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Comprehensive Invited Review

The Insulin-Like Growth Factor Family: Molecular Mechanisms, Redox Regulation, and Clinical Implications

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Abstract

Insulin-like growth factor (IGF)-induced signaling networks are vital in modulating multiple fundamental cellular processes, such as cell growth, survival, proliferation, and differentiation. Aberrations in the generation or action of IGF have been suggested to play an important role in several pathological conditions, including metabolic disorders, neurodegenerative diseases, and multiple types of cancer. Yet the exact mechanism involved in the pathogenesis of these diseases by IGFs remains obscure. Redox pathways involving reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to the pathogenetic mechanism of various diseases by modifying key signaling pathways involved in cell growth, proliferation, survival, and apoptosis. Furthermore, ROS and RNS have been demonstrated to alter IGF production and/or action, and *vice versa*, and thereby have the ability to modulate cellular functions, leading to clinical manifestations of diseases. In this review, we provide an overview on the IGF system and discuss the potential role of IGF-1/IGF-1 receptor and redox pathways in the pathophysiology of several diseases. *Antioxid. Redox Signal.* 11, 1165–1190.

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I. Introduction

The family of insulin-like growth factors (IGFs), composed of insulin, IGF-1, and IGF-2, and their signaling networks, are fundamental for normal fetal and postnatal growth, development, and metabolism in mammals. The effects of IGFs are modulated and affected by multiple factors, such as insulin-like growth factor-binding proteins (IGFBPs) (13), reactive oxygen species (ROS) (76), and reactive nitrogen species (RNS) (51). Alterations in IGF levels and/or IGF-1-induced signaling networks have been associated with multiple pathological conditions. For example, circulating IGF-1 levels have been suggested as important physiological regulators of brain amyloid (involved in the pathogenesis of Alzheimer's disease) (47), and a reduction in IGF signaling is associated with a longer lifespan, as well as augmented stress resistance in invertebrates and mammals (148).

In addition, multiple investigations have established a connection between high serum concentrations of IGF-1 and IGFBP-3, with increased risk of prostate, colorectal, and lung cancer (214), and elevated IGF-2 levels with heightened risk of colorectal cancer (213). Moreover, high circulating IGF-1 levels have been suggested to confer a greater breast cancer risk in premenopausal women, with a similar effect on prostate cancer (52). Furthermore, multiple studies have revealed that IGF-1 receptor (IGF-1R) is overexpressed in cancer cells, compared to cells from normal tissues (243, 281). Although some studies have reported a decreased expression of IGF-1R in some tumors (234, 253), a majority of reports have demonstrated an enhanced expression of IGF-1R in a variety of tumors (126), as well as in contributing to a metastatic phenotype in some experimental models (165). In addition, an aberration of IGF-1 function has been attributed to decreased insulin sensitivity and diabetes (60), and, consequently, improvements in patients suffering from diabetes mellitus, with severe insulin resistance, as well as Rabson Mendenhall syndrome, have been achieved with recombinant human IGF-1 (235, 262).

The precise mechanism by which ROS contribute to pathophysiological states remains unclear. Nonetheless, many studies with H_2O_2 and ROS metabolites have suggested that aberrant activation of several growth-promoting and proliferating signaling pathways by these agents may play an important part in this process (22, 23, 178). Recent studies have also demonstrated that ROS generation is critical to trigger the signaling responses of IGF-1 in vascular smooth muscle cells (VSMCs) (180). In fact, an involvement of NAD(P)H oxidase NOX4 in IGF-1-induced generation of superoxide anion in VSMCs was also demonstrated recently (180). In addition, transactivation of IGF-1R has been suggested to mediate the downstream effects of H_2O_2 (6, 7). There are also reports that vasoacative peptides, such as angiotensin II (Ang II), activate NAD(P)H oxidase via IGF-1R protein tyrosine kinase (PTK) activity. IGF-1 has been shown to stimulate endothelial nitric oxide synthase (eNOS) activity and/or expression in many cell types (208, 215, 250). In addition, NO was also found to attenuate some of the migratory and growth promoting effects of IGF-1 in VSMC (51, 289), suggesting that in addition to ROS, the NO system also plays an important role in modulating the action of IGF-1. Since aberrant IGF-1-induced signaling pathways and ROS/RNS systems have recently been implicated in multiple pathological states, in this review, we will focus on the crucial role of IGF and ROS/RNS signaling in various pathophysiological states.

II. IGFs: Structure, Synthesis, and Regulation

IGFs, also known as somatomedins, consist of a family of peptides with high sequence similarity to insulin, that are central in mammalian growth and development. IGFs are part of a complex system that the cell needs to communicate with its physiological environment. This complex system includes two ligands (IGF-1 and IGF-2), three cell surface receptors (insulin receptor (IR), IGF-1R, and IGF-2R), a family of six high affinity binding proteins (IGFBP1-6), and IGFBP-degrading proteases.

IGF-1 (somatomedin C) is a single chain polypeptide of 70 amino acids (74, 169). It is a trophic factor that circulates at high levels in the bloodstream (150–400 ng/ml in plasma), yet only a small fraction of that amount (1%) is found in its unbound active form (61). IGF-1 synthesis is stimulated by binding of GH, released by the pituitary gland, to its receptor located on multiple tissues (69). Whereas IGF-1 is mainly secreted by the liver, most other tissues, known as peripheral tissues, including bone, cartilage, and skeletal muscle, also synthesize it, albeit in low concentrations. This peripheral IGF-1 acts through both autocrine and paracrine means to regulate cell growth of these tissues (141), and contributes to normal somatic growth, because shutting down liver-specific IGF-1 synthesis in mice decreases serum IGF-1 by 80%, yet the total adult size of these mice is reduced by $< 10\%$, whereas total IGF-1 gene deletion causes a 70% diminution in adult size, compared to control animals (reviewed in ref. 60).

Mature IGF-1 peptide has four domains (Fig. 1). The first 29 residues are homologous to the B chain of insulin (B region, 1–29), followed by 12 residues that are analogous to the C peptide of proinsulin (C region, 30–41), and a 21-residue region that is homologous to the A chain of insulin (A region, 42–62). The carboxyl terminal octapeptide (D region, 36–70) has no complement to insulin (74). It is produced as pro-IGF-1, containing an additional carboxyl terminal E region, which may have limited biological tissue-specific functions. Many studies have demonstrated that the structure of the IGF-1 gene is very complex. The IGF-1 gene is on the long arm of chromosome 12q23 (30, 74). The human IGF-1 gene consists of six exons, with two leader exons and two promoters (74, 169, 220). Human IGF-1 is a single copy gene with two precursor peptides, the 153-amino acid IGF-1A and the 195-amino acid IGF-1B (221). These two peptides are synthesized from two separate mRNAs produced by alternative splicing of the primary gene transcript. IGF-1A consist of exons 1, 2, 3, 4, and 6, while IGF-1B is encoded by the set of 1, 2, 3, 4, and 5 exons (221).

IGF-2 (somatomedin A) displays nearly 50% homology with insulin and 70% homology with IGF-1 (200). The human IGF-2 gene is found on chromosome 11p15, and is adjacent to the insulin gene (30). The 180-amino acid IGF-2 preprohorm1 contains a carboxy-terminal peptide of 89 amino acids and a signal peptide of 24 amino acids, both of which are cleaved post-translationaly to produce a 67-amino acid plasma protein (200) (Fig. 1). The latter is thought to be important in fetal and placental development, having mitogenic effects through its interaction with IGF-2R (200).

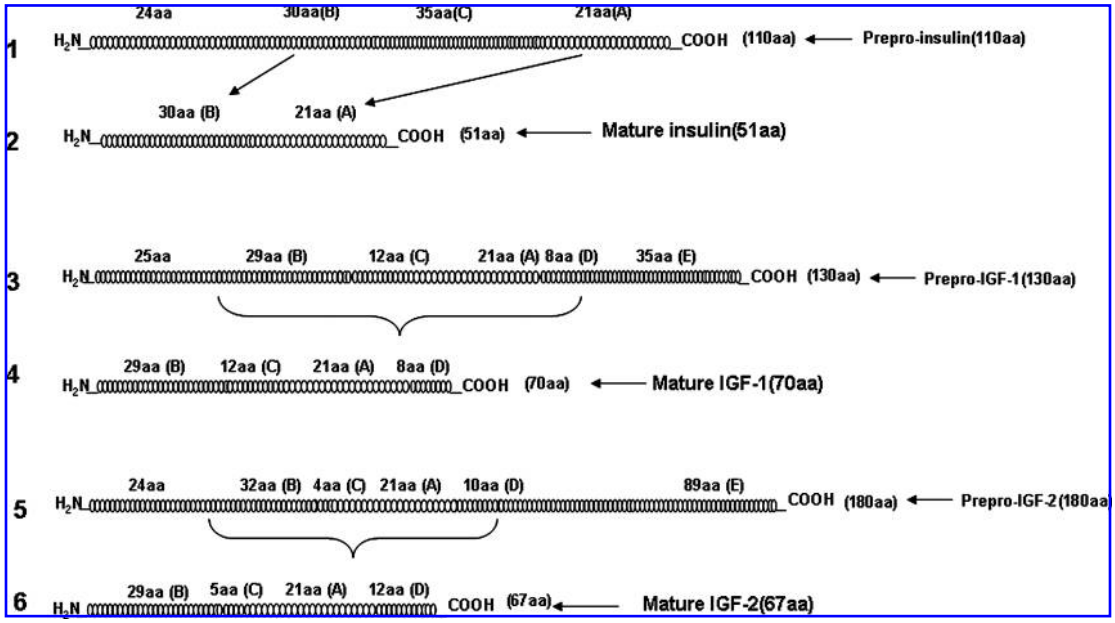

FIG. 1. Schematic representation of insulin, IGF-1 and IGF-2 as pro- and mature peptides. The active insulin molecule is comprised of two polypeptide domains; domain A containing 21 amino acids, and domain B containing 30 amino acids. The mature insulin molecule is produced by proteolytic cleavage of the 110-amino acid prepro-insulin (**1**; **2**). Both IGF-1 (70 amino acids) and IGF-2 (67 amino acids) are highly homologous small single chain peptides derived from prepro-IGF-1 (130 amino acids) and -2 (180 amino acids), respectively (**3**; **5**) and are $\sim$7.5 kDa in size. Prepro-IGF-1 and -2 are both 70% identical to each other and 50% identical to pro-insulin, mostly due to their B domains (residues 3–29 in IGF-1 and residues 3–32 in IGF-2). Mature IGF-1 is organized into four peptide domains; A (21 amino acids), B (29 amino acids), C (12 amino acids), and D (8 amino acids) (**4**). Domains A and B are similar in structure to those of mature insulin. Mature IGF-2 is also a single polypeptide composed of A and B domains, homologous to matuare insulin and IGF-1, and a 12-amino acid D domain (**6**). Both IGF-1 and IGF-2 C-terminal E peptides are cleaved before secretion.

III. IGFBPs

IGFBPs are a family of proteins which regulate IGF-1 and IGF-2 in a multifaceted way, including IGF inhibition through IGF-IGFBP complex formation, not allowing IGF to bind to the receptor, and promoting action by increasing the half-life of IGFs (Fig. 2). IGFs are found in the circulation and cellular environment primarily in bound form with IGFBPs. In normal subjects, 99% of circulating IGF-1 occurs in bound form (101). At present, six IGF-binding proteins, numbered IGFBP-1 to IGFBP-6, have been characterized (13). IGFBPs share sequence homology and are likely to have closely related IGF-binding sites. All six IGFBPs can be divided into three separate domains: N-terminal, C-terminal, and the central domain. The N- and C-terminal domains, both cysteine-rich, are thought to participate in IGF binding (98). The central domain of all six IGFBPs has an exclusive sequence and is believed to be linked between the N- and C-terminal regions. This central domain serves as the site for most post-translational modifications and proteolysis (37), as well as for associating with the acid labile subunit (ALS) and extracellular matrix (98). Several mutagenesis studies have disclosed that the N-terminal region of IGFBP-3 and -5 serves as a binding site for IGFs (36, 131). In comparison to the N-terminal region, the role of the C-terminal region in IGF-binding is not very clear (131). Mutations in both the N- and C-terminal regions of IGFBP-1 or -5 lead to the total loss of their ability to bind IGF-1 (240). Also, when the mutation is performed exclusively in the N-terminal region of IGFBP-3 and -5, IGF-binding is totally eliminated (36). These studies indicate that basal IGF-binding does not require the C-terminal region, yet the high affinity binding of IGFBPs to IGFs warrants significant involvement of the C-terminal region (283).

