldfine ID, Roth RA, Rutter WJ** 1985 The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. Cell 40:747–758
2. **Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J** 1985 Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313:756–761
3. **Mosthaf L, Vogt B, Häring HU, Ullrich A** 1991 Altered expression of insulin receptor types A and B in the skeletal muscle of non-insulin-dependent diabetes mellitus patients. Proc Natl Acad Sci USA 88:4728–4730

4. **Sesti G, Marini MA, Tullio AN, Montemurro A, Borboni P, Fusco A, Accili D, Lauro R** 1991 Altered expression of the two naturally occurring human insulin receptor variants in isolated adipocytes of non-insulin-dependent diabetes mellitus patients. Biochem Biophys Res Commun 181:1419–1424
5. **Kellerer M, Sesti G, Seffer E, Obermaier-Kusser B, Pongratz DE, Mosthaf L, Häring HU** 1993 Altered pattern of insulin receptor isotypes in skeletal muscle membranes of type 2 (non-insulin-dependent) diabetic subjects. Diabetologia 36:628–632
6. **Benecke H, Flier JS, Moller DE** 1992 Alternatively spliced variants of the insulin receptor protein. Expression in normal and diabetic human tissues. J Clin Invest 89:2066–2070
7. **Anderson CM, Henry RR, Knudson PE, Olefsky JM, Webster NJ** 1993 Relative expression of insulin receptor isoforms does not differ in lean, obese, and noninsulin-dependent diabetes mellitus subjects. J Clin Endocrinol Metab 76:1380–1382
8. **Hansen T, Bjørbaek C, Vestergaard H, Grønskov K, Bak JF, Pedersen O** 1993 Expression of insulin receptor spliced variants and their functional correlates in muscle from patients with non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 77:1500–1505
9. **Norgren S, Zierath J, Galuska D, Wallberg-Henriksson H, Luthman H** 1993 Differences in the ratio of RNA encoding two isoforms of the insulin receptor between control and NIDDM patients. The RNA variant without exon 11 predominates in both groups. Diabetes 42:675–681
10. **Mosthaf L, Grako K, Dull TJ, Coussens L, Ullrich A, McClain DA** 1990 Functionally distinct insulin receptors generated by tissue-specific alternative splicing. EMBO J 9:2409–2413
11. **Yamaguchi Y, Flier JS, Benecke H, Ransil BJ, Moller DE** 1993 Ligand-binding properties of the two isoforms of the human insulin receptor. Endocrinology 132:1132–1138
12. **Yamaguchi Y, Flier JS, Yokota A, Benecke H, Backer JM, Moller DE** 1991 Functional properties of two naturally occurring isoforms of the human insulin receptor in Chinese hamster ovary cells. Endocrinology 129:2058–2066
13. **Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, Vigneri R** 1999 Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 19:3278–3288
14. **Stewart CE, Rotwein P** 1996 Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. Physiol Rev 76:1005–1026
15. **Bartke A, Chandrashekar V, Dominici F, Turyn D, Kinney B, Steger R, Kopchick JJ** 2003 Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. Biogerontology 4:1–8
16. **Frasca F, Pandini G, Sciacca L, Pezzino V, Squatrito S, Belfiore A, Vigneri R** 2008 The role of insulin receptors and IGF-I receptors in cancer and other diseases. Arch Physiol Biochem 114:23–37
17. **Baserga R, Peruzzi F, Reiss K** 2003 The IGF-1 receptor in cancer biology. Int J Cancer 107:873–877
18. **Samani AA, Yakar S, LeRoith D, Brodt P** 2007 The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev 28:20–47
19. **Belfiore A** 2007 The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer. Curr Pharm Des 13:671–686
20. **Ebina Y, Edery M, Ellis L, Standring D, Beaudoin J, Roth RA, Rutter WJ** 1985 Expression of a functional human insulin receptor from a cloned cDNA in Chinese hamster ovary cells. Proc Natl Acad Sci USA 82:8014–8018
21. **Seino S, Seino M, Nishi S, Bell GI** 1989 Structure of the human insulin receptor gene and characterization of its promoter. Proc Natl Acad Sci USA 86:114–118
22. **Whittaker J, Groth AV, Mynarcik DC, Pluzek L, Gadsbøll VL, Whittaker LJ** 2001 Alanine scanning mutagenesis of a type 1 insulin-like growth factor receptor ligand binding site. J Biol Chem 276:43980–43986
23. **Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J, Fujita Yamaguchi Y** 1986 Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 5:2503–2512
24. **Drakas R, Tu X, Baserga R** 2004 Control of cell size through phosphorylation of upstream binding factor 1 by nuclear phosphatidylinositol 3-kinase. Proc Natl Acad Sci USA 101:9272–9276
25. **Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E** 2001 An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. Curr Biol 11:213–221
26. **Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G** 1997 daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277:942–946
27. **Ruvkun G, Hobert O** 1998 The taxonomy of developmental control in *Caenorhabditis elegans*. Science 282:2033–2041
28. **De Pirro R, Forte F, Bertoli A, Greco AV, Lauro R** 1981 Changes in insulin receptors during oral contraception. J Clin Endocrinol Metab 52:29–33
29. **Shier P, Watt VM** 1989 Primary structure of a putative receptor for a ligand of the insulin family. J Biol Chem 264:14605–14608
30. **Nef S, Verma-Kurvari S, Merenmies J, Vassalli JD, Efstratiadis A, Accili D, Parada LF** 2003 Testis determination requires insulin receptor family function in mice. Nature 426:291–295
31. **Hernández-Sánchez C, Mansilla A, de Pablo F, Zardoya R** 2008 Evolution of the insulin receptor family and receptor isoform expression in vertebrates. Mol Biol Evol 25:1043–1053
32. **Hubbard SR, Till JH** 2000 Protein tyrosine kinase structure and function. Annu Rev Biochem 69:373–398
33. **Brandt J, Andersen AS, Kristensen C** 2001 Dimeric fragment of the insulin receptor α-subunit binds insulin with full holoreceptor affinity. J Biol Chem 276:12378–12384
34. **Araki E, Murakami T, Shirotani T, Kanai F, Shinohara Y, Shimada F, Mori M, Shichiri M, Ebina Y** 1991 A cluster of four Sp1 binding sites required for efficient expression of the human insulin receptor gene. J Biol Chem 266:3944–3948
35. **Standaert ML, Schimmel SD, Pollet RJ** 1984 The development of insulin receptors and responses in the differentiating nonfusing muscle cell line BC3H-1. J Biol Chem 259:2337–2345

36. **Brunetti A, Foti D, Goldfine ID** 1993 Identification of unique nuclear regulatory proteins for the insulin receptor gene, which appear during myocyte and adipocyte differentiation. J Clin Invest 92:1288–1295
37. **Cameron KE, Resnik J, Webster NJ** 1992 Transcriptional regulation of the human insulin receptor promoter. J Biol Chem 267:17375–17383
38. **Yoshizato K, Shirotani T, Furukawa N, Taguchi T, Motoshima H, Toyonaga T, Hirashima Y, Kawashima J, Ebina Y, Shichiri M, Araki E** 2001 Identification of a cis-acting element and a novel trans-acting factor of the human insulin receptor gene in HepG2 and rat liver cells. Biochem Biophys Res Commun 280:428–434
39. **Lee JK, Tam JW, Tsai MJ, Tsai SY** 1992 Identification of cis- and trans-acting factors regulating the expression of the human insulin receptor gene. J Biol Chem 267:4638–4645
40. **Cleynen I, Van de Ven WJ** 2008 The HMGA proteins: a myriad of functions. Int J Oncol 32:289–305
41. **Brunetti A, Manfioletti G, Chiefari E, Goldfine ID, Foti D** 2001 Transcriptional regulation of human insulin receptor gene by the high-mobility group protein HMGI(Y). FASEB J 15:492–500
42. **Webster NJ, Resnik JL, Reichart DB, Strauss B, Haas M, Seely BL** 1996 Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor overexpression in breast cancer. Cancer Res 56:2781–2788
43. **García-Arencibia M, Molero S, Dávila N, Carranza MC, Calle C** 2005 17β-Estradiol transcriptionally represses human insulin receptor gene expression causing cellular insulin resistance. Leuk Res 29:79–87
44. **Kaplan SA** 1984 The insulin receptor. J Pediatr 104:327–336
45. **Beck-Nielsen H, Kühl C, Pedersen O, Bjerre-Christensen C, Nielsen TT, Klebe JG** 1979 Decreased insulin binding to monocytes from normal pregnant women. J Clin Endocrinol Metab 49:810–814
