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Defects in growth hormone receptor signaling

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Severe growth failure and insulin-like growth factor (IGF) deficiency were first reported 40 years ago in patients who ultimately proved to have mutations in the gene encoding the growth hormone receptor (GHR). So far, over 250 similar patients, encompassing more than 60 different mutations of *GHR*, have been reported. The GHR is a member of the cytokine receptor superfamily and has been shown to signal, at least in part, through the Janus-family tyrosine kinase–signal transducer and activator of transcription (JAK–STAT) pathway. Six patients, from five distinct families, have been reported to have phenotypes similar to that of patients with GHR defects but with wild-type receptors and homozygosity for five different mutations of the *STAT5b* gene. These patients define a new cause of GH insensitivity and primary IGF deficiency and confirm the crucial role of STAT5b in GH-mediated IGF-I gene transcription.

Introduction

Severe growth retardation and low serum concentrations of insulin-like growth factor (IGF) I, in the face of normal or increased serum growth hormone (GH), define the condition of GH insensitivity (GHI). Although initial cases have been linked to defects in the gene for the GH receptor (GHR), the recent discovery of patients with mutations affecting the signaling cascade of the GHR has provided a new basis for GHI, as well as helping to define the mechanisms involved in GH-mediated regulation of IGF-I production. Accordingly, we focus here on how molecular defects distal to GH binding by the GHR result in IGF deficiency and growth failure.

GHI: a historical perspective

In 1966, Laron *et al*. [1] reported the first cases of GHI, describing '...three siblings with hypoglycemia and other clinical and laboratory signs of GH deficiency, but with abnormally high levels of immunoreactive serum growth hormone'. In the ensuing 40 years, ~250 cases of GHI have been reported, encompassing all continents, except Antarctica, with the largest cohorts identified in the Mediterranean and in an isolated region of southern Ecuador [2].

Initial studies indicated that patients with GHI could not respond to either endogenous or exogenous GH in terms of growth, metabolic changes or significant elevations of serum concentrations of IGF-I [2]. Direct evidence of receptor dysfunction was provided by the failure of hepatic microsomes to bind to GH. With the cloning and sequencing of the gene for the GHR, it became possible to determine the molecular basis for GHI, and individual patients were shown to carry either significant gene deletions or point mutations [3,4].

The earliest described genetic abnormalities of the GHR involved the extracellular GH-binding domain [2,5]. These studies were supported by assays of serum concentrations of GH-binding protein (GHBP), the circulating extracellular domain of the GHR that results from proteolytic cleavage of the GHR [6]. The earliest such assays involved the binding of radiolabeled GH in the serum and, therefore, reflected reduced serum levels of GH-binding activity. Indeed, the majority of abnormalities of the GHR reported to date involve the extracellular domain of the receptor and are supported by the finding of decreased GHBP (or, at least, decreased GH-binding activity) in the serum.

As experience with abnormalities of the GHR deepened, it became apparent that a spectrum of phenotypic and biochemical abnormalities existed [2,5,7]. Thus, whereas the earliest reported cases of GHI were characterized by severe growth failure, frontal bossing, hypoplasia of the midfacies and the nasal bridge, sparse hair, high-pitched voices and blue sclerae, milder cases could also be seen, with more modest growth failure and a softening or absence of the characteristic facial features. Serum concentrations of the GH-dependent growth factors IGF-I, IGF-binding protein 3 (IGFBP-3) and acid-labile subunit (ALS), although still reduced (reflecting some degree of GH resistance), were not as severely affected, and serum concentrations of GHBP were, on occasion, normal or even increased. Some such cases reflected dominant negative mutations, defects in receptor dimerization, or mutations affecting the transmembrane or intracellular domains of the GHR.

Despite efforts to correlate phenotype and genotype, no clear linkage between specific mutations and clinical presentations has been found [8]. In the largest cohort of

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patients with mutations of the *GHR* gene, the height of affected Ecuadorian individuals homozygous for the same point mutation in exon 6 of the GHR ranged from −5.3 to −11.5 standard deviations from the mean [2]. Interestingly, although all affected individuals had low serum concentrations of IGF-I and IGFBP-3, a highly significant correlation was found between serum levels of these factors and height. These observations suggest that patients sharing the same mutation in the *GHR* gene might have some partial compensation, presumably at any of the following levels: (i) GH production; (ii) post-GHR signaling; or (iii) GH-independent IGF-I production.

GHR signaling

The cloning of the cDNA for the GHR, although yielding valuable information on its sequence, provided little insight concerning its basic signaling mechanisms [3,4]. It was soon evident, however, that the GHR, although constituting a transmembrane protein, lacked intrinsic kinase activity. Crystallization of the extracellular domain of the receptor revealed a 1:2 relationship of GH and GHRs [9], and monoclonal antibodies directed against the GHR were able to activate the receptor if the antibodies were divalent [10]. These findings were consistent with the belief that ligand-induced activation of the GHR required dimerization. Although it seemed at first that the binding of GH to two GHRs promoted receptor dimerization, recent studies have suggested that GH might actually induce GHR subunit rotation within a constitutive dimer [11].

The human *GHR* gene is located on chromosome 5p13.1-p12 and contains ten exons, encoding a 638 amino acid (aa) peptide that includes the mature 620 aa receptor plus an 18-residue leader sequence. The receptor is a transmembrane protein, with a 246 aa extracellular, GH-binding domain, a short, 24 aa transmembrane domain and a 350 aa intracellular domain. Mature GHRs are situated on the cell surface as loosely associated, preformed dimers; binding of a molecule of GH results in conformational changes leading to stabilization of the dimer and resulting signal transduction [12].