In tissues, IGFBPs are present predominantly in IGF-IGFBPs binary complexes, where IGF-1 or IGF-2 is bound to the different types of IGFBPs. In the circulation, IGFBPs exist as ternary complexes, composed of IGF-1 and IGF-2, bound primarily to IGFBP-3 and, to a much lesser extent, to IGFBP-5, and the 85-kDa glycoprotein ALS, forming 150-kDa complexes (13, 259). These complexes significantly enhance the circulating half-life of IGF-1, from $<$ 15 min up to 16 h (60), as they cannot cross the capillary endothelial barrier, and therefore stay in the circulation. In comparison to ternary complexes, plasma also contains complexes composed of the remaining IGFBPs (IGFBP-1, -2, -4, and -6) bound in an unsaturated manner to IGFs, forming 50-kDa binary complexes capable of crossing the endothelium and leaving the circulation faster, granting IGFs an albeit short, yet extended half-life of only 30–90 min (25, 118). In the cellular environment, when IGFs are in a ternary complex with IGFBP-3, -5, and ALS, they are unable to initiate IGF-1R-dependent signaling pathways, as it was shown that injection of the ternary complex of IGF-1 causes none of the insulin-like effects of IGF-1 in normal mice (287). For binary complexes to reach tissue, cleavage of IGFBPs is not necessary, yet the dissociation of ALS is important in ternary complexes. The mechanism of ternary complex dissociation is not very clear, but it may involve the interaction of these complexes with the endothelial

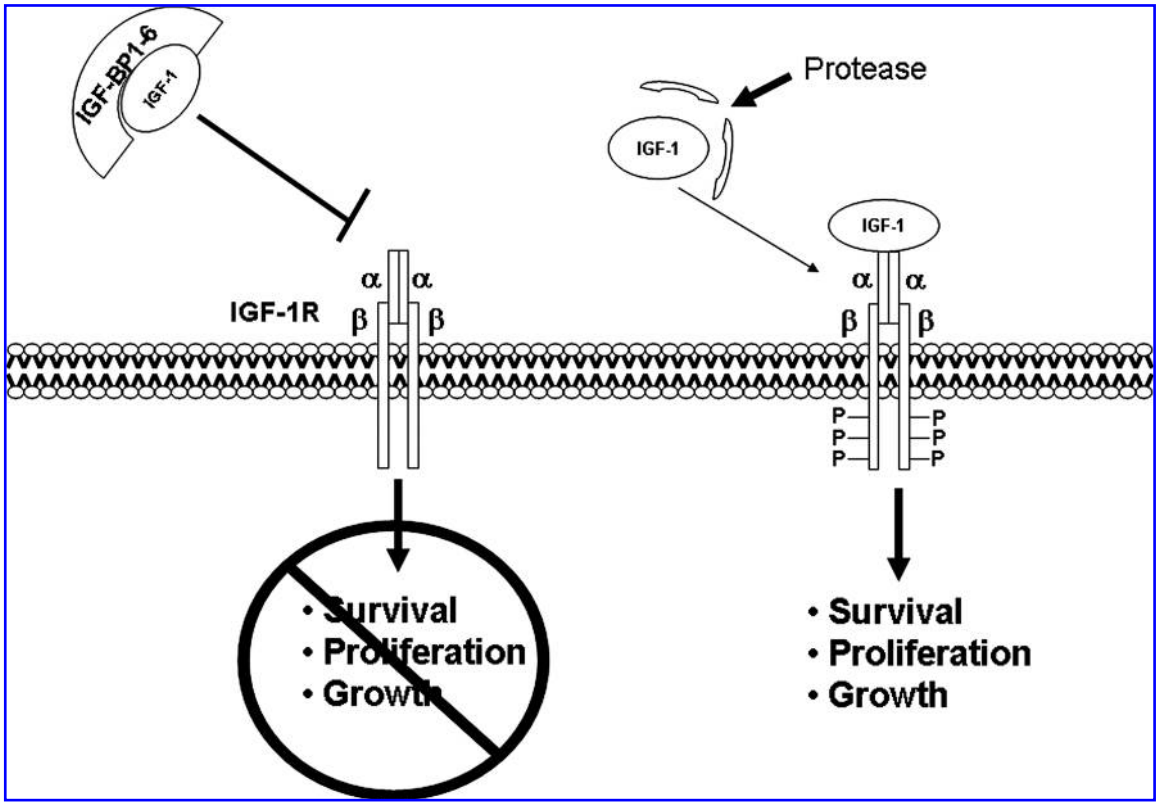

FIG. 2. Modulation of IGF-1 by IGFBPs. By binding to IGF, IGFBP 1-6 reduce free bioactive IGF-1 levels and decrease IGF-1R activation, consequently inhibiting cellular responses. IGF-1 binding to IGFBPs can be reversed through the action of proteases, which can cleave them into fragments, thus increasing free IGF-1, since fragmented IGFBPs have lower affinity for IGFs. IGF-1 binding and subsequent activation of the IGF-1R pathways are thus capable of leading to physiological responses, such as growth, proliferation, and survival.

cell surface and the amino acid sequence that interacts with ALS, as these sequences in IGFBP-3 are also responsible for cell surface binding (99). Glycosaminoglycans on the endothelial cell surface can act as receptors, binding ALS, and dissociating it from the ternary complex, thus allowing the smaller IGF–IGFBP complex to bind onto its proper receptor (12). Furthermore, proteases secreted by certain cells have also been observed to cleave IGFBPs, releasing IGFs into the circulation, allowing them to exert their metabolic and growth-promoting effects subsequent to interaction with the receptor (227) (Fig. 2).

IV. IGF-1R

IGF-1 acts in many ways to control diverse specific functions in cells, such as mitogenesis, substrate uptake, metabolic activity, and apoptosis. IGF-1 mediates its effect by binding and activating IGF-1R and IR. IGF-1R is a transmembrane protein, which shares homology with IR. It is a tetrameric protein consisting of two α- and two β-subunits, and belongs to a large family of receptor-protein tyrosine kinases (R-PTK) (133) that is activated by IGF-1 and associated growth factors, such as IGF-2, albeit with lower affinity. IR/IGF-1R differ from other R-PTKs in that they exist on the cell surface as covalent dimeric structures and require domain rearrangement for activation (261), whereas other R-PTKs dimerize or oligomerize on ligand binding to trigger receptor activation (125). The α-subunit of IGF-1R contains the IGF-1-binding site, whereas the β-subunit comprises an intracellular PTK domain that is critical for transducing most of the downstream signaling (Fig. 3). The receptor is synthesized as a single chain preproreceptor consisting of 30 residue single peptide, and is cleaved post-translationally to pro-receptor. The pro-receptor is glycosylated, folded, and dimerized with the help of chaperones, calnexin and calreticulin (11), before being transported to the Golgi apparatus, where it is processed at the tetrabasic Arg-Lys-Arg-Arg (R-K-R-R) furin protease cleavage site (amino acids 708–711) to yield the α-chain (1–707 residue) and β-chain (712–1337 residues). The α-chain and the 195 residues of the β-chain make up the extracellular part of the IGF-1R, which consists of 11 potential N-linked glycosylation sites for the α-chain, and 5 for the β-chain (261). The mature $\alpha 2\beta 2$ receptor also has a single transmembrane sequence (906–929 residues) and a 408-residue cytoplasmic domain, which possesses tyrosine kinase activity (Fig. 3). The N-terminal half of the IGF-1R also contains two homology domains, L1 and L2, separated by the Cys-rich region (Cys148 to Cys298). It has been shown that residues 131–315 (Cys-rich and L1 and L2 flanking regions) are required for binding IGF-1 (271). The C-terminal domain of the IGF-1R has three fibronectin-type domains (FnIII) (9, 175). The FnIII-1 domain is from residues 461–579, FnIII-2 is from 580–798, and Fn-III is from 799–901 in IGF-1R (Fig. 3). The key function of these domains is to mediate protein–protein interactions, which include ligand binding, but they also serve as spacers to place the functional domains appropriately. Each IGF-1R monomer contains an intracellular tyrosine kinase catalytic domain

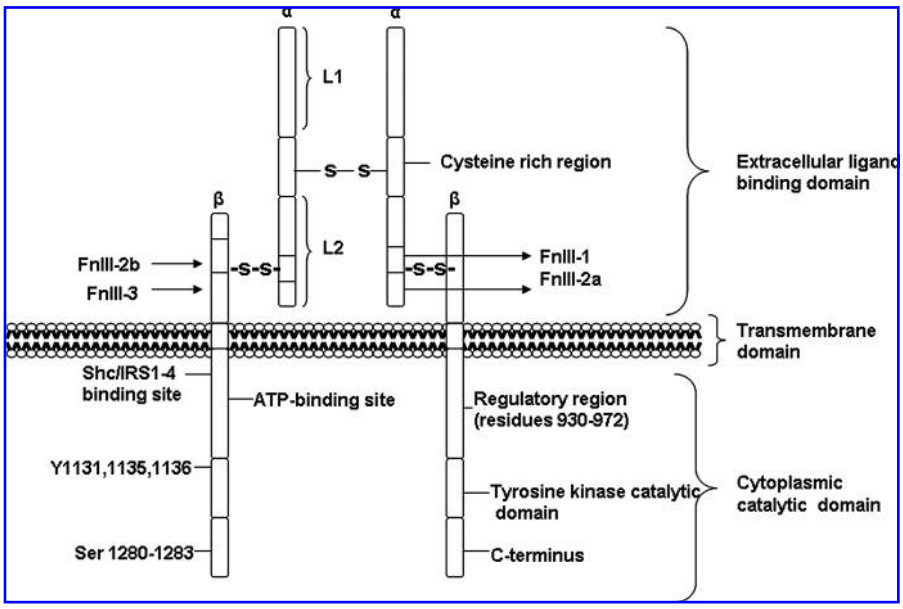

FIG. 3. Schematic representation IGF-1R structure. IGF-1R is a transmembrane receptor consisting of 2 α- and 2 β-subunits. The extracellular α-subunits contain IGF binding domains. The β-subunits are linked to the α-subunits by disulfide bonds, and are transmembrane proteins. The β-subunits also possess tyrosine kinase catalytic domains that are activated upon ligand binding to the α-subunits. The N-terminal half of the IGF-1R ectodomains contain 2 homologous L1 and L2 domains, separated by a Cys-rich region. The C-terminal half of the IGF-1R ectodomains consists of three fibronectin type III (FnIII) domains. The intracellular regions of IGF-1R contain tyrosine kinase catalytic domains flanked by a 42-residue (930–972) regulatory domain, containing Shc/IRS1-4 binding sites, and a 108-residue C-terminal domain, containing serine sites 1280–1283, important for the anti-apoptotic effects of IGF-1 signaling. The tyrosine kinase domain contains tyrosine sites Y1131, Y1135 and Y1136, which, similarly to IR, are autophosphorylated and may have physiological effects similar to those of IR.