46. **Mamula PW, McDonald AR, Brunetti A, Okabayashi Y, Wong KY, Maddux BA, Logsdon C, Goldfine ID** 1990 Regulating insulin-receptor-gene expression by differentiation and hormones. Diabetes Care 13:288–301
47. **Pedersen O, Beck-Nielsen H, Heding L** 1980 Increased IRs after exercise in patients with insulin-dependent diabetes mellitus. N Engl J Med 302:886–892
48. **Wigand JP, Anderson Jr JH, Jennings SS, Blackard WG** 1979 Effect of dietary composition on insulin receptors in normal subjects. Am J Clin Nutr 32:6–9
49. **Bailyes EM, Navé BT, Soos MA, Orr SR, Hayward AC, Siddle K** 1997 Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. Biochem J 327:209–215
50. **Havrankova J, Roth J, Brownstein M** 1978 Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272:827–829
51. **Havrankova J, Roth J, Brownstein MJ** 1979 Concentrations of insulin and insulin receptors in the brain are independent of peripheral insulin levels. Studies of obese and streptozotocin-treated rodents. J Clin Invest 64:636–642
52. **Park CR** 2001 Cognitive effects of insulin in the central nervous system. Neurosci Biobehav Rev 25:311–323
53. **Banks WA** 2004 The source of cerebral insulin. Eur J Pharmacol 490:5–12
54. **Cole GM, Frautschy SA** 2007 The role of insulin and neurotrophic factor signaling in brain aging and Alzheimer's disease. Exp Gerontol 42:10–21
55. **van der Heide LP, Ramakers GM, Smidt MP** 2006 Insulin signaling in the central nervous system: learning to survive. Prog Neurobiol 79:205–221
56. **Plum L, Schubert M, Brüning JC** 2005 The role of insulin receptor signaling in the brain. Trends Endocrinol Metab 16:59–65
57. **Watson GS, Craft S** 2006 Insulin resistance, inflammation, and cognition in Alzheimer's disease: lessons for multiple sclerosis. J Neurol Sci 245:21–33
58. **Plum L, Belgardt BF, Brüning JC** 2006 Central insulin action in energy and glucose homeostasis. J Clin Invest 116:1761–1766
59. **Bruning PF, Bonfrèr JM, van Noord PA, Hart AA, de Jong-Bakker M, Nooijen WJ** 1992 Insulin resistance and breast-cancer risk. Int J Cancer 52:511–516
60. **Stockhorst U, de Fries D, Steingrueber HJ, Scherbaum WA** 2004 Insulin and the CNS: effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans. Physiol Behav 83:47–54
61. **Brands AM, Biessels GJ, de Haan EH, Kappelle LJ, Kessels RP** 2005 The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 28:726–735
62. **Messier C, Teutenberg K** 2005 The role of insulin, insulin growth factor, and insulin-degrading enzyme in brain aging and Alzheimer's disease. Neural Plast 12:311–328
63. **Biessels GJ, Gispen WH** 2005 The impact of diabetes on cognition: what can be learned from rodent models? Neurobiol Aging 26(Suppl 1):36–41
64. **Van Schravendijk CF, Foriers A, Hooghe-Peters EL, Rogiers V, De Meyts P, Sodoyez JC, Pipeleers DG** 1985 Pancreatic hormone receptors on islet cells. Endocrinology 117:841–848
65. **Van Schravendijk CF, Foriers A, Van den Brande JL, Pipeleers DG** 1987 Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. Endocrinology 121:1784–1788
66. **Marincola F, Frank W, Clark W, Douglas M, Merrell R** 1983 The independence of insulin release and ambient insulin in vitro. Diabetes 32:1162–1167
67. **Stagner J, Samols E, Polonsky K, Pugh W** 1986 Lack of direct inhibition of insulin secretion by exogenous insulin in the canine pancreas. J Clin Invest 78:1193–1198
68. **Leibiger IB, Leibiger B, Moede T, Berggren PO** 1998 Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. Mol Cell 1:933–938
69. **Xu GG, Gao ZY, Borge Jr PD, Jegier PA, Young RA, Wolf BA** 2000 Insulin regulation of β-cell function involves a feedback loop on SERCA gene expression, Ca(2+) homeostasis, and insulin expression and secretion. Biochemistry 39:14912–14919
70. **Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR** 1999 Tissue-specific knockout of the insulin receptor in pancreatic β-cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96:329–339
71. **Kulkarni RN** 2002 Receptors for insulin and insulin-like growth factor-1 and insulin receptor substrate-1 mediate

pathways that regulate islet function. Biochem Soc Trans 30:317–322
72. **Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, Fisher PE, Morroni M, Cinti S, White MF, Herrera PL, Accili D, Efstratiadis A** 2002 Defective insulin secretion in pancreatic β cells lacking type 1 IGF receptor. J Clin Invest 110: 1011–1019
73. **Leahy JL, Vandekerkhove KM** 1990 Insulin-like growth factor-I at physiological concentrations is a potent inhibitor of insulin secretion. Endocrinology 126:1593–1598
74. **Pørksen N, Hussain MA, Bianda TL, Nyholm B, Christiansen JS, Butler PC, Veldhuis JD, Froesch ER, Schmitz O** 1997 IGF-I inhibits burst mass of pulsatile insulin secretion at supraphysiological and low IGF-I infusion rates. Am J Physiol 272:E352–E358
75. **Czech MP** 1982 Structural and functional homologies in the receptors for insulin and the insulin-like growth factors. Cell 31:8–10
76. **De Meyts P** 1994 The structural basis of insulin and insulin-like growth factor-I receptor binding and negative co-operativity, and its relevance to mitogenic versus metabolic signalling. Diabetologia 37(Suppl 2):S135–S148
77. **Okada T, Liew CW, Hu J, Hinault C, Michael MD, Krtzfeldt J, Yin C, Holzenberger M, Stoffel M, Kulkarni RN** 2007 Insulin receptors in β-cells are critical for islet compensatory growth response to insulin resistance. Proc Natl Acad Sci USA 104:8977–8982
78. **Smith FE, Rosen KM, Villa-Komaroff L, Weir GC, Bonner-Weir S** 1991 Enhanced insulin-like growth factor I gene expression in regenerating rat pancreas. Proc Natl Acad Sci USA 88:6152–6156
79. **Muller D, Huang GC, Amiel S, Jones PM, Persaud SJ** 2007 Gene expression heterogeneity in human islet endocrine cells in vitro: the insulin signalling cascade. Diabetologia 50:1239–1242
80. **Taylor SI, Roth J, Blizzard RM, Elders MJ** 1981 Qualitative abnormalities in insulin binding in a patient with extreme insulin resistance: decreased sensitivity to alterations in temperature and pH. Proc Natl Acad Sci USA 78:7157–7161
81. **Vella V, Pandini G, Sciacca L, Mineo R, Vigneri R, Pezzino V, Belfiore A** 2002 A novel autocrine loop involving IGF-II and the insulin receptor isoform-A stimulates growth of thyroid cancer. J Clin Endocrinol Metab 87:245–254
82. **Vella V, Sciacca L, Pandini G, Mineo R, Squatrito S, Vigneri R, Belfiore A** 2001 The IGF system in thyroid cancer: new concepts. Mol Pathol 54:121–124
83. **Lawrence MC, McKern NM, Ward CW** 2007 Insulin receptor structure and its implications for the IGF-1 receptor. Curr Opin Struct Biol 17:699–705
84. **De Meyts P** 2008 The insulin receptor: a prototype for dimeric, allosteric membrane receptors? Trends Biochem Sci 33:376–384
85. **Cohen P** 2006 The twentieth century struggle to decipher insulin signalling. Nat Rev Mol Cell Biol 7:867–873
86. **Taniguchi CM, Emanuelli B, Kahn CR** 2006 Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 7:85–96
87. **Sparrow LG, Gorman JJ, Strike PM, Robinson CP, McKern NM, Epa VC, Ward CW** 2007 The location and characterisation of the O-linked glycans of the human insulin receptor. Proteins 66:261–265
88. **Cheng I, Stram DO, Penney KL, Pike M, Le Marchand L, Kolonel LN, Hirschhorn J, Altshuler D, Henderson BE, Freedman ML** 2006 Common genetic variation in IGF1 and prostate cancer risk in the multiethnic cohort. J Natl Cancer Inst 98:123–134
89. **Zaid H, Antonescu CN, Randhawa VK, Klip A** 2008 Insulin action on glucose transporters through molecular switches, tracks and tethers. Biochem J 413:201–215
90. **McKern NM, Lawrence MC, Streltsov VA, Lou MZ, Adams TE, Lovrecz GO, Elleman TC, Richards KM, Bentley JD, Pilling PA, Hoyne PA, Cartledge KA, Pham TM, Lewis JL, Sankovich SE, Stoichevska V, Da Silva E, Robinson CP, Frenkel MJ, Sparrow LG, Fernley RT, Epa VC, Ward CW** 2006 Structure of the insulin receptor ectodomain reveals a folded-over conformation. Nature 443:218–221
