New molecular mechanisms of GH resistance

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Abstract

Primary growth hormone (GH) resistance describes growth failure in the presence of normal, or even elevated, GH secretion. In its classic form, the phenotype is identical to that of GH deficiency, and was originally described in association with defects of the GH receptor. With increasing understanding of the GH–insulin-like growth factor (IGF) axis, it has become apparent that GH resistance can result from either primary IGF deficiency (IGFD) or IGF resistance. Primary IGFD may be due to: (i) defects of the GH receptor, (ii) defects of post-GH receptor signaling or (iii) primary defects of IGF-I synthesis. IGF resistance may result from: (i) defects of the IGF receptor, (ii) defects of post-IGF receptor signaling, (iii) defects of IGF binding proteins or (iv) defects of the epiphyseal growth plate or of regulatory proteins involved in epiphyseal growth.

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Classical growth hormone (GH) insensitivity resulting from defects of the GH receptor

The clinical phenotype of severe growth failure associated with elevated serum concentrations of GH was first described by Laron and colleagues (1) in three siblings in 1966. Although initial hypotheses focused on the possibility of a biologically inactive or 'defective' GH molecule, serum GH from affected patients was found to be normal by chromatographic analysis, and bound normally to various antisera to GH and to GH receptors (2). Furthermore, not only were serum levels of sulfation factor exceedingly low, but concentrations failed to rise following administration of exogenous GH, suggesting that cellular unresponsiveness to GH was the underlying causative factor. Direct evidence of a GH receptor (GHR) abnormality was provided by Eshet *et al.* (3), who demonstrated that hepatic microsomes obtained from two patients failed to bind radiolabeled GH. Subsequent studies indicated that serum concentrations of GH binding protein (GHBP), the extracellular domain of the GHR, were reduced in patients, consistent with an underlying receptor defect (4).

The cloning of the cDNA for the human GHR provided the basis for understanding the molecular basis for classical GH insensitivity (GHI), as deletions or mutations of this gene have now been identified in hundreds of patients with the characteristic clinical and biochemical phenotype (5–7). The mature form of the human GHR is 620 amino acids in length,

consisting of a 245 amino acid extracellular, GH-binding domain, a single 30 amino acid transmembrane domain, and a 345 amino acid cytoplasmic domain. The translated and 3'-untranslated regions of the receptor are encoded by nine exons, numbered 2–10, with exon 2 corresponding to the secretion signal peptide, exons 3–7 encoding the extracellular, hormone-binding domain, exon 8 corresponding to the transmembrane domain, and exons 9 and 10 encoding the cytoplasmic domain and 3'-untranslated region. The human GHR gene has been localized to chromosome 5p13.1-p12, where it spans greater than 87 kb.

Although the initial reports of a molecular basis for GHI described complex gene deletions, involving all or parts of exons 3, 4, 5 and 6 (6), virtually all of the subsequently reported cases have had point mutations of the GHR gene (7). To date, over 50 different mutations of the GHR gene have been identified, including nonsense, frameshift, splicing and missense mutations. Every coding exon, with the exception of exon 3, has been reported with at least one molecular abnormality and little, if any, genotype–phenotype relationship has been established. Undoubtedly, this reflects the fact that the overwhelming majority of the molecular defects identified to date have been associated with severe (or 'classical') GHI and have usually involved the extracellular domain of the GHR. Such mutations have tended to result in severe, if not complete, loss of GH binding by the receptor, with the consequent loss of essentially all GH-mediated action. Interestingly, mutations affecting the extracellular domain which preserve GH binding have been reported,

but such mutations impact GH action, for example, by inhibiting dimerization of the receptor, a step necessary for GH signal transduction (8).

When abnormalities of the GHR result in decreased GH binding, it is reflected, typically, in a reduction of GHBP, the circulating extracellular domain of the GHR. Mutations affecting the transmembrane or intracellular domains of the GHR, however, are generally characterized by normal, or even increased, serum concentrations of GHBP, so care must be employed in excluding a diagnosis of GHI purely on the basis of measurement of serum GHBP. A homozygous splice site mutation resulting in a mutant GHR protein lacking the transmembrane and intracellular domains, leads to a clinical phenotype of severe GHI, accompanied by elevated serum concentrations of GHBP, presumably because the extracellular domain of the receptor can no longer be anchored (9). Some receptor abnormalities can even exert dominant negative effects, as in the GHR 1–277 mutants identified by Iida *et al.* (10) and by Ayling *et al.* (11). The affected allele lacked exon 9, and led to a cytoplasmic segment of the GHR encompassing only seven amino acids.