(973–1255 residues). The three tyrosine (Y1131, Y1135, and Y1136) sites in the IGF-1R kinase domain are homologous to those of the IR kinase domain, and phosphorylation of these sites contributes to IGF-1R activation (116). This tyrosine kinase domain is flanked by two regulatory regions: a juxtamembrane region that plays a major role in docking of IR substrates (IRSs), Shc, and receptor internalization (73), and a 108-residue long carboxy-terminal tail consisting of two phosphotyrosine-binding sites (2, 73). Ligands for IGF-1 (IGF-1, IGF-2, and insulin) are able to bind to IGF-1R in a competitive manner with varying affinities. Insulin has a 100-fold lower affinity for binding with IGF-1R than IGF-1, whereas IGF-2 has only 6- to 8-fold lower affinity to bind IGF-1R.

In addition to IGF-1R, IGF-2 has its own receptor, IGF-2R. IGF-2R is identical to the mannose 6-phosphate receptor, in that it is a transmembrane glycoprotein consisting of 4 structural domains (the amino-terminal signal sequence, the extracytoplasmic domain, the transmembrane region, and the carboxy-terminal cytoplasmic tail), and has no PTK activity (184). Insulin cannot bind to IGF-2R, but IGF-1 can, albeit with a much lower affinity than IGF-2 (124). It is thought that the ability of IGF-2 to bind to IR or IGF-1R, and not its interaction with IGF-2R, is responsible for its growth-promoting effects (68, 103, 149). IGF-2R has been implicated in the internalization and degradation of IGF-2, therefore decreasing its potential mitogenic effects (200). IGF-2R can also act as an IGFBP for IGF-2, as a cleaved form of IGF-2R has been found in the circulation (106).

V. Mechanism of Activated IGF-1R-Induced Signaling Pathways

IGF-1-binding to extracellular α-subunits triggers a conformational change in the β-subunit, resulting in its trans-autophosphorylation in multiple tyrosine residues (*e.g.*, Y1131, Y1135, Y1136) (Fig. 3), and evoking the PTK catalytic activity of the receptor. Activated IGF-1R phosphorylates several downstream substrates, such as Shc and IRSs 1-4, in multiple tyrosine residues (258, 275). Phosphorylated IRSs serve as docking proteins for many Src homology 2 (SH2) domain-containing molecules, including growth factor receptor-binding protein 2 (Grb2), the p85 subunit of phosphatidyl-inositol 3-kinase (PI3-K), NcK, and SH-phosphatase 2 Grb2 binding to the activated receptor, which recruits the son of sevenless (SOS), leading to the subsequent activation of the Ras/Raf/mitogen activated protein kinase (MAPK) pathway (160, 229) (Fig. 4).

MAPKs constitute a family of serine/threonine protein kinases which are widely conserved among eukaryotes, and are involved in many cellular responses, such as cell proliferation, cell differentiation, cell movement, and cell death (156, 237). In mammalian cells, 5 MAPK families have been identified, including ERK-1/2, Jun N-terminal kinase 1, 2, and 3 (JNK1/2/3), also called stress-activated protein kinase (SAPK), p38α/β/γ/δ, ERK5, and ERK7 (156, 237).

The groups of vertebrate MAPK studied most extensively to date are ERK-1/2, JNKs, and p38 kinases (156, 237). ERK-1/2 are stimulated by mitogens, such as polypeptide growth factors (IGF-1, platelet-derived growth factor (PDGF), colony stimulating factor-1 (CSF-1), *etc*.) as well as insulin and phorbol 12-myristate 13-acetate. In contrast, SAPKs and p38 MAPK are potently induced by a wide variety of stresses, including ultraviolet irradiation, gamma irradiation, anisomycin, heat shock, and chemotherapeutic drugs, but not mitogens. These two pathways are also activated by ischemia or reperfusion after ischemia and by inflammatory cytokines (58).

ERK-1/2 are the principal MAPK pathway activated by IGF-1. The mechanism of ERK-1/2 activation starts with signals derived from activated R-PTK to Raf/MEK/ERK through the small guanisine triphosphate (GTP)-binding protein Ras.

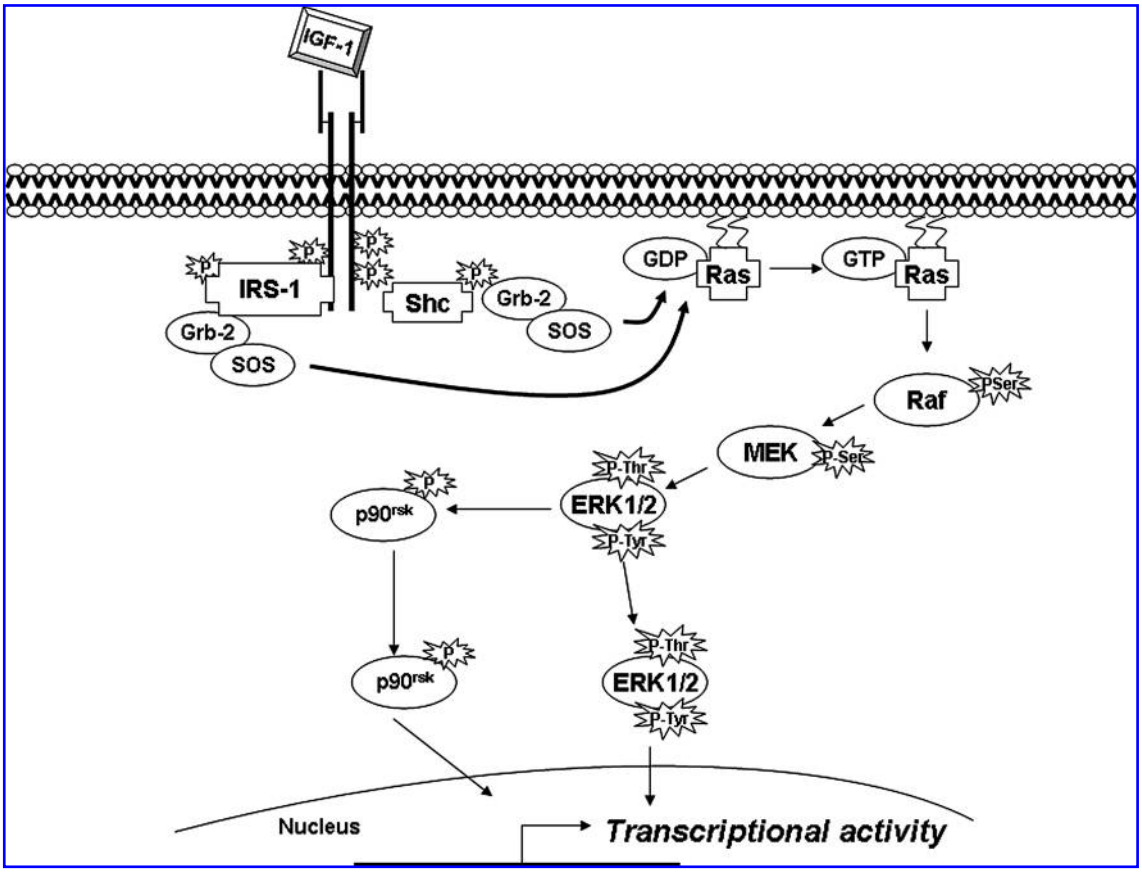

FIG. 4. Schematic diagram showing key steps involved in IGF-1-induced activation of ERK1/2. In response to IGF-1, Shc and/or IRS-1 become phosphorylated in tyrosine residues and bind the Grb2-SOS complex, leading to p21-Ras stimulation, with subsequent activation of Raf, MEK1/2, and two isozymic forms of MAPK, ERK1, and ERK2. Both ERK1/2 and other transcription factors phosphorylated by ERK1/2, such as $p90^{rsk}$, can be translocated to the nucleus, activating transcription and other cellular processes.

Ras is a 21-kDa molecular weight protein associated with the cytoplasmic face of the plasma membrane by a farnesyl group (45). The protein serves as a key "molecular switch" in cytoplasmic signaling pathways triggered by the activation of various membrane receptors. In its inactive state, in unstimulated cells, Ras is found to be linked with guanosine diphosphate (GDP) (Ras-GDP). After the activation of IGF-1R and its substrates (*e.g.*, Shc, IRSs), Grb-2 associates with guanine nucleotide exchange factors, such as SOS, causing GDP release, and GTP binding to Ras (forming Ras-GTP) (Fig. 4). This binding is accompanied by a conformational change in Ras, allowing it to bind to a wide range of downstream effector proteins, including isoforms of the Ser/Thr kinase Raf. Ras-bound active Raf phosphorylates the dual specificity protein kinases MEK-1 and -2, which, in turn, phosphorylate ERK-1/2 in Thr and Tyr within a conserved Thr-Glu-Tyr (TEY) motif in their activation loop. Once activated, ERK-1/2 can stimulate a number of cytosolic proteins, such as p90rsk through its proline-directed Ser/Thr kinase activity, or it can also translocate to the nucleus where it phosphorylates and activates a number of transcription factors implicated in immediate early gene transcription (53, 156) (Fig. 4).

The second pathway that radiates from the IRS complex upon IGF-1 stimulation involves PI3-K activation (275, 276). When bound to IRS-1, the p85 subunit of PI3-K activates the p110 catalytic subunit, which catalyzes the phosphorylation of phosphatidyl-inositol (PI) lipids at position 3 of the inositol ring, and generates 3-phosphorylated forms of PI, such as phosphatidylinositol 3, 4, 5 triphosphate (PIP3) (146, 241). Binding of PIP3 to the plekstrin homology (PH) domain of protein kinase B/Akt (PKB/Akt) results in its phosphorylation on Thr 308 and Ser 473 by two other PH domain-containing phospholipids-dependent kinases (PDK-1/2), respectively (46). PKB/Akt has multiple downstream targets, such as mammalian target of rapamycin (mTOR), p70 ribosomal S6 kinase ($p70^{s6k}$), forkhead box proteins (FOXO), and glycogen synthase kinase-3 (GSK-3) (Fig. 5). These downstream targets of PKB regulate multiple physiological functions, including metabolism, gene expression, protein synthesis, cell cycle, survival, and death (174, 228).

Many technical approaches, such as yeast 2-hybrid, fusion protein pulldown, and *in vitro* phosphorylation, have been used to explain how signaling effector molecules activated by IGF-1 stimulation can interact directly with the C-terminal domain of IGF-1R in an SH2-dependent manner. This includes the p85 subunit of PI3-K and Syp/SH-PTP2 (protein phosphatase 2) binding to p-Tyr1316. The guanine triphosphatase (GTPase)-activating protein GAP binds to p-Tyr950 (158, 236), the adapter SH2-B binds to p-Tyr950 and p-Tyr1316, and the C-terminal Src-kinase (CSK) binds to