91. **Lou M, Garrett TP, McKern NM, Hoyne PA, Epa VC, Bentley JD, Lovrecz GO, Cosgrove LJ, Frenkel MJ, Ward CW** 2006 The first three domains of the insulin receptor differ structurally from the insulin-like growth factor 1 receptor in the regions governing ligand specificity. Proc Natl Acad Sci USA 103:12429–12434
92. **Garrett TP, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, Elleman TC, Cosgrove LJ, Ward CW** 1998 Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. Nature 394:395–399
93. **Wan ZL, Huang K, Xu B, Hu SQ, Wang S, Chu YC, Katsoyannis PG, Weiss MA** 2005 Diabetes-associated mutations in human insulin: crystal structure and photocross-linking studies of a-chain variant insulin Wakayama. Biochemistry 44:5000–5016
94. **Chen WJ, Goldstein JL, Brown MS** 1990 NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. J Biol Chem 265:3116–3123
95. **Kohanski RA** 1993 Insulin receptor autophosphorylation. II. Determination of autophosphorylation sites by chemical sequence analysis and identification of the juxtamembrane sites. Biochemistry 32:5773–5780
96. **White MF, Livingston JN, Backer JM, Lauris V, Dull TJ, Ullrich A, Kahn CR** 1988 Mutation of the insulin receptor at tyrosine 960 inhibits signal transmission but does not affect its tyrosine kinase activity. Cell 54:641–649
97. **Lee J, O'Hare T, Pilch PF, Shoelson SE** 1993 Insulin receptor autophosphorylation occurs asymmetrically. J Biol Chem 268:4092–4098
98. **White MF, Shoelson SE, Keutmann H, Kahn CR** 1988 A cascade of tyrosine autophosphorylation in the β-subunit activates the phosphotransferase of the insulin receptor. J Biol Chem 263:2969–2980
99. **Maegawa H, Olefsky JM, Thies S, Boyd D, Ullrich A, McClain DA** 1988 Insulin receptors with defective tyrosine kinase inhibit normal receptor function at the level of substrate phosphorylation. J Biol Chem 263:12629–12637
100. **Myers MG, Backer JM, Siddle K, White MF** 1991 The insulin receptor functions normally in Chinese hamster ovary cells after truncation of the C terminus. J Biol Chem 266:10616–10623
101. **Takata Y, Webster NJ, Olefsky JM** 1992 Intracellular signaling by a mutant human insulin receptor lacking the carboxyl-terminal tyrosine autophosphorylation sites. J Biol Chem 267:9065–9070
102. **Maegawa H, McClain DA, Freidenberg G, Olefsky JM,**

Napier M, Lipari T, Dull TJ, Lee J, Ullrich A 1988 Properties of a human insulin receptor with a COOH-terminal truncation. II. Truncated receptors have normal kinase activity but are defective in signaling metabolic effects. J Biol Chem 263:8912–8917
103. **Thies RS, Ullrich A, McClain DA** 1989 Augmented mitogenesis and impaired metabolic signaling mediated by a truncated insulin receptor. J Biol Chem 264:12820–12825
104. **Ando A, Momomura K, Tobe K, Yamamoto-Honda R, Sakura H, Tamori Y, Kaburagi Y, Koshio O, Akanuma Y, Yazaki Y** 1992 Enhanced insulin-induced mitogenesis and mitogen-activated protein kinase activities in mutant IRs with substitution of two COOH-terminal tyrosine autophosphorylation sites by phenylalanine. J Biol Chem 267: 12788–12796
105. **Backer JM, Kahn CR, Cahill DA, Ullrich A, White MF** 1990 Receptor-mediated internalization of insulin requires a 12-amino acid sequence in the juxtamembrane region of the insulin receptor β-subunit. J Biol Chem 265: 16450–16454
106. **Rajagopalan M, Neidigh JL, McClain DA** 1991 Amino acid sequences Gly-Pro-Leu-Tyr and Asn-Pro-Glu-Tyr in the submembranous domain of the insulin receptor are required for normal endocytosis. J Biol Chem 266:23068–23073
107. **Backer JM, Schroeder GG, Kahn CR, Myers Jr MG, Wilden PA, Cahill DA, White MF** 1992 Insulin stimulation of phosphatidylinositol 3-kinase activity maps to insulin receptor regions required for endogenous substrate phosphorylation. J Biol Chem 267:1367–1374
108. **Taguchi A, White MF** 2008 Insulin-like signaling, nutrient homeostasis, and life span. Annu Rev Physiol 70:191–212
109. **Folsom AR, Kaye SA, Prineas RJ, Potter JD, Gapstur SM, Wallace RB** 1990 Increased incidence of carcinoma of the breast associated with abdominal adiposity in postmenopausal women. Am J Epidemiol 131:794–803
110. **Mora A, Komander D, van Aalten DM, Alessi DR** 2004 PDK1, the master regulator of AGC kinase signal transduction. Semin Cell Dev Biol 15:161–170
111. **Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME** 1997 Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231–241
112. **Barthel A, Schmoll D, Unterman TG** 2005 FoxO proteins in insulin action and metabolism. Trends Endocrinol Metab 16:183–189
113. **Myatt SS, Lam EW** 2007 The emerging roles of forkhead box (Fox) proteins in cancer. Nat Rev Cancer 7:847–859
114. **Zou Y, Tsai WB, Cheng CJ, Hsu C, Chung YM, Li PC, Lin SH, Hu MC** 2008 Forkhead box transcription factor FOXO3a suppresses estrogen-dependent breast cancer cell proliferation and tumorigenesis. Breast Cancer Res 10:R21
115. **Wang X, Proud CG** 2006 The mTOR pathway in the control of protein synthesis. Physiology (Bethesda) 21:362–369
116. **Levine AJ, Feng Z, Mak TW, You H, Jin S** 2006 Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. Genes Dev 20: 267–275
117. **Liu J, Kimura A, Baumann CA, Saltiel AR** 2002 APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3–L1 adipocytes. Mol Cell Biol 22:3599–3609
118. **Kanzaki M** 2006 Insulin receptor signals regulating GLUT4 translocation and actin dynamics. Endocr J 53: 267–293
119. **Frasca F, Pandini G, Malaguarnera R, Mandarino A, Messina RL, Sciacca L, Belfiore A, Vigneri R** 2007 Role of c-Abl in directing metabolic versus mitogenic effects in insulin receptor signaling. J Biol Chem 282:26077–26088
120. **Ceresa BP, Pessin JE** 1998 Insulin regulation of the Ras activation/inactivation cycle. Mol Cell Biochem 182:23–29
121. **Roux PP, Blenis J** 2004 ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68:320–344
122. **Moller DE, Yokota A, Caro JF, Flier JS** 1989 Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol 3:1263–1269
123. **Denley A, Wallace JC, Cosgrove LJ, Forbes BE** 2003 The insulin receptor isoform exon 11- (IR-A) in cancer and other diseases: a review. Horm Metab Res 35:778–785
124. **Kosaki A, Nelson J, Webster NJ** 1998 Identification of intron and exon sequences involved in alternative splicing of insulin receptor pre-mRNA. J Biol Chem 273:10331–10337
125. **Webster NJ, Evans LG, Caples M, Erker L, Chew SL** 2004 Assembly of splicing complexes on exon 11 of the human insulin receptor gene does not correlate with splicing efficiency in-vitro. BMC Mol Biol 5:7
126. **Cáceres JF, Stamm S, Helfman DM, Krainer AR** 1994 Regulation of alternative splicing in vivo by overexpression of antagonistic splicing factors. Science 265:1706–1709
127. **Mayeda A, Helfman DM, Krainer AR** 1993 Modulation of exon skipping and inclusion by heterogeneous nuclear ribonucleoprotein A1 and pre-mRNA splicing factor SF2/ASF. Mol Cell Biol 13:2993–3001
128. **Zerbe LK, Pino I, Pio R, Cosper PF, Dwyer-Nield LD, Meyer AM, Port JD, Montuenga LM, Malkinson AM** 2004 Relative amounts of antagonistic splicing factors, hnRNP A1 and ASF/SF2, change during neoplastic lung growth: implications for pre-mRNA processing. Mol Carcinog 41:187–196
129. **Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T** 1992 Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. Cell 68:799–808
130. **Taneja KL, McCurrach M, Schalling M, Housman D, Singer RH** 1995 Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. J Cell Biol 128:995–1002
131. **Miller JW, Urbinati CR, Teng-Umnuay P, Stenberg MG, Byrne BJ, Thornton CA, Swanson MS** 2000 Recruitment of human muscleblind proteins to (CUG) (n) expansions associated with myotonic dystrophy. EMBO J 19:4439–4448
132. **Fardaei M, Rogers MT, Thorpe HM, Larkin K, Hamshere MG, Harper PS, Brook JD** 2002 Three proteins, MBNL, MBLL and MBXL, co-localize in vivo with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells. Hum Mol Genet 11:805–814
133. **Timchenko NA, Cai ZJ, Welm AL, Reddy S, Ashizawa T,**

Timchenko LT 2001 RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. J Biol Chem 276:7820–7826