An expanded view of GH insensitivity

The initial reports by Laron and colleagues (1), followed by the elucidation of the multiple molecular defects resulting in abnormal GHRs provided the foundation for our current understanding of GH resistance. Perhaps most important was the realization that the clinical phenotype of classical GHI was indistinguishable from severe congenital GH deficiency: minimal growth retardation *in utero*, infantile facial appearance, and profound postnatal growth failure (2). Since both of these clinical conditions are associated with markedly reduced serum concentrations of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3, it became increasingly apparent that IGF deficiency (IGFD) was the shared underlying biochemical abnormality (12–15). Viewed in this light, a contemporary perspective of GHI would revolve around the issue of IGFD and/or IGF resistance (Table 1 and Fig. 1).

Secondary IGFD encompasses clinical situations where IGF-I deficiency is the result of GH deficiency, and is not the proper subject of this review. Primary IGFD can result from three recognized categories of defects: (i) abnormalities of the GHR, (ii) abnormalities of the GH signaling cascade, and (iii) molecular abnormalities of the IGF-I gene. IGF resistance could also manifest as GH insensitivity, with the added feature that the insensitivity to IGF actions would manifest itself *in utero*, as well as postnatally. Since IGF-I and IGF-II (but not GH) are critically involved in fetal growth, it is to be anticipated that such patients would be characterized by both intrauterine and postnatal growth failure. Abnormalities of the IGF-I

Table 1 GH insensitivity (resistance).

I. GH insensitivity resulting from primary IGF deficiency
A. Defects of the GH receptor
1. Defects of the extracellular domain (a)
a. Associated with decreased GHBP
b. Associated with normal or increased GHBP
1. Defects in receptor dimerization (b)
2. Defects of the transmembrane domain (c)
3. Defects of the intracellular (cytoplasmic) domain (d)
B. Defects of GH signaling
1. Abnormalities of JAK2 (theoretical) (e)
2. Abnormalities of STAT5b (f)
3. Abnormalities of STATa (theoretical) (g)
C. Primary defects of IGF-I synthesis
1. IGF-I gene mutations or deletions (h)
2. Defects of IGF-I secretion (theoretical) (i)
II. GH insensitivity resulting from IGF-I insensitivity (resistance)
A. Defects of the IGF-I receptor
1. Mutations or deletions of the <i>IGFR</i> gene (k)
2. Deletions of chromosome 15q (<i>IGFR</i> gene) (k)
B. Defects of IGF-I signaling (theoretical) (l)
C. Defects of IGF binding proteins (theoretical) (j)
D. Defects of the epiphyseal growth plate (m)
1. <i>SHOX</i> abnormalities
III. Secondary GH insensitivity
A. Circulating antibodies of GH that inhibit GH action
B. Antibodies to the GH receptor (theoretical)
C. Malnutrition
D. Liver disease
E. Chronic disease

Letters in parentheses refer to Fig. 1. The word (theoretical) means that growth failure associated with a molecular defect has not been demonstrated convincingly to date.

receptor have been reported in patients displaying this predicted phenotype.

Secondary GHI can result from circulating antibodies to GH or (at least theoretically) antibodies to the GHR. The most common causes of secondary GHI, however, are malnutrition, liver disease, and other chronic diseases, especially when they are associated with catabolic states.

GH insensitivity resulting from defects of GH signaling

Elucidation of the GH signaling cascade has indicated that GH signal transduction is mediated through at least three pathways: phosphatidylinositol-3 kinase (PI3K), extracellular signal related kinases (ERK) 1/2, and Janus kinase-signal transducers and activators of transcription (JAK-STAT) (16). Recent studies have indicated that the last pathway, JAK-STAT, is centrally involved in the growth-mediating actions of GH. The binding of GH to the GHR induces the activation of JAK2, which then undergoes tyrosine autophosphorylation and concurrently phosphorylates the GHR. These phosphorylation steps provide a docking site for STATs, which are phosphorylated, dissociate from the GHR, dimerize, translocate to the nucleus, bind to