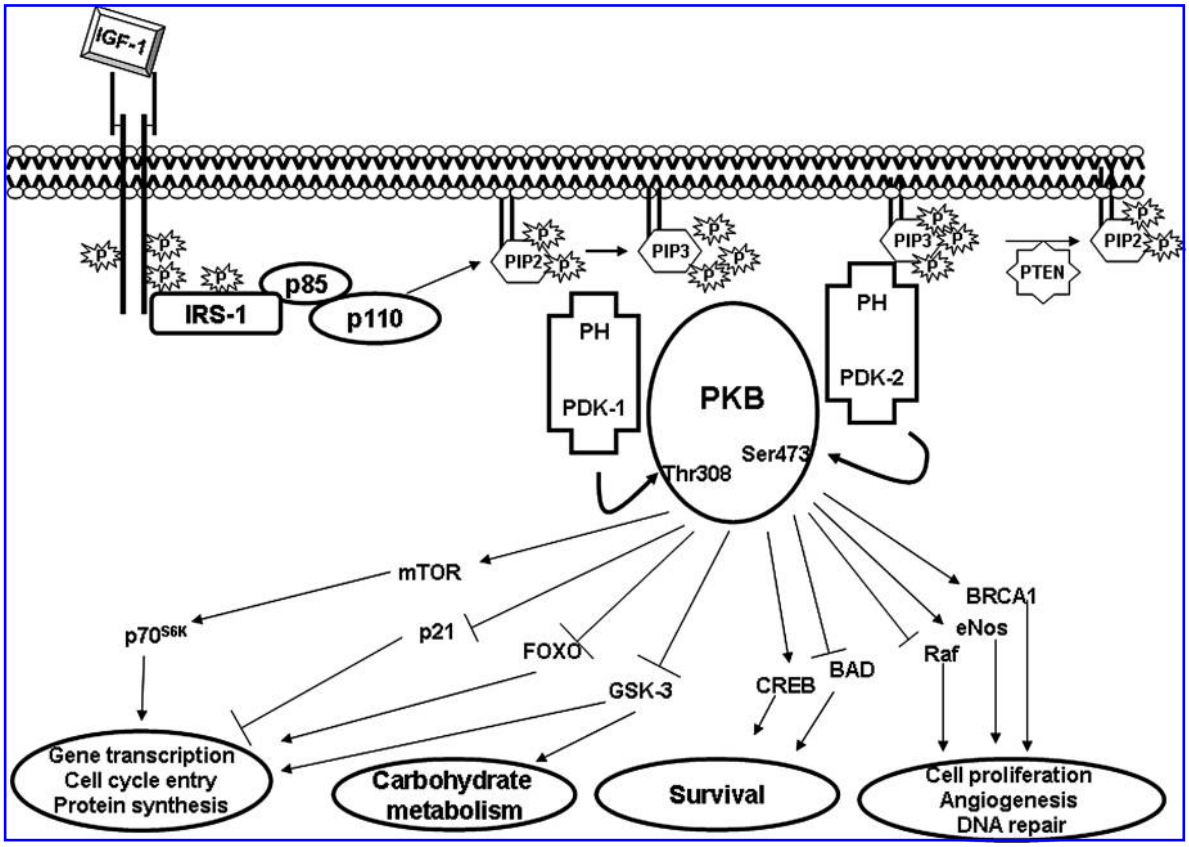

FIG. 5. Schematic representation of IGF-1 signaling of PKB activation and its physiological role. Phosphorylated IRS-1 recruits PI3-K, which catalyzes PIP2 phosphorylation, leading to PIP3 formation. PIP3 recruits PH domain-containing proteins to the plasma membrane, including PDK-1/2 and PKB, where PKB is phosphorylated and activated, exerting its physiological effects through phosphorylation of multiple downstream substrates, including other protein kinases, as well as transcription factors. PTEN, a lipid phosphatase, dephosphorylates PIP3 to PIP2 and thus inhibits PKB activation.

p-Tyr943 and p-Tyr1316 (5). Some additional proteins, such as Grb10, can bind directly to the cytoplasmic domain of the activated receptor (81). Furthermore, protein 14-3-3 links IGF-1R to many signaling pathways. This protein is comprised of three isoforms, β, ε, and ζ. The binding of each isofrom is specific to the phosphorylation of serine residues in the cytoplasmic tail of IGF-1R. Isoforms β and ζ require Ser1283 phosphorylation, while ε requires Ser1272 or Ser1283 phosphorylation to activate multiple cellular responses, including cell progression, vascular trafficking, and defense against cell death (65, 80, 102, 288).

VI. ROS and the IGF-1 System

Evidence has also indicated a role of ROS in IGF-1 synthesis and in transducing its downstream effects (76, 181). Moreover, an involvement of IGF-1R activation in mediating the actions of ROS, such as H_2O_2, has also been documented recently (6), supporting the existence of a cross-talk between ROS and the IGF-1 system.

ROS are formed as intermediates in redox reactions, leading from molecular oxygen (O_2) to water (H_2O). These small, quickly diffusible, and highly reactive molecules are classified into superoxide anion ($^{\cdot}O_2^-$), hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2) (285). A major intracellular source of ROS is the mitochondria, which converts 1%–2% of consumed O_2 to $^{\cdot}O_2^-$ (27). A univalent reduction of O_2 leads to $^{\cdot}O_2^-$, which is relatively unstable and short-lived because of its unpaired electron (Fig. 6).

NAD(P)H oxidase is among the primary enzymes responsible for the generation of $^{\cdot}O_2^-$ (15), and is composed of many subunits, including p22phox, p47phox, gp91phox, the GTPase Rac, and the recently identified Nox1 and Nox4 (115, 120, 128, 157). NAD(P)H oxidase catalyzes $^{\cdot}O_2^-$ production by the one electron reduction of O_2, where NAD(P)H is the electron donor. In addition to NAD(P)H oxidases, O_2^- can also be generated by xanthine/xanthine oxidase, lipooxygenase, and cyclooxygenase (82, 86) (Fig. 6).

Under physiological conditions, $^{\cdot}O_2^-$ undergoes dismutation either spontaneously or by a reaction catalyzed by superoxide dismutase (SOD) to produce H_2O_2. Dismutation of $^{\cdot}O_2^-$ by SOD is favored at low concentrations of $^{\cdot}O_2^-$ and at high concentrations of SOD, which occurs under physiological conditions. H_2O_2 is much more stable than $^{\cdot}O_2^-$, can cross cell membranes, and has a longer half-life. Normally, it is scavenged by catalase and glutathione peroxidase to produce H_2O (232) (Fig. 6). In the presence of metal-containing molecules such as Fe^{2+}, H_2O_2 can also be reduced to generate the extremely active hydroxyl radical ($^{\cdot}OH$) that causes damage to cell components (100). In the glutathione peroxidase reaction, glutathione (GSH) is oxidized to glutathione disulfide (GSSG), which can be converted back to GSH by glutathione

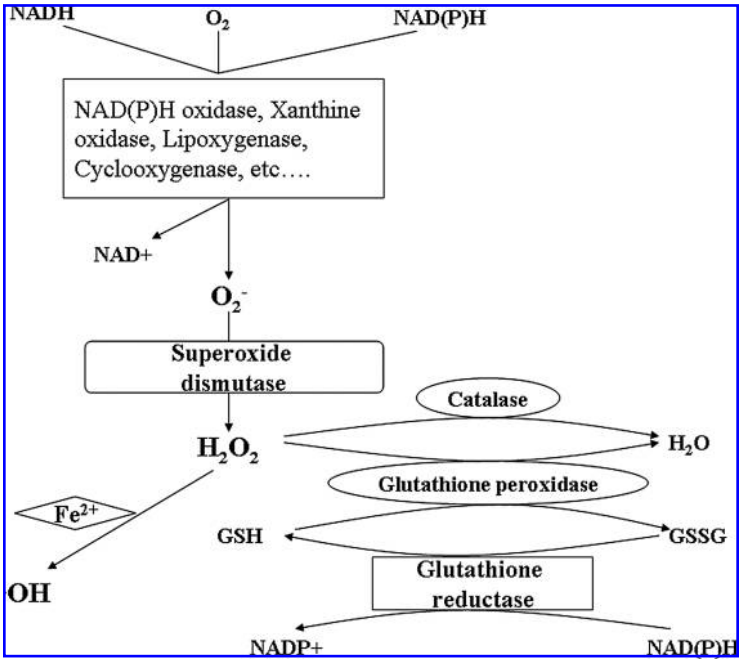

FIG. 6. Production and elimination of $\cdot O_2^-$ and H_2O_2. O_2 is converted to $\cdot O_2^-$ by NAD(P)H oxidase. $\cdot O_2^-$ can then undergo dismutation by SOD to produce H_2O_2. H_2O_2 is scavenged by catalase or glutathione peroxidase to produce water (H_2O) while in the same reaction; glutathione (GSH) is converted to glutathine disulfide (GSSG), which can be converted back to GSH by glutathine peroxidase. In presence of metals, H_2O_2 can be converted to hydroxyl anion ($\cdot OH$).

reductase in a NAD(P)H-consuming process. Several forms of SOD are known: copper-zinc SOD (Cu/Zn-SOD), mitochondrial or manganese SOD (Mn-SOD), extracellular SOD type C (EC SOD C) and iron-containing SOD (Fe-SOD) (1, 136) (Fig. 6). Normally, the rate of ROS production is balanced by the rate of their elimination. However, in pathological conditions, a disequilibrium between ROS generation and elimination results in increased ROS bioavailability, leading to oxidative stress (286). Under these conditions, $\cdot O_2^-$ can react with nitric oxide (NO) to produce peroxynitrite ONOO- (71) (Fig. 7), which is reduced to form its conjugate acid, peroxynitrous acid (ONOOH), a reactive and unstable oxidizing species. NO is also implicated in the production of nitrite (NO_2^-) that can be oxidized to produce nitrogen dioxide (NO_2), a nitrogen intermediate, which along with an $\cdot OH$ group forms nitrate (NO_3^-) (Fig. 7). $ONOO^-$ and its metabolites are capable of tyrosine nitration in multiple proteins, evoking changes in their conformation, structure, and catalytic activity (19).

Studies showing that treatment of vascular smooth muscle cells (VSMCs) with H_2O_2 enhanced the synthesis of IGF-1 and reduced the levels of IGFBP4 (79) suggest that ROS-induced oxidative stress can potentially upregulate IGF-1 action in key target tissues. Further support for a role of ROS in modulating IGF-1 function has been provided by reports where Ang II was shown to enhance the expression of IGF-1R through ROS-dependant mechanism (87).

A potential role of IGF-1R transactivation in triggering Ang II-induced generation of ROS has been proposed by studies in which inhibition of IGF-1R-PTK activity blocked the formation of ROS in response to Ang II (66). In these experiments, IGF-1R blockade also inhibited the activity of NAD(P)H oxidase in VSMCs (66). H_2O_2 treatment was also found to induce the Tyr phosphorylation of IGF-1R in VSMC, and pharmacological inhibition of IGF-1R-PTK attenuated the stimulatory effect of H_2O_2 on phosphorylation of ERK-1/2 and PKB in VSMC (6, 7). Furthermore, IGF-1R activation has also been shown to induce ROS generation through the activation of NOX4 in VSMCs (181). Thus, it appears that ROS generation is essential to trigger IGF-1-evoked signaling events, and ROS-induced oxidative stress is capable of upregulating IGF-1 action by enhancing the expression of IGF-1 and IGF-1R, especially in VSMC (76, 88, 171).

VII. RNS

NO, a small molecule containing an unpaired electron in the orbit, is produced by specific nitric oxide synthases (NOS). NOS catalyze the oxidation of one of the guanido terminal nitrogen atoms of L-arginine, whereby the precursor, arginine is metabolized to citrulline in a five-electron oxidative reaction to produce NO (108, 203) (Fig. 7).

Physiological NO levels are generated primarily by the two constitutively-active forms of NOS, endothelial NOS (eNOS or NOS3) and neuronal NOS (nNOS or NOS1), which are activated by an increase in intracellular Ca^{2+}. nNOS is found in multiple tissues, such as skeletal and cardiac muscles, neuronal cells, endothelial tissue, and macrophages (28, 56, 70), while in addition to endothelial tissue, eNOS also occurs in neurons, cardiac myocytes and blood platelets, erythrocytes, and leukocytes (72, 92, 151, 166). Inducible NOS (iNOS or NOS2) plays an important role in mediating host-inflammatory responses, since the expression of this isoform is evoked by pro-inflammatory cytokines and is Ca^{2+} independent (245).