134. **Ladd AN, Charlet N, Cooper TA** 2001 The CELF family of RNA binding proteins is implicated in cell-specific and developmentally regulated alternative splicing. Mol Cell Biol 21:1285–1296
135. **Paul S, Dansithong W, Kim D, Rossi J, Webster NJ, Comai L, Reddy S** 2006 Interaction of muscleblind, CUG-BP1 and hnRNP H proteins in DM1-associated aberrant IR splicing. EMBO J 25:4271–4283
136. **Benyoucef S, Surinya KH, Hadaschik D, Siddle K** 2007 Characterization of insulin/IGF hybrid receptors: contributions of the insulin receptor L2 and Fn1 domains and the alternatively spliced exon 11 sequence to ligand binding and receptor activation. Biochem J 403:603–613
137. **Whittaker J, Sørensen H, Gadsbøll VL, Hinrichsen J** 2002 Comparison of the functional insulin binding epitopes of the A and B isoforms of the insulin receptor. J Biol Chem 277:47380–47384
138. **Denley A, Bonython ER, Booker GW, Cosgrove LJ, Forbes BE, Ward CW, Wallace JC** 2004 Structural determinants for high-affinity binding of insulin-like growth factor II to insulin receptor (IR)-A, the exon 11 minus isoform of the IR. Mol Endocrinol 18:2502–2512
139. **Vogt B, Carrascosa JM, Ermel B, Ullrich A, Häring HU** 1991 The two isotypes of the human IR (HIR-A and HIR-B) follow different internalization kinetics. Biochem Biophys Res Commun 177:1013–1018
140. **Li Calzi S, Choice CV, Najjar SM** 1997 Differential effect of pp120 on insulin endocytosis by two variant insulin receptor isoforms. Am J Physiol 273:E801–E808
141. **Leibiger B, Leibiger IB, Moede T, Kemper S, Kulkarni RN, Kahn CR, de Vargas LM, Berggren PO** 2001 Selective insulin signaling through A and B IRs regulates transcription of insulin and glucokinase genes in pancreatic β-cells. Mol Cell 7:559–570
142. **Uhles S, Moede T, Leibiger B, Berggren PO, Leibiger IB** 2003 Isoform-specific insulin receptor signaling involves different plasma membrane domains. J Cell Biol 163:1327–1337
143. **Nevado C, Valverde AM, Benito M** 2006 Role of insulin receptor in the regulation of glucose uptake in neonatal hepatocytes. Endocrinology 147:3709–3718
144. **Nevado C, Benito M, Valverde AM** 2008 Role of insulin receptor and balance in insulin receptor isoforms A and B in regulation of apoptosis in simian virus 40-immortalized neonatal hepatocytes. Mol Biol Cell 19:1185–1198
145. **Sciacca L, Prisco M, Wu A, Belfiore A, Vigneri R, Baserga R** 2003 Signaling differences from the A and B isoforms of the insulin receptor (IR) in 32D cells in the presence or absence of IR substrate-1. Endocrinology 144:2650–2658
146. **Wu Y, Cui K, Miyoshi K, Hennighausen L, Green JE, Setser J, LeRoith D, Yakar S** 2003 Reduced circulating insulin-like growth factor I levels delay the onset of chemically and genetically induced mammary tumors. Cancer Res 63:4384–4388
147. **Morrione A, Valentinis B, Xu SQ, Yumet G, Louvi A, Efstratiadis A, Baserga R** 1997 Insulin-like growth factor II stimulates cell proliferation through the insulin receptor. Proc Natl Acad Sci USA 94:3777–3782
148. **Sciacca L, Mineo R, Pandini G, Murabito A, Vigneri R, Belfiore A** 2002 In IGF-I receptor-deficient leiomyosarcoma cells autocrine IGF-II induces cell invasion and protection from apoptosis via the insulin receptor isoform A. Oncogene 21:8240–8250
149. **Pandini G, Medico E, Conte E, Sciacca L, Vigneri R, Belfiore A** 2003 Differential gene expression induced by insulin and insulin-like growth factor-II through the insulin receptor isoform A. J Biol Chem 278:42178–42189
150. **Sacco A, Morcavallo A, Pandini G, Vigneri R, Belfiore A** 2009 Differential signaling activation by insulin and insulin-like growth factors I and II upon binding to insulin receptor isoform A. Endocrinology 150:3594–3602
151. **Claeys I, Simonet G, Poels J, Van Loy T, Vercammen L, De Loof A, Vanden Broeck J** 2002 Insulin-related peptides and their conserved signal transduction pathway. Peptides 23:807–816
152. **Garofalo RS** 2002 Genetic analysis of insulin signaling in *Drosophila*. Trends Endocrinol Metab 13:156–162
153. **Rulifson EJ, Kim SK, Nusse R** 2002 Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296:1118–1120
154. **Louvi A, Accili D, Efstratiadis A** 1997 Growth-promoting interaction of IGF-II with the insulin receptor during mouse embryonic development. Dev Biol 189:33–48
155. **Efstratiadis A** 1998 Genetics of mouse growth. Int J Dev Biol 42:955–976
156. **Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A** 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59–72
157. **Wertheimer E, Lu SP, Backeljauw PF, Davenport ML, Taylor SI** 1993 Homozygous deletion of the human insulin receptor gene results in leprechaunism. Nat Genet 5:71–73
158. **Psiachou H, Mitton S, Alaghband-Zadeh J, Hone J, Taylor SI, Sinclair L** 1993 Leprechaunism and homozygous nonsense mutation in the insulin receptor gene. Lancet 342:924
159. **Krook A, Brueton L, O'Rahilly S** 1993 Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism. Lancet 342:277–278
160. **Jospe N, Kaplowitz PB, Furlanetto RW** 1996 Homozygous nonsense mutation in the insulin receptor gene of a patient with severe congenital insulin resistance: leprechaunism and the role of the insulin-like growth factor receptor. Clin Endocrinol (Oxf) 45:229–235
161. **Danan C, Amselem S, Dassieu G, Cohen R, Janaud JC** 1994 [Physiopathological approach and antenatal diagnosis of diabetes mellitus insulin resistant: apropos of a case with leprechaunism] (in French). Arch Pediatr 1:268–272
162. **DeChiara TM, Efstratiadis A, Robertson EJ** 1990 A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345:78–80
163. **Han VK, D'Ercole AJ, Lund PK** 1987 Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 236:193–197
164. **Nakae J, Kido Y, Accili D** 2001 Distinct and overlapping functions of insulin and IGF-I receptors. Endocr Rev 22:818–835
165. **Han VK, Carter AM** 2000 Spatial and temporal patterns of expression of messenger RNA for insulin-like growth factors and their binding proteins in the placenta of man and laboratory animals. Placenta 21:289–305

166. **Sibley CP, Turner MA, Cetin I, Ayuk P, Boyd CA, D'Souza SW, Glazier JD, Greenwood SL, Jansson T, Powell T** 2005 Placental phenotypes of intrauterine growth. Pediatr Res 58:827–832
167. **Cullen KJ, Smith HS, Hill S, Rosen N, Lippman ME** 1991 Growth factor messenger RNA expression by human breast fibroblasts from benign and malignant lesions. Cancer Res 51:4978–4985
168. **Milio LA, Hu J, Douglas GC** 1994 Binding of insulin-like growth factor I to human trophoblast cells during differentiation in vitro. Placenta 15:641–651
169. **Lacey H, Haigh T, Westwood M, Aplin JD** 2002 Mesenchymally-derived insulin-like growth factor 1 provides a paracrine stimulus for trophoblast migration. BMC Dev Biol 2:5
170. **Constância M, Angiolini E, Sandovici I, Smith P, Smith R, Kelsey G, Dean W, Ferguson-Smith A, Sibley CP, Reik W, Fowden A** 2005 Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems. Proc Natl Acad Sci USA 102:19219–19224
171. **Serrano J, Bevins CL, Young SW, de Pablo F** 1989 Insulin gene expression in chicken ontogeny: pancreatic, extrapancreatic, and prepancreatic. Dev Biol 132:410–418
172. **Moore GE, Abu-Amero SN, Bell G, Wakeling EL, Kingsnorth A, Stanier P, Jauniaux E, Bennett ST** 2001 Evidence that insulin is imprinted in the human yolk sac. Diabetes 50:199–203
173. **Morales AV, Serna J, Alarcón C, de la Rosa EJ, de Pablo F** 1997 Role of prepancreatic (pro)insulin and the insulin receptor in prevention of embryonic apoptosis. Endocrinology 138:3967–3975
174. **Díaz B, Pimentel B, de Pablo F, de La Rosa EJ** 1999 Apoptotic cell death of proliferating neuroepithelial cells in the embryonic retina is prevented by insulin. Eur J Neurosci 11:1624–1632
175. **Scavo LM, Serrano J, Roth J, de Pablo F** 1991 Genes for the insulin receptor and the insulin-like growth factor I receptor are expressed in the chicken embryo blastoderm and throughout organogenesis. Biochem Biophys Res Commun 176:1393–1401