The GHR belongs to the type I class in the superfamily of cytokine receptors. Similarly to other members of this family, including receptors for prolactin, erythropoietin and several interleukins (ILs), GHRs form functional homodimers but lack intrinsic kinase activity (Figure 1). Each subunit of the dimeric GHR associates noncovalently (through its box one motif) with a molecule of cytosolic Janus-family tyrosine kinase 2 (JAK2). Following binding of one GH molecule, the dimeric GHR undergoes conformational changes that induce transphosphorylation of JAK2 and initiation of GHR signaling. Ligand-activated JAK2 phosphorylates multiple tyrosines on the intracellular domain of the GHR, which then serve as docking sites for cytosolic components of at least three distinct

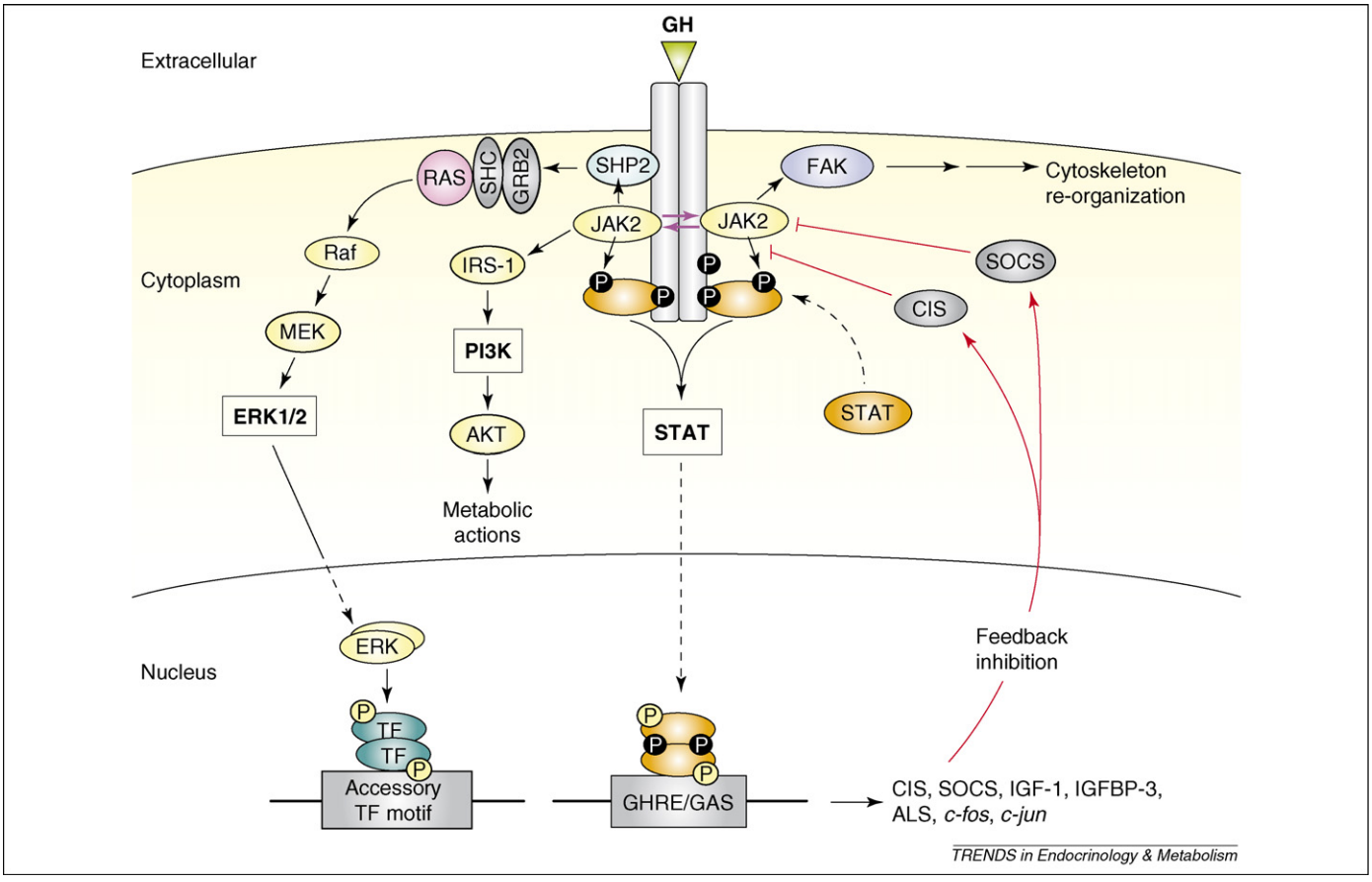

Figure 1. GH signaling pathways. After associating with a homodimeric GHR complex, which might be predimerized, JAK2 is recruited, with resulting activation of the ERK1 and -2, PI3K and STAT pathways. STAT5b seems to be the crucial mediator of GH-induced *IGF-I* gene transcription. Abbreviations: CIS, cytokine-inducible SH2-containing protein; FAK, focal adhesion kinase; GAS, gamma-interferon-activated sites, GHRE, growth hormone response element; GRB, growth factor receptor-bound protein; IRS, insulin receptor substrate; MEK, MAPK or ERK kinase; RAS, small GTP-binding protein; SHC, SH2-containing collagen-related protein; F, transcription factor.