NO is a chemically active molecule, primarily bound to metals at the regulatory site of guanylate cyclase and in hemoglobin. Activation of guanylate cyclase produces cyclic guanosine monophosphate (cGMP), which, in turn, activates cGMP-dependent protein kinase G (PKG), modulating intracellular calcium levels, and initiating multiple reactions in different target tissues. NO serves as an important signaling molecule, involved in many physiological processes, for example, neurotransmission, blood pressure regulation, smooth muscle relaxation, and immune control (18). The requirement

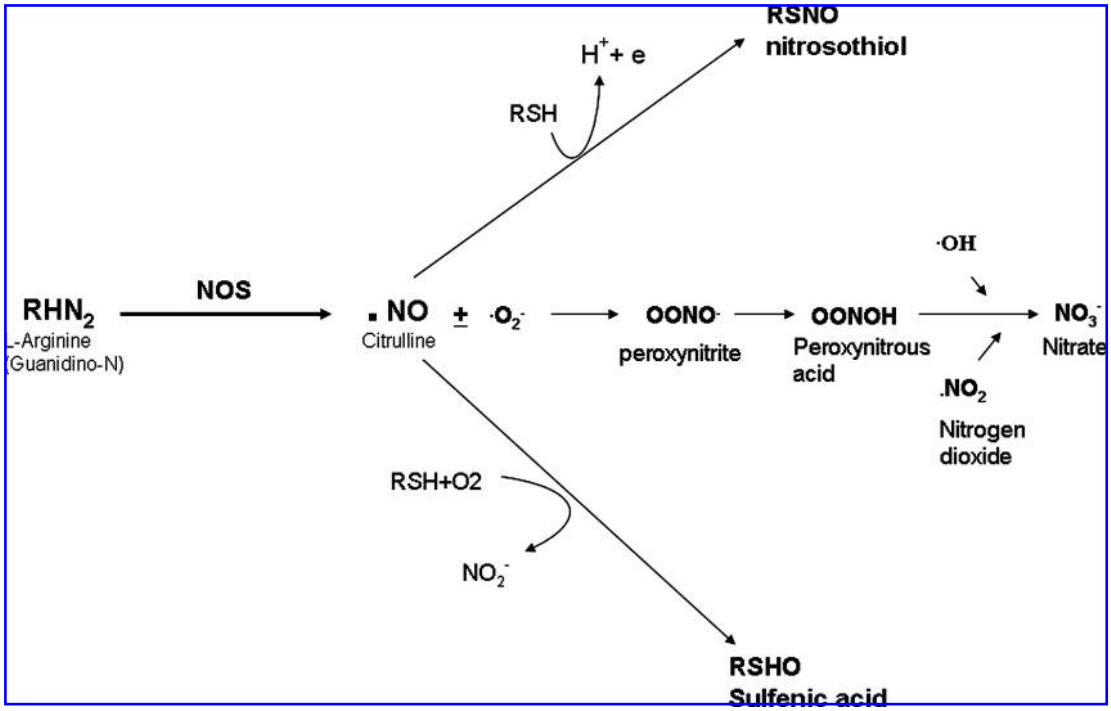

FIG. 7. Pathway of RNS generation. NO is formed from the guanidine nitrogen of L-arginine, via NOS. When exposed to strong oxidizing agents (*e.g.*, NO) thiols (RSH) are converted to sulfenic acid through disulfide intermediates. In addition, thiols of redox proteins may also be oxidized by RNS to form nitrosated species, such as nitrosothiol (RNSOx). Furthermore, O_2^- interacts with $^{\cdot}NO$ to form peroxynitrite (OONO-), a reaction that is extremely fast (6.7×10^9 mol^{-1} s^{-1}). OONO- is not highly reactive; however, its acid form, peroxynitrous acid (OONOH), is very unstable and reactive, having properties similar to $^{\cdot}OH$ and $^{\cdot}NO_2$, and can react with nitrogen dioxide (NO_2) and $^{\cdot}OH$ to form nitrate (NO_3^-).

of NOS in mediating IGF-1-induced vasodilation has also been suggested (123). This is achieved by PKB-catalyzed phosphorylation of eNOS in Ser 1177, resulting in its activation (250) in response to IGF-1. IGF-1 has also been shown to enhance eNOS expression, as well as NO production in glomeruli and aortic tissues (250,268). In a normal physiological state, NO diffuses quickly through tissues and into red blood cells, and, through its reaction with oxyhemoglobin, is converted to nitrate (41). Depending on the microenvironment, NO can generate other RNS, such as nitrosonium cation (NO+), nitroxyl anion (NO-), and ONOO-, which is formed when NO interacts with O_2^- (246). ONOO- is generally formed when NO and superoxide are formed at the same time, in very close proximity to each other (Fig. 7). $ONOO^-$ and other RNS contribute to the reversible inhibition of several enzymes, including catalase, cytochrome P450, and cytochrome-c oxidase (31, 35, 59, 278). Inhibition of the latter increases the flow of O_2^- from the electron transport chain, which can react with NO to generate more ONOO-, causing irreversible mitochondrial damage (200). Since IGF-1-induced signaling events are able to induce the generation of both ROS and RNS, a fine balance between these two events is critical for the regulation of the physiological effects of IGF-1.

VIII. Redox Signaling

As with key regulatory factors in many diseases, it is important to understand the molecular mechanisms and interactions of ROS/RNS with different redox-responsive signaling components. The key free radicals regulating biological signaling at the redox level are superoxide and NO, which are formed mainly by two classes of enzymes, NAD(P)H oxidase and NOS, respectively. When the delicate concentration-dependent balance is disrupted, free radicals incite a biochemical stress response through the free radical-dependent modification of intracellular proteins, culminating in the activation of signaling pathways involved in cell growth, survival, hypertrophy, proliferation, and apoptosis (86).

As mentioned earlier, ROS/RNS are capable of activating receptor, as well as nonreceptor PTKs, such as Src and Janus Kinase (JAK), which have been shown to play a role in H_2O_2-induced activation of p21Ras (3), as well as several transcription factors, such as NF-κB and activator protein-1, a transcription complex consisting of dimers of Fos-Jun or Jun-Jun proteins (114), leading to cell growth and differentiation by activation of the PI3-K-Akt/PKB signaling pathway. Ang II-mediated MAPK and apoptosis signaling-regulated kinase 1 (ASK1) activation may also be involved (256). ROS may directly target growth factor receptors such as IGF-1R, platelet-derived growth factor receptor (PDGFR), and epidermal growth factor receptor (EGFR) (86), which can induce intracellular ROS generation (8). Receptor tyrosine kinases have been implicated in Ang II signaling by Ang II type 1 receptor (AT_1R)-mediated transactivation (226) (Fig. 8). This transactivation may involve Ang II-mediated NAD(P)H-dependent ROS formation, followed by MAPK activation. Furthermore, transactivation of IGF-1R and EGFR via Ang II has been shown to activate p38 MAPK, ERK5 and ERK1/2 in VSMCs (255). Recent studies have also suggested that IGF-1

signals through ROS-dependent transactivation of EGFR (181) (Fig. 9), and a role of NAD(P)H oxidase 4 (NOX4) in IGF-induced production has been demonstrated in VSMC (180).

In addition to its effects on receptor and nonreceptor PTKs, IGF-1-induced ROS can potentially oxidize and inactivate multiple PTPs *in vitro*, as well as *in vivo* (182). PTPases catalyze the rapid dephosphorylation and inactivation of IR and the IGF-1R β-subunit, and their substrates (110,198), and have the ability to change the tyrosine phosphorylation status of key signaling intermediates involved in transducing growth-promoting proliferative or cell survival signals (216). PTPs can respond to oxidative stresses from the environment, as well as to intracellular ROS, generated in response to physiological activation of growth factor receptors (182).

Because of the ability of IGF-1 to activate signaling pathways that are critical to normal cellular function and the existence of a cross-talk between IGF-1 signaling and ROS/RNS system (6, 7, 51, 79, 87, 180, 181, 289), IGF-1 levels and signaling have been implicated in the clinical manifestations of various pathophysiologies, including cancer, diabetes, obesity, cardiovascular disease, neurological disorders, bone disease, and age-related diseases.

IX. The IGF-1 System in Pathophysiology

A. Cancer

Defects or alterations in the proliferative and/or survival signaling pathways have been implicated in the pathogenesis of malignancies associated with multiple forms of cancers. Since IGF-1-induced signal transduction pathways are involved in cell growth, proliferation, and survival, IGF-1 has been studied extensively in the context of cancer research. Increased IGF-1 plasma levels have been linked to an elevated risk of colon, prostate, and breast cancers, as well as many other types of malignant tumors (117, 201). Conversely, higher serum IGFBP-3 concentrations have been associated with a lower risk of cancers and malignancies, such as colon cancer (170). Moreover, an overexpression of IGF-1R has been demonstrated in cancer cells, as compared to normal cells (117, 201, 281). IGF-1 may augment cell growth, replication, and susceptibility to malignancy. It may also have anti-apoptotic properties, thus preventing cancerous cells from undergoing apoptosis (129). The probable mechanism by which IGF-1 contributes to enhanced survival of cancer cells is by activating the pathways involved in cell survival. Protein families, such as Bcl-2, the caspase family or Apaf, are the main regulators of cell survival. IGF-1-induced activation of the PKB signaling cascade plays an important role in this process by phosphorylating Bcl-2-associated death promother (BAD). Phosphorylated BAD loses the capacity to interact with Bcl-xL or Bcl-2, and thereby contributes to the inhibition of cytochrome c release from mitochondria (75, 289). Studies have also revealed that IGF-1 signaling through PKB promotes cell survival by inhibiting FOXO3, a transcriptional regulator of Bcl-2-interacting mediator of cell death (163).

It has been determined that normal cells show increased proliferation and expression of growth-related genes when exposed to H_2O_2 and superoxide (38), supporting the growing notion that ROS are important factors in carcinogenesis and malignancy (38, 280). Cell growth regulation is a complex process, and therefore the role of ROS depends on the type of radical and its concentration in the cell. It has been demon-

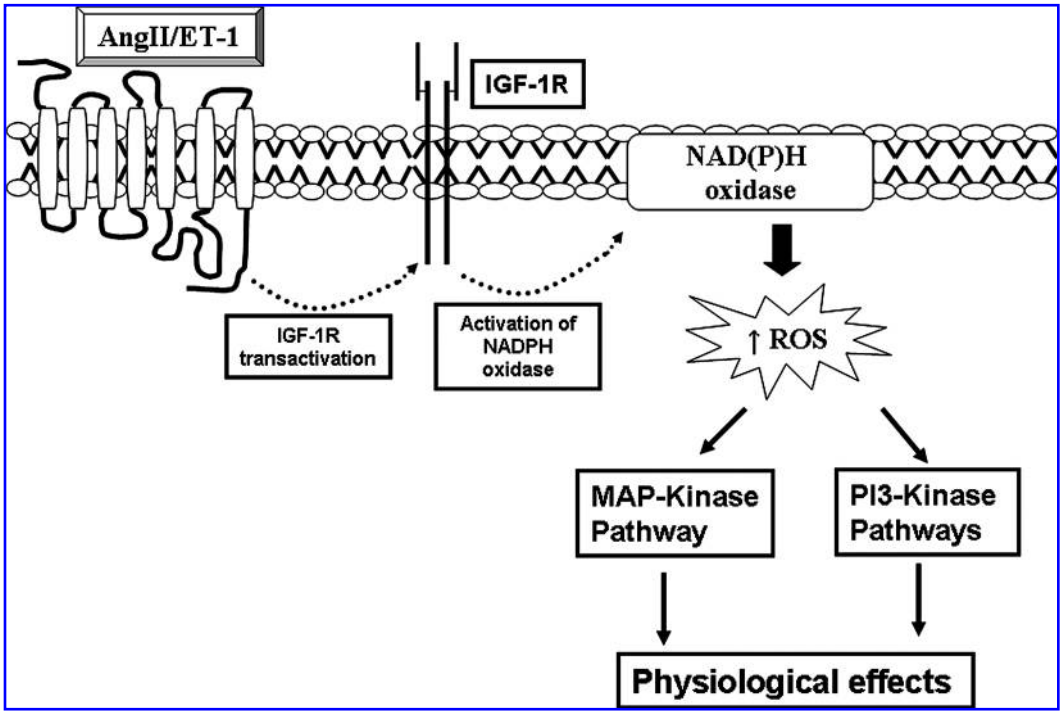

FIG. 8. Regulation of ROS generation by vasoactive peptides. The vasoactive peptides Ang II and endothelin-1 (ET-1) are known to increase ROS generation in many cell types. Ang II and ET-1-induced transactivation of R-PTK, such as IGF-1R, which serve as intermediates to activate NAD(P)H oxidase activity, results in increased levels of cellular ROS. Ros activates multiple signaling pathways, including MAPK, as well as the PI3–K/PKB pathways, which regulate physiological responses in the target cells.