176. **Girbau M, Bassas L, Alemany J, de Pablo F** 1989 In situ autoradiography and ligand-dependent tyrosine kinase activity reveal IRs and insulin-like growth factor I receptors in prepancreatic chicken embryos. Proc Natl Acad Sci USA 86:5868–5872
177. **Chang SG, Kim DY, Choi KD, Shin JM, Shin HC** 1998 Human insulin production from a novel mini-proinsulin which has high receptor-binding activity. Biochem J 329: 631–635
178. **García-de Lacoba M, Alarcón C, de la Rosa EJ, de Pablo F** 1999 Insulin/insulin-like growth factor-I hybrid receptors with high affinity for insulin are developmentally regulated during neurogenesis. Endocrinology 140:233–243
179. **Entingh AJ, Taniguchi CM, Kahn CR** 2003 Bi-directional regulation of brown fat adipogenesis by the insulin receptor. J Biol Chem 278:33377–33383
180. **Maggi D, Laurino C, Andraghetti G, Cordera R** 1994 The overexpression of insulin receptor makes CHO cells resistant to the action of IGF-1: role of IRS-1. Biochem Biophys Res Commun 205:693–699
181. **Kosaki A, Webster NJ** 1993 Effect of dexamethasone on the alternative splicing of the insulin receptor mRNA and insulin action in HepG2 hepatoma cells. J Biol Chem 268: 21990–21996
182. **Accili D, Taylor SI** 1991 Targeted inactivation of the insulin receptor gene in mouse 3T3–L1 fibroblasts via homologous recombination. Proc Natl Acad Sci USA 88:4708–4712
183. **Serrano R, Villar M, Martínez C, Carrascosa JM, Gallardo N, Andrés A** 2005 Differential gene expression of insulin receptor isoforms A and B and insulin receptor substrates 1, 2 and 3 in rat tissues: modulation by aging and differentiation in rat adipose tissue. J Mol Endocrinol 34:153–161
184. **Pandini G, Frasca F, Mineo R, Sciacca L, Vigneri R, Belfiore A** 2002 Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 277:39684–39695
185. **Valtieri M, Tweardy DJ, Caracciolo D, Johnson K, Mavilio F, Altmann S, Santoli D, Rovera G** 1987 Cytokine-dependent granulocytic differentiation. Regulation of proliferative and differentiative responses in a murine progenitor cell line. J Immunol 138:3829–3835
186. **Zecevic M, Amos CI, Gu X, Campos IM, Jones JS, Lynch PM, Rodriguez-Bigas MA, Frazier ML** 2006 IGF1 gene polymorphism and risk for hereditary nonpolyposis colorectal cancer. J Natl Cancer Inst 98:139–143
187. **Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R** 1993 A *C. elegans* mutant that lives twice as long as wild type. Nature 366:461–464
188. **Carter CS, Ramsey MM, Sonntag WE** 2002 A critical analysis of the role of growth hormone and IGF-1 in aging and lifespan. Trends Genet 18:295–301
189. **Tatar M, Yin C** 2001 Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. Exp Gerontol 36:723–738
190. **Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ, Partridge L** 2001 Extension of lifespan by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science 292:104–106
191. **Holzenberger M, Dupont J, Ducos B, Leneuve P, Géloën A, Even PC, Cervera P, Le Bouc Y** 2003 IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421:182–187
192. **Barbieri M, Bonafè M, Franceschi C, Paolisso G** 2003 Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. Am J Physiol Endocrinol Metab 285:E1064–E1071
193. **Bartke A, Chandrashekar V, Bailey B, Zaczek D, Turyn D** 2002 Consequences of growth hormone (GH) overexpression and GH resistance. Neuropeptides 36:201–208
194. **Brown-Borg HM, Rakoczy SG, Romanick MA, Kennedy MA** 2002 Effects of growth hormone and insulin-like growth factor-1 on hepatocyte antioxidative enzymes. Exp Biol Med (Maywood) 227:94–104
195. **Sonntag WE, Lynch CD, Cefalu WT, Ingram RL, Bennett SA, Thornton PL, Khan AS** 1999 Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. J Gerontol A Biol Sci Med Sci 54:B521–B538

196. **Parr T** 1996 Insulin exposure controls the rate of mammalian aging. Mech Ageing Dev 88:75–82
197. **Longo VD, Finch CE** 2003 Evolutionary medicine: from dwarf model systems to healthy centenarians? Science 299: 1342–1346
198. **Bonkowski MS, Pamenter RW, Rocha JS, Masternak MM, Panici JA, Bartke A** 2006 Long-lived growth hormone receptor knockout mice show a delay in age-related changes of body composition and bone characteristics. J Gerontol A Biol Sci Med Sci 61:562–567
199. **Paolisso G, Gambardella A, Ammendola S, D'Amore A, Balbi V, Varricchio M, D'Onofrio F** 1996 Glucose tolerance and insulin action in healthy centenarians. Am J Physiol 270:E890–E894
200. **Juul A** 2003 Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res 13:113–170
201. **Janssen JA, Lamberts SW** 2004 Igf-I and longevity. Horm Res 62(Suppl 3):104–109
202. **Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P** 2008 Functionally significant insulin-like growth factor I receptor mutations in centenarians. Proc Natl Acad Sci USA 105:3438–3442
203. **Wiersma MM, Auboeuf D, Nieuwenhuizen-Bakker IM, Radder JK, Riou JP, Vidal H** 1997 Insulin receptor mRNA splicing and altered metabolic control in aged and mildly insulin-deficient rats. Am J Physiol 272:E607–E615
204. **Vidal H, Auboeuf D, Beylot M, Riou JP** 1995 Regulation of insulin receptor mRNA splicing in rat tissues. Effect of fasting, aging, and diabetes. Diabetes 44:1196–1201
205. **Kaaks R, Lukanova A** 2001 Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc 60:91–106
206. **Denley A, Carroll JM, Brierley GV, Cosgrove L, Wallace J, Forbes B, Roberts Jr CT** 2007 Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated IRs. Mol Cell Biol 27:3569–3577
207. **Taguchi A, Wartschow LM, White MF** 2007 Brain IRS2 signaling coordinates life span and nutrient homeostasis. Science 317:369–372
208. **Machuca-Tzili L, Brook D, Hilton-Jones D** 2005 Clinical and molecular aspects of the myotonic dystrophies: a review. Muscle Nerve 32:1–18
209. **Moxley RT, Corbett AJ, Minaker KL, Rowe JW** 1984 Whole body insulin resistance in myotonic dystrophy. Ann Neurol 15:157–162
210. **Savkur RS, Philips AV, Cooper TA** 2001 Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. Nat Genet 29:40–47
211. **Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, Day JW, Ranum LP** 2001 Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 293:864–867
212. **Ranum LP, Day JW** 2002 Myotonic dystrophy: clinical and molecular parallels between myotonic dystrophy type 1 and type 2. Curr Neurol Neurosci Rep 2:465–470
213. **Philips AV, Timchenko LT, Cooper TA** 1998 Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. Science 280:737–741
214. **Sbraccia P, Giaccari A, D'Adamo M, Caiola S, Morviducci L, Zorretta D, Maroccia E, Buongiorno A, Tamburrano G** 1998 Expression of the two insulin receptor isoforms is not altered in the skeletal muscle and liver of diabetic rats. Metabolism 47:129–132
215. **Huang Z, Bodkin NL, Ortmeyer HK, Hansen BC, Shuldiner AR** 1994 Hyperinsulinemia is associated with altered insulin receptor mRNA splicing in muscle of the spontaneously obese diabetic rhesus monkey. J Clin Invest 94:1289–1296
216. **Huang Z, Bodkin NL, Ortmeyer HK, Zenilman ME, Webster NJ, Hansen BC, Shuldiner AR** 1996 Altered insulin receptor messenger ribonucleic acid splicing in liver is associated with deterioration of glucose tolerance in the spontaneously obese and diabetic rhesus monkey: analysis of controversy between monkey and human studies. J Clin Endocrinol Metab 81:1552–1556
217. **Norgren S, Zierath J, Wedell A, Wallberg-Henriksson H, Luthman H** 1994 Regulation of human insulin receptor RNA splicing in vivo. Proc Natl Acad Sci USA 91:1465–1469
218. **Sell SM, Reese D, Ossowski VM** 1994 Insulin-inducible changes in insulin receptor mRNA splice variants. J Biol Chem 269:30769–30772
219. **Sbraccia P, D'Adamo M, Leonetti F, Caiola S, Iozzo P, Giaccari A, Buongiorno A, Tamburrano G** 1996 Chronic primary hyperinsulinaemia is associated with altered insulin receptor mRNA splicing in muscle of patients with insulinoma. Diabetologia 39:220–225