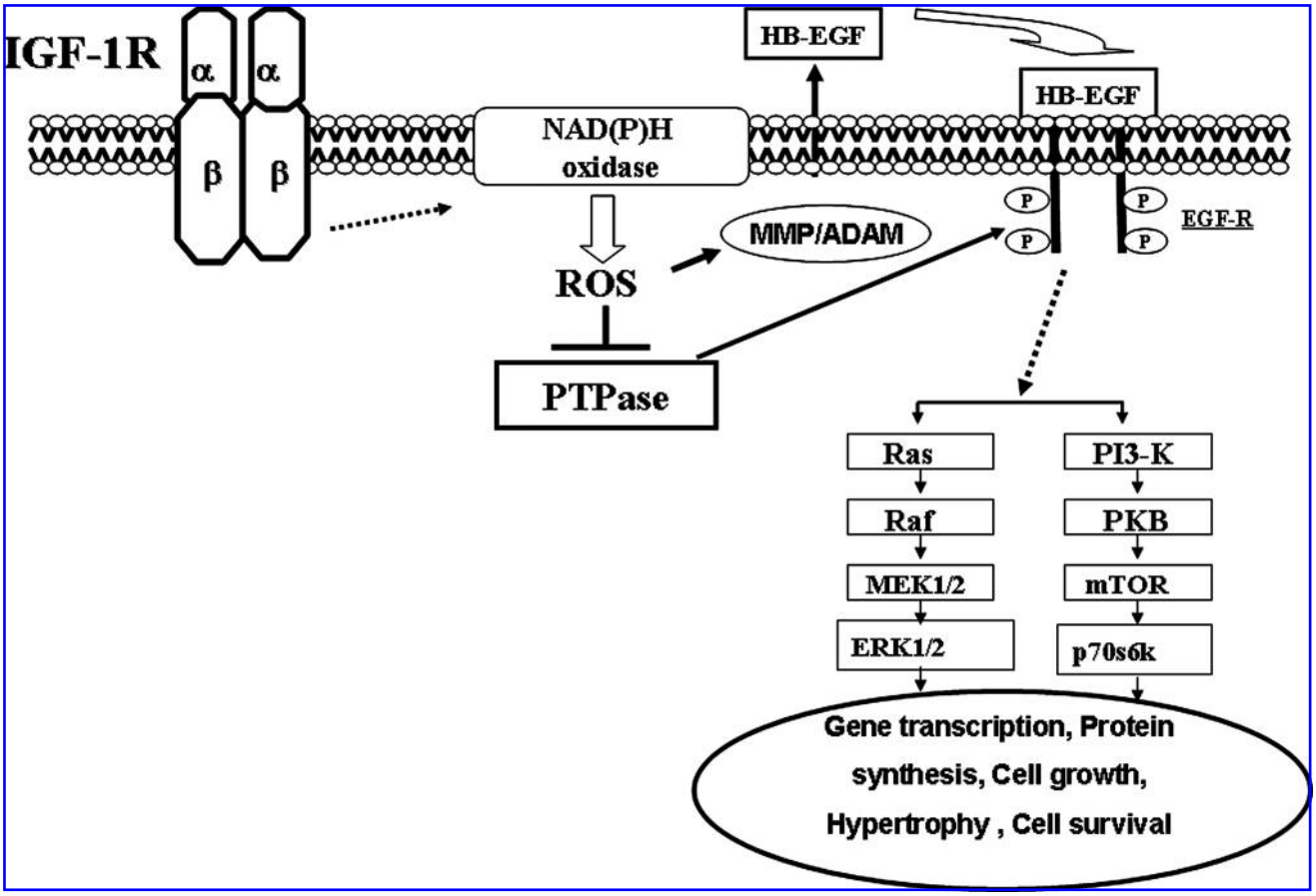

FIG. 9. Potential mechanism of EGFR transactivation by IGF-1R. IGF-1R activation causes ROS generation via NAD(P)H oxidase activation. ROS-dependent or -independent stimulation of matrix metalloproteinases, such as MMP2/9 and/or ADAMs, leads to the transformation of pro-heparin-binding (HB) EGF to HB-EGF. The interaction of HB-EGF with EGFR leads to its phosphorylation (P) and subsequent activation of Ras/Raf/MEK1/2 and ERK1/2, as well as the PI3-K/PKB signaling cascades. ROS production also inhibits PTPases, which increases EGF-P, thereby activating the MAPK and PI3-K signaling cascades. These signaling intermediates participate in various cellular processes, including protein synthesis, hypertrophy, cell growth, survival and gene transcription.

strated that ROS contributes to carcinogenesis through multiple mechanisms, such as interference with multiple genes, as well as with signal transduction (263). ROS can easily cause multiple irreversible mutations in DNA, leading to gene instability and cancer progression (138). Furthermore, recent studies have linked IGF-1 signaling and ROS production in carcinogenesis, by suggesting that IGF-1 may contribute to proliferation and transformation of breast carcinoma cells through ROS-dependent activation of MAPK pathways (162). It should be noted that IGF-1-induces ROS generation through the activation of the NOX family of NADPH oxidases in some cell types (180).

In addition to the regulation of apoptosis, IGF-1 also contributes to malignant phenotypes through its effects on regulation of the cell cycle (93). It increases cyclin D1 and cyclin-dependent kinase 4 (CDK4) gene expression and activation of cyclin E (93). IGF-1 activates CDK4 by inhibiting transcriptional inhibitors like p27/KiP1 (63). It also inhibits phosphatases, such as phosphatase and tensin homologue (PTEN), whose improper regulation has been noted in cancer (199). This inhibition of PTEN, and other tyrosine phosphatases, during mitogenic stimulation, is due in large part to ROS generation, and facilitates the prolonged activation of R-PTK (143).

Epidemiological and experimental evidence linking an upregulated IGF-1 system to the increased risk of cancer is growing (224), therefore, attenuation of IGF-1 system has emerged as an attractive target to develop new therapies for many forms of malignancies. These strategies include decreasing the IGF-1 bioavailability by increasing the expression of IGFBPs, and the inhibition of IGF-1R and the molecular components of the IGF-1 signaling pathway. A variety of these inhibitors include chemical compounds, monoclonal antibodies, antisense oligonucleotides, or small interfering RNA (siRNA) and PTK inhibitors, which block the enzymatic activity of receptors (Table 1). PTK inhibitors have been designed to compete with adenosine triphosphate (ATP) binding domains of these kinases, thereby preventing the catalytic activity of IGF-1R and turning off the downstream signaling events (42, 219). These inhibitors have been very effective in inhibiting the growth of multiple tumors under experimental settings (Table 1). However, none of these molecules have entered clinical trials. Apart from other signaling component inhibitors, rapamycin and its derivatives, inhibitors of mTOR,

Table 1. Potential IGF-1 Targeting Therapies for Multiple Pathophysiological States*

Type of potential treatment targeting IGFs	*Models studied*	*Major effects*	*Diseases targeted*	*Current status of potential therapy*
Antibodies Targeting IGF-1R				
αIR3	Athymic mice	Decreased tumor size	Breast cancer, rhabdomyosarcoma, lung cancer, Ewing sarcoma	Some monoclonal antibodies for anti-IGF-1R are in Phase I trials; no studies have been reported for Phase II (20,39,65, 142,167,173,225,230,269,270,284)
CP-751,871	Athymic mice	Inhibition of tumor growth, IGF-1R downregulation	Breast cancer, colorectal cancer, multiple myeloma	
A12	Athymic mice, SCID mice	Decreased tumor size	Prostate cancer, lung cancer, Multiple myeloma, anaplastic thyroid carcinoma	
KM1468	Athymic mice	Blocked colon cancer metastasis	Colon cancer	
Chemical Compounds as IGF-1R Inhibitors				
Picropodophyllin	Syngeneic mice, SCID mice, 5T33MM mice, human primary uveal melanomas	Decreased tumor growth, increased survival,	Multiple myeloma, uveal melanoma	No small molecule has entered clinical trial (60,94,104,140,183,272)
PQ401	Syngeneic mice	Decreased tumor growth	Breast cancer	
PQIP	Athymic mice, human colorectal cancer cells	Inhibition of tumor growth	Colorectal, non-small cell lung, pediatric	
NVP-ADW742	SCLC cells	Enhances effect of chemotherapeutic agents	Small cell lung cancer	
Oligonucleotides Targeting the IGF-1 System				
Antisense oligonucleotides	Human mylenoma cells, athymic mice, spontaneously hypertensive rat	Decreased tumor growth; decreased IGF-1R expression, reduced AT1R, lowering resting blood pressure	Multiple myeloma, glioblastoma, hepatic cancers, hypertension	Multiple *in vivo* and *in vitro* studies (185,193,195,196,242,251)
Small interfering RNA	Human mylenoma cells, athymic mice	Inhibition of tumor growth, enhanced radio- and chemo sensitivity, blocked IGF signaling	Breast, lung, prostate, and ovarian cancer	
Triple helix-forming oligonucleotides	Glioblastoma cell line, athymic mice	Suppress IGF-IR gene transcription, inhibition of tumor growth	Multiple myeloma	
Recombinant human IGF-1 with IGFBP-3 combination	Type 1 and 2 diabetes human subjects	Blood glucose lowering, Insulin sensitization	Diabetes, hyperglycemia	Numerous clinical trials (62,155,204,206)

*Numerous strategies have been used to target the IGF-1 system in treatments for multiple diseased states. These include the use of oligonucleotides or short-interference RNA (siRNA), which reduce the number of IGF-1 receptors. By using monoclonal antibodies, binding of IGF-1 to IGF-1R can be inhibited, as can the enzymatic activity of the IGF-1R by using chemical inhibitors. IGFBPs have also been used to decrease free IGF serum levels in combination with other types of treatments. Several of the aforementi1d treatments have been or are being used in clinical trials, yet others have only been used in in-vitro studies.

Abbreviations: αIR3, monoclonal antibody against IGF-1R ligand binding domain; CP751,871, a dimeric antibody against IGF-1R; A12, antibody against IGF-1R; KM1468, anti-human IGF-1 and IGF-2 antibody; PPP, picropodophyllin developed by molecular modeling to mimic the 3-dimensional structure of the IGF-1R tyrosine kinase domain; PQIP, PQ401, diaryl urea compounds, antagonizing IGF-IR signaling.

an important substrate of PI3-K/PKB, is coming to light as an anticancer agent (267).

Several studies have employed monoclonal antibodies to target IGF-1R (Table 1). These antibodies have very high affinity for the hormone-binding domain of the phosphorylated tyrosine kinase receptor and directly block IGF-1. Many mouse and human antibodies have been investigated in a wide range of tumor cell types (290). It has also been shown that inhibition of IGF-1 activity by monoclonal antibodies enhances the effect of anti-tumor therapies (230). Many of these antibodies are in Phase I trials, some of which have entered into Phase II clinical trials (17, 109, 219, 254). Another approach, the anti-gene approach, can be divided into three groups: a) Antisense molecules that target complementary sequence in mRNA (antisense RNA, antisense oligodeoxynucleotides and ribozymes) (20, 142), b) Triple helix-forming oligomers (242), targeting the double-stranded DNA gene, and c) Sense oligodeoxynucleotides have also been engineered, and act directly as decoys to inhibit regulatory proteins (185). Furthermore, increased cell death and heightened susceptibility to chemotherapeutic agents in tumor cells have been demonstrated by this approach (251). This approach has mostly been used in cultured cells and lacks *in vivo* data. It thus appears that IGF-1 system is an emerging new target to develop novel therapies to treat many forms of cancer. Most of these therapies are targeting IGF-1R either by using monoclonal antibodies or small molecule inhibitors of IGF-1R-PTK activity.