220. **Rousseau MC, Parent ME, Pollak MN, Siemiatycki J** 2006 Diabetes mellitus and cancer risk in a population-based case-control study among men from Montreal, Canada. Int J Cancer 118:2105–2109
221. **Phy JL, Conover CA, Abbott DH, Zschunke MA, Walker DL, Session DR, Tummon IS, Thornhill AR, Lesnick TG, Dumesic DA** 2004 Insulin and messenger ribonucleic acid expression of insulin receptor isoforms in ovarian follicles from nonhirsute ovulatory women and polycystic ovary syndrome patients. J Clin Endocrinol Metab 89:3561–3566
222. **Kellerer M, Lammers R, Ermel B, Tippmer S, Vogt B, Obermaier-Kusser B, Ullrich A, Häring HU** 1992 Distinct α-subunit structures of human insulin receptor A and B variants determine differences in tyrosine kinase activities. Biochemistry 31:4588–4596
223. **Kosaki A, Pillay TS, Xu L, Webster NJ** 1995 The B isoform of the insulin receptor signals more efficiently than the A isoform in HepG2 cells. J Biol Chem 270:20816–20823
224. **Blanquart C, Achi J, Issad T** 2008 Characterization of IRA/IRB hybrid IRs using bioluminescence resonance energy transfer. Biochem Pharmacol 76:873–883
225. **Papa V, Pezzino V, Costantino A, Belfiore A, Giuffrida D, Frittitta L, Vannelli GB, Brand R, Goldfine ID, Vigneri R** 1990 Elevated insulin receptor content in human breast cancer. J Clin Invest 86:1503–1510
226. **Pezzino V, Papa V, Trischitta V, Brunetti A, Goodman PA, Treutelaar MK, Williams JA, Maddux BA, Vigneri R, Goldfine ID** 1989 Human insulin receptor radioimmunoassay: applicability to insulin-resistant states. Am J Physiol 257:E451–E457
227. **Frittitta L, Vigneri R, Papa V, Goldfine ID, Grasso G, Trischitta V** 1993 Structural and functional studies of IRs in human breast cancer. Breast Cancer Res Treat 25:73–82
228. **Sciacca L, Costantino A, Pandini G, Mineo R, Frasca F,**

Scalia P, Sbraccia P, Goldfine ID, Vigneri R, Belfiore A 1999 Insulin receptor activation by IGF-II in breast cancers: evidence for a new autocrine/paracrine mechanism. Oncogene 18:2471–2479

229. **Pandini G, Vigneri R, Costantino A, Frasca F, Ippolito A, Fujita-Yamaguchi Y, Siddle K, Goldfine ID, Belfiore A** 1999 Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: evidence for a second mechanism of IGF-I signaling. Clin Cancer Res 5:1935–1944
230. **Kalli KR, Falowo OI, Bale LK, Zschunke MA, Roche PC, Conover CA** 2002 Functional IRs on human epithelial ovarian carcinoma cells: implications for IGF-II mitogenic signaling. Endocrinology 143:3259–3267
231. **Belfiore A, Pandini G, Vella V, Squatrito S, Vigneri R** 1999 Insulin/IGF-I hybrid receptors play a major role in IGF-I signaling in thyroid cancer. Biochimie 81:403–407
232. **Altieri A, Franceschi S, Ferlay J, Smith J, La Vecchia C** 2003 Epidemiology and aetiology of gestational trophoblastic diseases. Lancet Oncol 4:670–678
233. **Diaz LE, Chuan YC, Lewitt M, Fernandez-Perez L, Carrasco-Rodríguez S, Sanchez-Gomez M, Flores-Morales A** 2007 IGF-II regulates metastatic properties of choriocarcinoma cells through the activation of the insulin receptor. Mol Hum Reprod 13:567–576
234. **Kricker JA, Towne CL, Firth SM, Herington AC, Upton Z** 2003 Structural and functional evidence for the interaction of insulin-like growth factors (IGFs) and IGF binding proteins with vitronectin. Endocrinology 144:2807–2815
235. **Li Y, Chang Q, Rubin BP, Fletcher CD, Morgan TW, Mentzer SJ, Sugarbaker DJ, Fletcher JA, Xiao S** 2007 Insulin receptor activation in solitary fibrous tumours. J Pathol 211: 550–554
236. **Arcaro A, Doepfner KT, Boller D, Guerreiro AS, Shalaby T, Jackson SP, Schoenwaelder SM, Delattre O, Grotzer MA, Fischer B** 2007 Novel role for insulin as an autocrine growth factor for malignant brain tumour cells. Biochem J 406:57–66
237. **Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, Kim JY, Goumnerova LC, Black PM, Lau C, Allen JC, Zagzag D, Olson JM, Curran T, Wetmore C, Biegel JA, Poggio T, Mukherjee S, Rifkin R, Califano A, Stolovitzky G, Louis DN, Mesirov JP, Lander ES, Golub TR** 2002 Prediction of central nervous system embryonal tumour outcome based on gene expression. Nature 415:436–442
238. **Grabowski PJ, Black DL** 2001 Alternative RNA splicing in the nervous system. Prog Neurobiol 65:289–308
239. **Venables JP** 2004 Aberrant and alternative splicing in cancer. Cancer Res 64:7647–7654
240. **Pisani P** 2008 Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. Arch Physiol Biochem 114: 63–70
241. **Papa V, Milazzo G, Goldfine ID, Waldman FM, Vigneri R** 1997 Sporadic amplification of the insulin receptor gene in human breast cancer. J Endocrinol Invest 20:531–536
242. **Smith ML, Fornace Jr AJ** 1995 Genomic instability and the role of p53 mutations in cancer cells. Curr Opin Oncol 7:69–75
243. **Frasca F, Rustighi A, Malaguarnera R, Altamura S, Vigneri P, Del Sal G, Giancotti V, Pezzino V, Vigneri R, Manfioletti G** 2006 HMGA1 inhibits the function of p53 family members in thyroid cancer cells. Cancer Res 66:2980–2989
244. **Sgarra R, Rustighi A, Tessari MA, Di Bernardo J, Altamura S, Fusco A, Manfioletti G, Giancotti V** 2004 Nuclear phosphoproteins HMGA and their relationship with chromatin structure and cancer. FEBS Lett 574:1–8
245. **Foti D, Iuliano R, Chiefari E, Brunetti A** 2003 A nucleoprotein complex containing Sp1, C/EBP β, and HMGI-Y controls human insulin receptor gene transcription. Mol Cell Biol 23:2720–2732
246. **Frittitta L, Cerrato A, Sacco MG, Weidner N, Goldfine ID, Vigneri R** 1997 The insulin receptor content is increased in breast cancers initiated by three different oncogenes in transgenic mice. Breast Cancer Res Treat 45:141–147
247. **Giorgino F, Belfiore A, Milazzo G, Costantino A, Maddux B, Whittaker J, Goldfine ID, Vigneri R** 1991 Overexpression of IRs in fibroblast and ovary cells induces a ligand-mediated transformed phenotype. Mol Endocrinol 5:452–459
248. **Frittitta L, Vigneri R, Stampfer MR, Goldfine ID** 1995 Insulin receptor overexpression in 184B5 human mammary epithelial cells induces a ligand-dependent transformed phenotype. J Cell Biochem 57:666–669
249. **Drejer K** 1992 The bioactivity of insulin analogues from in vitro receptor binding to in vivo glucose uptake. Diabetes Metab Rev 8:259–285
250. **Strickler HD, Wylie-Rosett J, Rohan T, Hoover DR, Smoller S, Burk RD, Yu H** 2001 The relation of type 2 diabetes and cancer. Diabetes Technol Ther 3:263–274
251. **Vigneri P, Frasca F, Sciacca L, Frittitta L, Vigneri R** 2006 Obesity and cancer. Nutr Metab Cardiovasc Dis 16:1–7
252. **Pandini G, Genua M, Frasca F, Squatrito S, Vigneri R, Belfiore A** 2007 17β-Estradiol up-regulates the insulin-like growth factor receptor through a nongenotropic pathway in prostate cancer cells. Cancer Res 67:8932–8941
253. **Pandini G, Mineo R, Frasca F, Roberts Jr CT, Marcelli M, Vigneri R, Belfiore A** 2005 Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. Cancer Res 65:1849–1857
254. **Pandini G, Genua M, Frasca F, Vigneri R, Belfiore A** 2009 Sex steroids upregulate the IGF-1R in prostate cancer cells through a nongenotropic pathway. Ann NY Acad Sci 1155:263–267
255. **Vajo Z, Fawcett J, Duckworth WC** 2001 Recombinant DNA technology in the treatment of diabetes: insulin analogs. Endocr Rev 22:706–717
256. **Drejer K, Kruse V, Larsen UD, Hougaard P, Bjørn S, Gammeltoft S** 1991 Receptor binding and tyrosine kinase activation by insulin analogues with extreme affinities studied in human hepatoma HepG2 cells. Diabetes 40:1488–1495
257. **Milazzo G, Sciacca L, Papa V, Goldfine ID, Vigneri R** 1997 ASPB10 insulin induction of increased mitogenic responses and phenotypic changes in human breast epithelial cells: evidence for enhanced interactions with the insulin-like growth factor-I receptor. Mol Carcinog 18:19–25
258. **Kurtzhals P, Schäffer L, Sørensen A, Kristensen C, Jonassen I, Schmid C, Trüb T** 2000 Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. Diabetes 49:999–1005
259. **Vincent MT, Carroll RJ, Hammer RE, Chan SJ, Guz Y, Steiner DF, Teitelman G** 1995 A transgene coding for a

human insulin analog has a mitogenic effect on murine embryonic β-cells. Proc Natl Acad Sci USA 92:6239–6243