B. Diabetes and obesity

Diabetes mellitus is a major global health problem characterized by hyperglycemia, polyurea, polydipsia, and polyphagia (210). Two major forms of the disease exist: Type 1 diabetes, or insulin-dependent diabetes mellitus (IDDM), formerly also known as juvenile diabetes, and type 2 diabetes, or noninsulin-dependent diabetes mellitus (NIDDM). In general, diabetes can be characterized by decreased uptake of glucose in muscle and adipose tissue, resulting in chronic extracellular hyperglycemia, with eventual pathological consequences, including cardiovascular complications, arthrosclerosis, and retinopathy (33).

Impaired insulin secretion and signaling are among the major causes of diabetes. Furthermore, IGF-1 has also been shown to play a role in beta cell growth and function, controlling blood glucose levels, and the development of diabetes (60, 260). It has also been reported to stimulate glucose transport in muscle cells (60, 84), and IGF-1R deletion in skeletal muscle leads to glucose intolerance and impaired insulin action, followed by the development of type 2 diabetes in mice (97). Studies have identified an independent connection between low serum IGF-1 levels and the future development of diabetes (229). IGF-1 concentrations affect postprandial glucose disposal in humans, as it has been demonstrated that IGF-1 administration to patients with extreme insulin resistance or type A insulin resistance results in improved postprandial glucose uptake (155).

Also, treatment of diabetic patients with recombinant human IGF-1 has been shown to reduce insulin dose requirements by 50%, and to decrease serum glucose levels by almost 25% in diabetic patients (62, 235). Recombinant IGF-1 administration also improved insulin sensitivity (135), plasma free fatty acid, and fasting triglyceride levels (26), and increased oxidative and nonoxidative metabolism in both type 1 and type 2 diabetic patients (67).

Blood glucose-lowering effects have been observed with the administration of IGF-1 alone or in combination with IGFBP-3 in patients with IR mutations (186, 187). This may be due to suppression of GH secretion, which can function as an insulin antagonist (282).

Multiple clinical trials have been conducted in patients with type 1 and type 2 diabetes to evaluate the efficacy of IGF-1 (49, 50, 67, 135, 235). In type 1 diabetes, insulin sensitization was the likely result of GH suppression and inhibition of its effects in the liver, as well as increased insulin sensitivity due to decreased GH levels after recombinant IGF-1 treatment (49). IGF-1 reduced hemoglobin A1c levels by 1.2% in these patients, and insulin sensitivity was enhanced by 3.4-fold (209). Other strategies have been evaluated to improve insulin sensitivity, and include increasing free IGF-1 levels by introducing small molecules that can inhibit the interaction between IGF-1 and IGFBPs, resulting in heightened insulin action (164). It is also of interest to note here that in a phase I clinical trial of cancer subjects using humanized monoclonal antibodies of IGF-1R, hyperglycemia appeared to be the most common toxic effect (127).

The mechanism by which IGF-1 improves glucose homeostasis is not yet clear, but many mechanisms have been proposed, such as inhibition of hepatic and renal gluconeogenesis (206). Since IGF-1R and IR activate overlapping signaling pathways, a cross-talk between insulin and IGF-1 signal transduction pathways may be one the mechanisms involved in the glucoregulatory response of IGF-1. More notably, the stimulation of PI3-K/PKB signaling, which plays a key role in regulating insulin-stimulated glucose transport and inhibition of gluconeogenesis may be attributed to these responses (190).

The IGF-1 system has also been implicated in b-cell development and function (260). Therefore, an upregulation of IGF-1 system has been suggested to contribute to glucose homeostasis by improved b-cell function and insulin secretion (107). This notion is further reinforced by recent studies showing that mice overexpressing IGF-1 are protected from STZ-induced diabetes (218). Thus, IGF-1 based therapies have the potential to serve in the treatment of diabetes. However, due to the emerging role of an upregulated IGF-1 system in the development of cancer, a cautious approach needs to be taken before introducing IGF-1 in the clinics.

ROS are also being closely studied greatly with regard to their potential role in the pathogenesis of diabetes and obesity. Elevated glucose levels can stimulate ROS production through a variety of sources, such as oxidative phosphorylation, glucose auto-oxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenases, and NOS (15, 195). Superoxide generation by the glucose auto-oxidation process was found to be linked with the formation of glycated proteins in diabetic patients (279). In addition, high glucose levels also stimulated cell-mediated peroxidation of low density lipoproteins (LDL) (177). There are also multiple studies suggesting that increased oxidative stress may contribute to the pathogenesis of hyperglycemia/diabetes-induced complications, as well as insulin resistance in type 2 diabetes (95, 176, 274) Furthermore, an involvement of ROS in diabetic complications, such as diabetic neuropathy and

macrovascular disease (34, 44) has been implicated from studies showing treatment with antioxidants improved these diabetic complications. For example, antioxidants, such as α-lipoic acid vitamins C and E have been shown to improve hyperglycemia (152, 179). Also, *N*-acetylcysteine (NAC) treatment, in combination with creatine, was found to significantly reduce glucose intolerance, as well as body fat content (248). Furthermore, it has been shown that pancreatic islets have small amounts of antioxidant enzymes, such as Cu/Zn-SOD, Mn-SOD, catalase, and glutathione peroxidase (GPx) (111). These low levels of antioxidants make islets more prone to oxidative damage. Apoptosis of *β*-cells can be suppressed by antioxidant treatment, with no change in *β*-cell proliferation, and improve insulin and insulin mRNA content, favoring the notion that hyperglycemia-induced oxidative stress and apoptosis reduce *β*-cell mass (145). Antioxidants have also been demonstrated to suppress the cyclin-dependent kinase (CDK) inhibitor p21 (172, 191), which can be stimulated by ROS and can suppress *β*-cell proliferation and insulin production (144).

C. Cardiovascular diseases

Several studies have indicated that IGF-1 plays a critical role in cardiovascular complications, such as hypertension, atherosclerosis, restenosis, and even congestive heart failure (14, 85, 112, 113, 212, 265). IGF-1 can be synthesized and secreted in cultured VSMCs (78). Elevated mRNA levels of IGF-1 and IGF-1R have been found in VSMCs from *de novo* restenotic coronary plaques, compared to normal coronary arteries (79). Furthermore, increased IGF-1 mRNA expression has been observed in rat models of hypertrophying aorta, portal vein, and urinary bladder (55). As well, IGF-1-induced relaxation in pre-contracted phenylephrine aortic rings was impaired in spontaneously hypertensive rats (SHR) (137, 266). The vasorelaxant properties of IGF-1 were impaired in SHR even before the onset of hypertension, suggesting that this could play a role in the development of hypertension (266). On the contrary, a considerable increase in arterial pressure was seen in homozygous mice with a site-specific mutation in *IGF-1* exon3 (159), suggesting that IGF-1 and the components comprising its signaling pathway may play an important role in blood pressure regulation, and should not be excluded as candidate genes in genetic studies of hypertension (159). Enhanced levels of IGFBP-4 mRNA have also been demonstrated in the SHR aorta after abdominal coarctation, showing that IGBP-4 is limited to hypertensive blood vessels (4). The mechanism by which IGF-1 mediates its vasorelaxation effects involves an increase in endothelial cell NO production, increasing Na(+)-K(+)-ATPase activity, reducing Ca^{2+} concentrations and modifying Ca^{2+}-myosin light chain (MLC) phosphorylation (244). Many of the metabolic and vasomotor effects of IGF-1 are mediated by PI3-K/PKB and MAPK pathways (53, 60, 90, 276). ERK-1/2 promotes IGF-1-induced VSMC survival and proliferation and leads to mitogenic effects of the MAPK pathway (89). Ang II has been shown to increase the levels of IGF-1 mRNA and protein in heart and VSMC (29, 77). Conversely, IGF-1 was also found to upregulate the expression of AT_1R in VSMCs (188), suggesting the existence of a potential cross-talk between Ang II and IGF-1 system. This notion is further supported by the studies showing that IGF-1R antisense-induced reduction in IGF-1R was associated with an inhibition of Ang II-induced vascular responses in SHR and Wistar Kyoto rats (WKY) (193, 194). In addition, native unmodified LDL has been found to increase IGF-1 mRNA, whereas oxidatively-modified LDL (oxy-LDL) decreases IGF-1 mRNA and protein expression in a dose-dependent fashion (233). IGF-1 also stimulates ROS-mediated transactivation of EGFR via Src activation, leading to ERK-1/2 phosphorylation, which plays a critical role in VSMC proliferation (181). Antioxidant treatment can inhibit IGF-1-induced EGFR transactivation by lowering H_2O_2 production (181). In VSMCs, H_2O_2 can enhance IGF-1R and Src-PTK-dependent PKB activation (6) (Fig. 10). Thus, significant reductions in IGF-1 effects appear advantageous in cardiovascular diseases, including hypertension, and the early

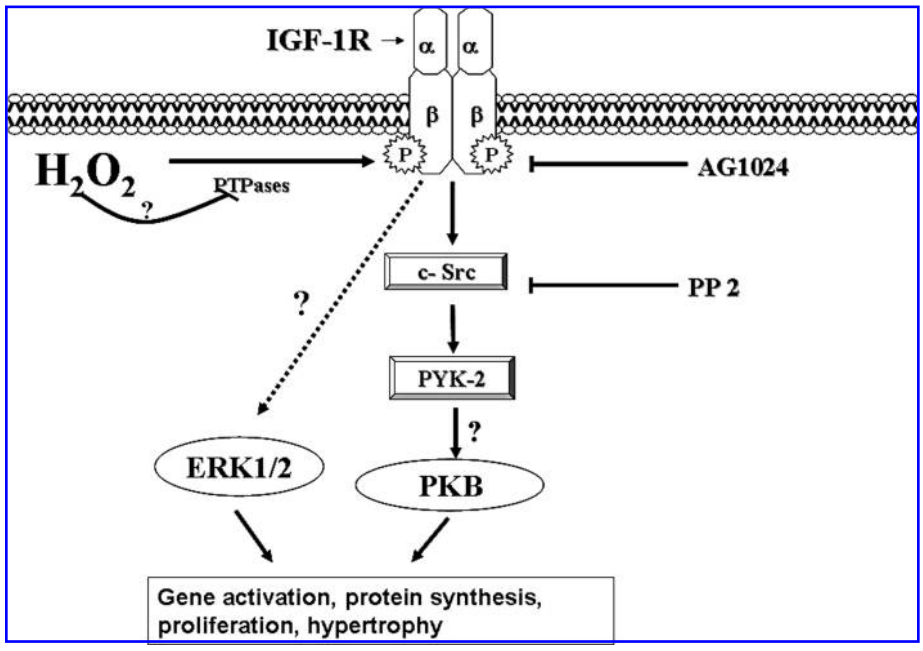

FIG. 10. Schematic model showing the potential key steps in H_2O_2-evoked responses. IGF-1R activation seems to be necessary for H_2O_2-induced phosphorylation of PKB, ERK1/2, c-Src, and Pyk2, as all these events were blocked by AG 1024. Src appears to act upstream of Pyk2 and PKB, since its pharmacological inhibition by PP2 decreases both Pyk2 and PKB phosphorylation. Whether Pyk2 is acting upstream of PKB and/or ERK1/2 remains to be seen. Furthermore, the mechanism by which H_2O_2 stimulates IGF-1R*β* phosphorylation is still unknown, but the ability of H_2O_2 to inhibit PTPases may contribute to this effect.

phase of atherosclerosis plaque formation in VSMCs. However, in contrast to proatherogenic effects of an upregulated IGF-1 system, there is an increasing body of evidence to invoke a beneficial effect of IGF-1 signaling in advanced plaque conditions associated with atherosclerosis (79). This is exemplified by recent studies showing that infusion of IGF-1 in ApoE-deficient mice fed a high-fat diet resulted in a decreased atherosclerotic plaque progression (250). These studies also demonstrated that IGF-1 infusion was associated with decreased vascular expression of inflammatory cytokines, such as interleukin-6 (IL-6) and TNF-α, as well as a reduction in superoxide formation, and an upregulation of eNOS. Thus, it is possible that the IGF-1 axis has both pro- and antiatherogenic effects dependant on the early and/or late stages of vascular abnormalities (250).

D. Neurological disorders

IGF-1 is a key molecule involved in normal brain growth and function, both as a neurotrophic peptide and neural survival factor (91, 223, 257). It may also be involved in the modulation of house-keeping processes (48), controlling such tasks as the removal of toxic brain amyloid-β peptide (AβP) and stimulation of neural excitability (139). Furthermore, altered PKB signaling observed in neurodegenerative diseases, such as spinocerebellar ataxia (SCA) and Huntington's disease, suggests the possibility that IGF-1-induced activation of PKB signaling confers a neuroprotective effect by inhibiting neuronal death (54, 134).

Variations in serum and brain levels of IGF-1 have been associated with several neurodegenerative diseases in animals, as well as in humans (40). IGF-1-deficient mice exhibit decreased motor and sensory nerve conduction, and treatment with recombinant human IGF-1 restores both motor and sensory nerve conduction velocities in these mice (105). High (late onset type of disease) and low (familial Alzheimer) levels of IGF-1 have been reported in studies of Alzheimer patients (189).

Low IGF-1 levels may result in attenuated IGF-1R activation and downstream PKB signaling, as well as compromised neuronal survival (249). In fact, IGF-induced activation of ERK1/2 and PI3-K/PKB pathways has recently been suggested to confer protection against AβP-induced death of neuroblastoma cells (273). Another mechanism by which PKB could contribute to neuroprotection is via its effect on GSK-3 activity, which has been postulated to be upregulated in the frontal cortex and hippocampus of Alzheimer patients (21, 132, 161, 205). GSK-3 catalyzes the phosphorylation of the microtubule-associated protein tau. Tau hyperphosphorylation has been implicated in the pathogenesis of Alzheimer's disease (121), and PKB-induced inhibition of GSK-3 has the ability to potentially reduce tau hyperphosphorylation.

IGF-1 treatment in animal models of cerebellar ataxia has also been shown to be very effective, demonstrating that it could be a possible therapeutic target for neurological disorders (96). Many clinical studies have revealed that long-term IGF-1 administration is safe, despite the anti-apoptotic properties of IGF-1 associated with tumorigenesis (96).

ROS are other important factors associated with brain pathology. ROS have been linked with accumulation of amyloid in Alzheimer's disease (264). Both AβP and amyloid precursor protein (APP) have strong copper (Cu)-reducing activity, which generates H_2O_2 as a by-product. AβP and APP-related-Cu^+ may contribute to the increased oxidative stress found in Alzheimer disease (43). Dysfunction of mitochondria in aging neurons also generates a large amount of ROS, causing inflammation, reduced efficiency in cellular respiration, and cell death (211). It has also been reported that IGF-1 can protect from oxidative stress and neural injury by influencing the inner mitochondrial membrane, involving uncoupling protein-3, so that IGF-1 could act as a regulator of oxidative stress in the brain (119).

Since many neurodegenerative conditions are associated with enhanced generation of ROS/RNS, antioxidant-based therapies can potentially be quite helpful. In clinical trials, vitamin E had beneficial effects in Alzheimer's disease (153). It has also been found that the antioxidant deprenyl is effective in improving cognitive function in clinical trials of dementia with advanced HIV infection, but thioctic acid (α-lipoic acid) had no impact (83). Although a great deal of clinical evidence endorses IGF-1 and antioxidants for the treatment of neurological disorders, their true therapeutic potential still remains unclear, and warrants further investigation.

E. Aging

A great deal of knowledge on aging and lifespan extension has been generated in lower organisms, such as the nematode *Caenorhabditis elegans* (*C. elegans*) and the fly *Drosophila melanogaster* (*D. melanogaster*). These organisms reproduce quickly, are easy to handle, and have very short lifespans, making it easier to evaluate changes in a short period of time. For example, *C. elegans* has a lifespan of only $\sim$2 weeks, while the average life expectancy of *D. melanogaster* is $\sim$28 days.

Aging is generally associated with an overall decline in the function of multiple systems and organs. One of the major systems affected by aging is the endocrine system (222), and since the GH and IGF-1 systems play crucial roles in regulation of growth, development, and metabolism, these systems have been studied extensively for their involvement in aging and longevity (217).

Early evidence for a role of insulin/IGF-1 was provided by studies showing that mutations in daf-2, a homologue of insulin/IGF-1R, resulted in the extension of life span in *C. elegans* (147, 150). An involvement of the insulin/IGF-1R pathway in the regulation of lifespan extension was also demonstrated in *D. melanogaster*, where loss of the IR and IRS homologue CHICO resulted in longer lifespan by reducing juvenile hormone (JH) production, which is upregulated through IR and CHICO activation (57). These studies in *D. melanogaster* and *C. elegans* raise the question of whether insulin/IGF-1 signaling also plays a role in lifespan extension in higher species. As such, multiple studies have been undertaken to investigate the impact of the insulin/IGF-1 pathway on longevity in several animal models. One of the first reports showing a role of IGF-1 in lifespan extension in mammals comes from the studies in GH-deficient and GH-resistant mice, which exhibit low levels of IGF-1 (32, 57). Further proof for a role of IGF-1 in longevity was derived from studies in heterozygous IGF-1R knockout mice that live 26% longer than wild-type mice (130). More recently, a study conducted in a cohort of centenarians has shown that functionally significant mutations in IGF-1R may be associated with increased lifespan in humans (249). Other studies (10) have suggested that

IGF-1 plays an important role in regulating the lifespan in higher species as well. The precise mechanism by which a decreased IGF-1R function contributes to longevity in mammals remains elusive, however, a potential role of the down regulation of the IR/IGF-1 signaling cascade has been proposed to play a role in this process (10, 207).

In this regard, it is interesting to note that fat-specific IR knockout (FIRKO) mice also exhibit an increase in lifespan (24). These animals show a fat-specific decrease in insulin-signaling and have lower levels of insulin, as compared to control animals. Similarly, knockout of IRS-1 and IRS-2, two effectors of IR/IGF-1 signaling, in mouse models, also resulted in enhanced lifespan (238, 252), and although the claim of enhanced longevity in brain-specific IRS-2 knockout mouse model has not been universally confirmed (239), these studies have provided further support to the notion that a decrease in the signaling events downstream of IRS may contribute to life extension.

In fact, recent human studies have also shown that IGF-1-induced phosphorylation of PKB was decreased in lymphocytes isolated from long-lived centenarians, as compared to controls (249). Although in these studies no attempts to monitor the phosphorylation of FOXO transcription factors, which are downstream substrates of PKB, was made, a recent report has indicated that a genetic variation in the FOXO3a gene was associated with longevity in long-lived humans (277). PKB-mediated phosphorylation of FOXO results in its translocation from the nucleus to the cytoplasm, causing the arrest of the transcription of several key genes, some of which are involved in redox-regulation, such as catalase and Mn-SOD (154, 192). It is thus possible that a decreased activity of PKB would cause nuclear translocation of FOXO and an enhanced transcription of antioxidant genes. In view of the free radical theory of aging (16, 122), and emerging role of oxidative stress in the aging process (168), one of the mechanisms by which decreased IGF-1 signaling may contribute to longevity and lifespan extension may involve an upregulation of antioxidant systems.

X. Conclusions

A growing body of evidence suggests an important role of the IGF-1 system in modulating multiple cellular functions. IGF-1 regulates these functions by activating signal transduction pathways that mediate cell growth, migration, survival, apoptosis, and metabolism. Aberrant IGF-1 production and/or signaling have been implicated in the pathogenetic mechanisms of several diseases, which include metabolic disorders, neurodegenerative diseases, and multiple types of cancers.

In view of the upregulation of IGF-1 levels/signaling in multiple forms of cancer and cardiovascular diseases, inhibition of the IGF-1 system is being targeted as a potential therapy of these disorders. In this regard, IGF-1R antibodies, siRNA/antisense oligonucleotides, and small molecular weight inhibitors of IGF-1R are being utilized in experimental models for treatment. Most therapies are directed towards enhancing the apoptosis of cancerous cells. A decreased IGF-1R function has also been suggested to contribute to longevity in many organisms, including humans. In contrast, in the case of neurological disorders where neuronal survival is compromised, the focus is on enhancing IGF-induced pro-survival pathways. Similarly, upregulation of insulin-signaling pathways would be beneficial in the treatment of metabolic disorders. In fact, IGF-1 and its analogues have recently been shown to improve insulin sensitivity in animal models of insulin resistance. There is also increasing awareness of cross-talk between the IGF-1 system and ROS/RNS pathways. ROS have been identified both as activators and mediators of IGF-1-induced signaling events; therefore, they have the ability to aggravate the clinical manifestations of vascular and neurological disorders as well as aging. On the other hand, RNS have been suggested to serve as attenuators of IGF-1-induced responses. Thus, antioxidant therapy and agents that raise NO levels could also exert beneficial effects in some disease states.

It should be noted that multiple antioxidants have successfully been used in conjunction with other medications to treat various vascular and cardiovascular diseases, as well as in multiple types of cancers, yet their exact mechanisms of action and how they affect ROS/RNS generation and metabolism are not yet fully understood. Furthermore, negative implications of long-term antioxidant exposure also remain uncharacterized.

It may be possible to develop therapies of multiple diseases by specifically targeting IGF-1 production/circulation and IGF-1R activation, and by reducing oxidative stress. However, caution needs to be exercised in maintaining a fine balance between up- and downregulation of IGF-1 signaling in devising therapies that target the IGF system to correct pathologies, such as cancer and diabetes.

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Abbreviations

AβP, amyloid β peptide; ALS, acid labile subunit; Ang II, angiotensin II; APP, amyloid precursor protein; ASK, apoptosis signaling-regulated kinase; AT_1R, angiotensin II receptor type 1; ATP, adenosine triphosphate; BAD, Bcl-2-associated death promoter; CDK, cyclin-dependent kinase; cGMP, cyclic guanosine monophosphate; ET 1, endothelin-1; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FnIII, fibr1ctine type III domain; FOXO, forkhead box protein; G6Pase, glucose-6-phosphatase; GDP, guanosine diphosphate; GH, growth horm1; GPx, glutathione peroxidase; Grb-2, growth factor receptor-binding protein 2; GSH, glutathione; GSK-3, glycogen synthase kinase-3; GSSG, glutathione disulfide; GTP, guanosine triphosphate; GTPase, guanine triphosphatase; HB, heparin binding; H_2O_2, hydrogen peroxide; HIV, human immunodeficiency virus; IDDM, insulin-dependent diabetes mellitus; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor type 1 receptor; IGFBP, insulin-like growth factor-binding protein; IR, insulin receptor; IRS, insulin receptor substrate; JH, juvenile horm1; JNK, Jun N-terminal kinase; LDL, low density lipoprotein; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; MLC, myosin light chain; NAC, *N*-acetylcysteine; NAD(P)H, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear

factor kappa B; NIDDM, non-insulin-dependent diabetes mellitus; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NAD(P)H oxidase; $p70^{s6k}$, p70 ribosomal s6 kinase; PDK, phosphoinositide-dependent kinase; PH, plekstrin homology; PI, phosphatidyl-inositol; PI3-K, phosphatidyl-inositol 3-kinase; PIP3, phosphatidyl-inositol 3, 4, 5 triphosphate; PKB, protein kinase B; PKG, protein kinase G; PDGF, platlet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PTEN, phosphatase and tensin homologue; PTP, protein tyrosine phosphatase; RNS, reactive nitrogen species; ROS, reactive oxygen species; R-PTK, receptor-protein tyrosine kinase; SAPK, stress-activated protein kinase; SCA, spinocerebellar ataxia; SH2, Src homology 2 domain; SHR, spontaneous hypertensive rat; Sir2, silent mating type information regulation-2; Sirtuin, Sir2 homologue; siRNA, small interfering RNA; SOD, superoxide dismutase; SOS, son of sevenless; VSMCs, vascular smooth muscle cells.

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