260. **Hansen BF, Danielsen GM, Drejer K, Sørensen AR, Wiberg FC, Klein HH, Lundemose AG** 1996 Sustained signalling from the insulin receptor after stimulation with insulin analogues exhibiting increased mitogenic potency. Biochem J 315:271–279
261. **Dideriksen LH, Jorgensen JN, Drejer K** 1992 Carcinogenic effect on female rats after 12 months administration of the insulin analogue B10 ASP. Diabetes 41:143A
262. **Ciaraldi TP, Carter L, Seipke G, Mudaliar S, Henry RR** 2001 Effects of the long-acting insulin analog insulin glargine on cultured human skeletal muscle cells: comparisons to insulin and IGF-I. J Clin Endocrinol Metab 86: 5838–5847
263. **Hennige AM, Lehmann R, Weigert C, Moeschel K, Schäuble M, Metzinger E, Lammers R, Häring HU** 2005 Insulin glulisine: insulin receptor signaling characteristics in vivo. Diabetes 54:361–366
264. **Slieker LJ, Brooke GS, DiMarchi RD, Flora DB, Green LK, Hoffmann JA, Long HB, Fan L, Shields JE, Sundell KL, Surface PL, Chance RE** 1997 Modifications in the B10 and B26–30 regions of the B chain of human insulin alter affinity for the human IGF-I receptor more than for the insulin receptor. Diabetologia 40(Suppl 2):S54–S61
265. **Gammeltoft S, Hansen BF, Dideriksen L, Lindholm A, Schäffer L, Trüb T, Dayan A, Kurtzhals P** 1999 Insulin aspart: a novel rapid-acting human insulin analogue. Expert Opin Investig Drugs 8:1431–1442
266. **Stammberger I, Seipke G, Bartels T** 2006 Insulin glulisine—a comprehensive preclinical evaluation. Int J Toxicol 25:25–33
267. **Mayer D, Shukla A, Enzmann H** 2008 Proliferative effects of insulin analogues on mammary epithelial cells. Arch Physiol Biochem 114:38–44
268. **Staiger K, Hennige AM, Staiger H, Häring HU, Kellerer M** 2007 Comparison of the mitogenic potency of regular human insulin and its analogue glargine in normal and transformed human breast epithelial cells. Horm Metab Res 39:65–67
269. **Hemkens LG, Grouven U, Bender R, Günster C, Gutschmidt S, Selke GW, Sawicki PT** 2009 Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study. Diabetologia 52:1732–1744
270. **Jonasson JM, Ljung R, Talbäck M, Haglund B, Gudbjörnsdòttir S, Steineck G** 2009 Insulin glargine use and short-term incidence of malignancies—a population-based follow-up study in Sweden. Diabetologia 52: 1745–1754
271. **Currie CJ, Poole CD, Gale EA** 2009 The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 52:1766–1777
272. **Colhoun HM** 2009 Use of insulin glargine and cancer incidence in Scotland: a study from the Scottish Diabetes Research Network Epidemiology Group. Diabetologia 52: 1755–1765
273. **Rosenstock J, Fonseca V, McGill JB, Riddle M, Hallé JP, Hramiak I, Johnston P, Davis M** 2009 Similar risk of malignancy with insulin glargine and neutral protamine Hagedorn (NPH) insulin in patients with type 2 diabetes: findings from a 5 year randomised, open-label study. Diabetologia 52:1971–1973
274. **Jones HE, Gee JM, Taylor KM, Barrow D, Williams HD, Rubini M, Nicholson RI** 2005 Development of strategies for the use of anti-growth factor treatments. Endocr Relat Cancer 12 Suppl 1:S173–S182
275. **Turner BC, Haffty BG, Narayanan L, Yuan J, Havre PA, Gumbs AA, Kaplan L, Burgaud JL, Carter D, Baserga R, Glazer PM** 1997 Insulin-like growth factor-I receptor overexpression mediates cellular radioresistance and local breast cancer recurrence after lumpectomy and radiation. Cancer Res 57:3079–3083
276. **Yu D, Watanabe H, Shibuya H, Miura M** 2003 Redundancy of radioresistant signaling pathways originating from insulin-like growth factor I receptor. J Biol Chem 278:6702–6709
277. **Peretz S, Jensen R, Baserga R, Glazer PM** 2001 ATM-dependent expression of the insulin-like growth factor-I receptor in a pathway regulating radiation response. Proc Natl Acad Sci USA 98:1676–1681
278. **Canman CE, Lim DS** 1998 The role of ATM in DNA damage responses and cancer. Oncogene 17:3301–3308
279. **Shahrabani-Gargir L, Pandita TK, Werner H** 2004 Ataxia-telangiectasia mutated gene controls insulin-like growth factor I receptor gene expression in a deoxyribonucleic acid damage response pathway via mechanisms involving zinc-finger transcription factors Sp1 and WT1. Endocrinology 145:5679–5687
280. **Hay N, Sonenberg N** 2004 Upstream and downstream of mTOR. Genes Dev 18:1926–1945
281. **Sekuliæ A, Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, Abraham RT** 2000 A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. Cancer Res 60:3504–3513
282. **Choo AY, Blenis J** 2006 TORgeting oncogene addiction for cancer therapy. Cancer Cell 9:77–79
283. **Wan X, Harkavy B, Shen N, Grohar P, Helman LJ** 2007 Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene 26: 1932–1940
284. **Tamburini J, Chapuis N, Bardet V, Park S, Sujobert P, Willems L, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D** 2008 Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways. Blood 111:379–382
285. **Kelly K, Averbuch S** 2004 Gefitinib: phase II and III results in advanced non-small cell lung cancer. Semin Oncol 31: 93–99
286. **Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M** 2001 Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst 93:1852–1857
287. **Liu B, Fang M, Lu Y, Mendelsohn J, Fan Z** 2001 Fibroblast growth factor and insulin-like growth factor differentially modulate the apoptosis and G1 arrest induced by anti-epidermal growth factor receptor monoclonal antibody. Oncogene 20:1913–1922
288. **Chakravarti A, Loeffler JS, Dyson NJ** 2002 Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human

glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer Res 62:200–207
289. **Camirand A, Zakikhani M, Young F, Pollak M** 2005 Inhibition of insulin-like growth factor-1 receptor signaling enhances growth-inhibitory and proapoptotic effects of gefitinib (Iressa) in human breast cancer cells. Breast Cancer Res 7:R570–R579
290. **Jones HE, Goddard L, Gee JM, Hiscox S, Rubini M, Barrow D, Knowlden JM, Williams S, Wakeling AE, Nicholson RI** 2004 Insulin-like growth factor-I receptor signalling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells. Endocr Relat Cancer 11: 793–814
291. **Knowlden JM, Hutcheson IR, Barrow D, Gee JM, Nicholson RI** 2005 Insulin-like growth factor-I receptor signaling in tamoxifen-resistant breast cancer: a supporting role to the epidermal growth factor receptor. Endocrinology 146:4609–4618
292. **Jones HE, Gee JM, Barrow D, Tonge D, Holloway B, Nicholson RI** 2006 Inhibition of insulin receptor isoform-A signalling restores sensitivity to gefitinib in previously de novo resistant colon cancer cells. Br J Cancer 95:172–180
293. **Hofmann F, García-Echeverría C** 2005 Blocking the insulin-like growth factor-I receptor as a strategy for targeting cancer. Drug Discov Today 10:1041–1047
294. **Zhang H, Pelzer AM, Kiang DT, Yee D** 2007 Down-regulation of type I insulin-like growth factor receptor increases sensitivity of breast cancer cells to insulin. Cancer Res 67:391–397
295. **Fulzele K, DiGirolamo DJ, Liu Z, Xu J, Messina JL, Clemens TL** 2007 Disruption of the insulin-like growth factor type 1 receptor in osteoblasts enhances insulin signaling and action. J Biol Chem 282:25649–25658
296. **Girnita A, Girnita L, del Prete F, Bartolazzi A, Larsson O, Axelson M** 2004 Cyclolignans as inhibitors of the insulin-like growth factor-1 receptor and malignant cell growth. Cancer Res 64:236–242
297. **Miyamoto S, Nakamura M, Shitara K, Nakamura K, Ohki Y, Ishii G, Goya M, Kodama K, Sangai T, Maeda H, Shi-Chuang Z, Chiba T, Ochiai A** 2005 Blockade of paracrine supply of insulin-like growth factors using neutralizing antibodies suppresses the liver metastasis of human colorectal cancers. Clin Cancer Res 11:3494–3502
298. **Goya M, Miyamoto S, Nagai K, Ohki Y, Nakamura K, Shitara K, Maeda H, Sangai T, Kodama K, Endoh Y, Ishii G, Hasebe T, Yonou H, Hatano T, Ogawa Y, Ochiai A** 2004 Growth inhibition of human prostate cancer cells in human adult bone implanted into nonobese diabetic/severe combined immunodeficient mice by a ligand-specific antibody to human insulin-like growth factors. Cancer Res 64:6252–6258
299. **Pandey V, Perry JK, Mohankumar KM, Kong XJ, Liu SM, Wu ZS, Mitchell MD, Zhu T, Lobie PE** 2008 Autocrine human growth hormone stimulates oncogenicity of endometrial carcinoma cells. Endocrinology 149:3909–3919
300. **Feng Y, Zhu Z, Xiao X, Choudhry V, Barrett JC, Dimitrov DS** 2006 Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potently inhibit the IGF receptor type I signal transduction function. Mol Cancer Ther 5:114–120
301. **Pandini G, Wurch T, Akla B, Corvaia N, Belfiore A, Goetsch L** 2007 Functional responses and in vivo anti-tumour activity of h7C10: a humanised monoclonal antibody with neutralising activity against the insulin-like growth factor-1 (IGF-1) receptor and insulin/IGF-1 hybrid receptors. Eur J Cancer 43:1318–1327
302. **Haluska P, Carboni JM, Loegering DA, Lee FY, Wittman M, Saulnier MG, Frennesson DB, Kalli KR, Conover CA, Attar RM, Kaufmann SH, Gottardis M, Erlichman C** 2006 In vitro and in vivo antitumor effects of the dual insulin-like growth factor-I/insulin receptor inhibitor, BMS-554417. Cancer Res 66:362–371
303. **Olson TS, Bamberger MJ, Lane MD** 1988 Post-translational changes in tertiary and quaternary structure of the insulin proreceptor. Correlation with acquisition of function. J Biol Chem 263:7342–7351
304. **Peruzzi F, Prisco M, Dews M, Salomoni P, Grassilli E, Romano G, Calabretta B, Baserga R** 1999 Multiple signaling pathways of the insulin-like growth factor 1 receptor in protection from apoptosis. Mol Cell Biol 19:7203–7215
305. **Soos MA, Field CE, Siddle K** 1993 Purified hybrid insulin/insulin-like growth factor-I receptors bind insulin-like growth factor-I, but not insulin, with high affinity. Biochem J 290:419–426
306. **Soos MA, Siddle K** 1989 Immunological relationships between receptors for insulin and insulin-like growth factor I. Evidence for structural heterogeneity of insulin-like growth factor I receptors involving hybrids with IRs. Biochem J 263:553–563
307. **Moxham CP, Duronio V, Jacobs S** 1989 Insulin-like growth factor I receptor β-subunit heterogeneity. Evidence for hybrid tetramers composed of insulin-like growth factor I and insulin receptor heterodimers. J Biol Chem 264: 13238–13244
308. **Soos MA, Whittaker J, Lammers R, Ullrich A, Siddle K** 1990 Receptors for insulin and insulin-like growth factor-I can form hybrid dimers. Characterisation of hybrid receptors in transfected cells. Biochem J 270:383–390
309. **Kasuga M, Fujita-Yamaguchi Y, Blithe DL, White MF, Kahn CR** 1983 Characterization of the insulin receptor kinase purified from human placental membranes. J Biol Chem 258:10973–10980
310. **De Meyts P** 2004 Insulin and its receptor: structure, function and evolution. Bioessays 26:1351–1362
311. **Denley A, Cosgrove LJ, Booker GW, Wallace JC, Forbes BE** 2005 Molecular interactions of the IGF system. Cytokine Growth Factor Rev 16:421–439
312. **Modan-Moses D, Janicot M, McLenithan JC, Lane MD, Casella SJ** 1998 Expression and function of insulin/insulin-like growth factor I hybrid receptors during differentiation of 3T3–L1 preadipocytes. Biochem J 333:825–831
313. **Whittaker J, Soos MA, Siddle K** 1990 Hybrid insulin receptors. Molecular mechanisms of negative-dominant mutations in receptor-mediated insulin resistance. Diabetes Care 13:576–581
314. **Soos MA, Navé BT, Siddle K** 1993 Immunological studies of type I IGF receptors and insulin receptors: characterisation of hybrid and atypical receptor subtypes. Adv Exp Med Biol 343:145–157
315. **Frattali AL, Treadway JL, Pessin JE** 1992 Insulin/IGF-1 hybrid receptors: implications for the dominant-negative phenotype in syndromes of insulin resistance. J Cell Biochem 48:43–50

316. **Kasuya J, Paz IB, Maddux BA, Goldfine ID, Hefta SA, Fujita-Yamaguchi Y** 1993 Characterization of human placental insulin-like growth factor-I/insulin hybrid receptors by protein microsequencing and purification. Biochemistry 32:13531–13536
317. **Garofalo RS, Barenton B** 1992 Functional and immunological distinction between insulin-like growth factor I receptor subtypes in KB cells. J Biol Chem 267:11470–11475
318. **Langlois WJ, Sasaoka T, Yip CC, Olefsky JM** 1995 Functional characterization of hybrid receptors composed of a truncated insulin receptor and wild type insulin-like growth factor 1 or IRs. Endocrinology 136:1978–1986
319. **Slaaby R, Schäffer L, Lautrup-Larsen I, Andersen AS, Shaw AC, Mathiasen IS, Brandt J** 2006 Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. J Biol Chem 281:25869–25874
320. **Koval AP, Blakesley VA, Roberts Jr CT, Zick Y, Leroith D** 1998 Interaction in vitro of the product of the c-Crk-II proto-oncogene with the insulin-like growth factor I receptor. Biochem J 330:923–932
321. **Jonas HA, Eckardt GS, Clark S** 1990 Expression of atypical and classical IRs in Chinese hamster ovary cells transfected with cloned cDNA for the human insulin receptor. Endocrinology 127:1301–1309
322. **Hernández-Sánchez C, López-Carranza A, Alarcón C, de La Rosa EJ, de Pablo F** 1995 Autocrine/paracrine role of insulin-related growth factors in neurogenesis: local expression and effects on cell proliferation and differentiation in retina. Proc Natl Acad Sci USA 92:9834–9838
323. **Papa V, Gliozzo B, Clark GM, McGuire WL, Moore D, Fujita-Yamaguchi Y, Vigneri R, Goldfine ID, Pezzino V** 1993 Insulin-like growth factor-I receptors are overexpressed and predict a low risk in human breast cancer. Cancer Res 53:3736–3740
324. **Cox ME, Gleave ME, Zakikhani M, Bell RH, Piura E, Vickers E, Cunningham M, Larsson O, Fazli L, Pollak M** 2009 Insulin receptor expression by human prostate cancers. Prostate 69:33–40
325. **Avnet S, Sciacca L, Salerno M, Gancitano G, Cassarino MF, Longhi A, Zakikhani M, Carboni JM, Gottardis M, Giunti A, Pollak M, Vigneri R, Baldini N** 2009 Insulin receptor isoform A and insulin-like growth factor II as additional treatment targets in human osteosarcoma. Cancer Res 69:2443–2452
326. **Serrano ML, Sánchez-Gómez M, Bravo MM, Yakar S, LeRoith D** 2008 Differential expression of IGF-I and insulin receptor isoforms in HPV positive and negative human cervical cancer cell lines. Horm Metab Res 40:661–667
327. **Dou T, Ji C, Gu S, Xu J, Xu J, Ying K, Xie Y, Mao Y** 2006 Co-evolutionary analysis of insulin/insulin like growth factor 1 signal pathway in vertebrate species. Front Biosci 11:380–388
328. **Bowker SL, Majumdar SR, Veugelers P, Johnson JA** 2006 Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 29:254–258
329. **Coughlin SS, Calle EE, Teras LR, Petrelli J, Thun MJ** 2004 Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. Am J Epidemiol 159: 1160–1167
330. **Kaaks R, Lukanova A** 2002 Effects of weight control and physical activity in cancer prevention: role of endogenous hormone metabolism. Ann NY Acad Sci 963:268–281
331. **Vainio H, Kaaks R, Bianchini F** 2002 Weight control and physical activity in cancer prevention: international evaluation of the evidence. Eur J Cancer Prev 11(Suppl 2):S94–S100
332. **Lev-Ran A** 1998 Mitogenic factors accelerate later-age diseases: insulin as a paradigm. Mech Ageing Dev 102:95–113
333. **Giovannucci E** 2001 Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr 131: 3109S–3120S
334. **Ma J, Giovannucci E, Pollak M, Leavitt A, Tao Y, Gaziano JM, Stampfer MJ** 2004 A prospective study of plasma C-peptide and colorectal cancer risk in men. J Natl Cancer Inst 96:546–553
335. **Hamelers IH, Steenbergh PH** 2003 Interactions between estrogen and insulin-like growth factor signaling pathways in human breast tumor cells. Endocr Relat Cancer 10:331–345
336. **Surmacz E, Bartucci M** 2004 Role of estrogen receptor α in modulating IGF-I receptor signaling and function in breast cancer. J Exp Clin Cancer Res 23:385–394
337. **Mur C, Valverde AM, Kahn CR, Benito M** 2002 Increased insulin sensitivity in IGF-I receptor-deficient brown adipocytes. Diabetes 51:743–754