Figure 1 Schematic diagram of the GH-IGF axis, showing identified and theoretical defects: (a) defects of the extracellular domain of the GHR, affecting binding of GH; (b) defects in GHR dimerization; (c) defects of the transmembrane domain of the GHR; (d) defects of the intracellular domain of the GHR; (e) defects of JAK2 (theoretical at this time); (f) defects of STAT5b; (g) defects of STAT5a (theoretical at this time); (h) defects of transcriptional regulation of IGF-I (theoretical at this time); (i) defects of the IGF-I gene; (j) defects of IGFBPs, affecting IGF availability (theoretical at this time); (k) defects of the IGF receptor; (l) defects of IGF receptor signal transduction (theoretical at this time); (m) defects at the epiphyseal growth plate, potentially affecting IGF action. GH, growth hormone; GHR, growth hormone receptor; JAK2, janus-family tyrosine kinase 2; PI3K, phosphatidylinositol-3 kinase; ERK, extracellular signal-regulated kinase; STAT, signal transducer and activator of transcription; ISRE, interferon-stimulated response element; GAS, interferon-gamma-activated sequences; IGF, insulin-like growth factor; IGFBP, IGF binding proteins; IGF-IR, IGF-I receptor; IRS, insulin receptor substrate.

specific transcription elements on DNA and regulate transcription of critical genes, including IGF-I.

Seven STATs have been identified to date (STATs 1–6 include two STAT5 genes), all ranging between 750 and 900 amino acids, and probably all arising from an ancestral gene involved in fundamental developmental processes. The STATs all preserve five discrete domains, including: (i) an amino-terminus domain involved in nuclear translocation and DNA binding, (ii) a coiled-coil domain, (iii) a DNA binding domain, which recognizes members of the interferon-gamma-activated sequences (GAS) family of enhancers, (iv) a highly conserved SH2 domain, involved in receptor-specific recruitment and STAT dimerization, and (v) a COOH-terminal transcriptional activation domain. STAT5a and 5b appear to have particular relevance for GH and prolactin actions; although structurally highly related, they are not fully redundant. Mice with knockouts of STAT5b demonstrate a loss of sexually dimorphic growth, with males experiencing a 27% decrease in body size, resulting in reduction to the size of wild-type females; knock-outs in females do not appear to affect growth (17).

Ambrosio *et al.* (18) evaluated STAT5b as a candidate gene in children with 'idiopathic short stature,' but no base alterations were observed other than two polymorphisms. A recent report, however, of a 16-year-old

girl from consanguineous Argentine parents, associated severe postnatal growth failure (height – 7.5 S.D.), marked IGF-I and IGFBP-3 deficiency, and failed responsiveness to GH, with homozygosity for a missense mutation of the *STAT5b* gene, resulting in a substitution of proline for alanine in the SH2 domain (19). The resulting protein has proven to be incapable of phosphorylation upon stimulation with either GH or gamma interferon (20). Since this patient, in addition to growth failure and GHI, has abnormal immune function, a critical role for STAT5b in mediating the actions of both GH and other cytokines is indicated. In addition to providing the first demonstration of a post-GHR signaling defect, and defining a new syndrome combining GHI and immune dysfunction, this case presents intriguing new insights into the regulation of sexually dimorphic growth in mammals, since, in this human female case, a 'knockout' of *STAT5b* resulted in pronounced growth failure and IGF deficiency.

Given that the growth characteristics of the patient with the *STAT5b* mutation mirrored those observed in patients with severe GHI resulting from *GHR* mutations or deletions, one would infer that most, if not all, of the growth promoting actions of the GHR are mediated through STAT5b, and that the other STATs activated by GH, as well as the other pathways (ERK and PI3K) play little role in this aspect of GH action. If this were

to be the case, it would have important implications for our understanding of human growth, and how growth in our species parallels and deviates from that of other mammals (17, 19). Several other potential defects downstream of the GHR have been reported in patients with growth failure and apparent GHI, but underlying molecular defects have not been identified to date. Nevertheless, in two of the studies, GH failed to induce tyrosine phosphorylation of the STAT proteins, further underscoring the importance of this protein in GH action (21, 22).

GH insensitivity resulting from primary defects of IGF-I synthesis

In 1996, Woods *et al.* (23) reported the first case of growth failure associated with a primary defect of IGF-I synthesis, shown to be the result of homozygosity for deletion of exons 4 and 5 of the IGF-I gene. As anticipated from knockout studies in mice, the patient had both intrauterine growth retardation and postnatal growth failure, indicating that, while GH may not be required for fetal growth, the IGF system is operative *in utero* and critical for normal intrauterine growth (24). In an additional departure from the phenotype of classical GHI, with its postnatal IGF deficiency, this patient was also characterized by microcephaly, mental retardation and sensory-neural deafness. In sum, these observations, although restricted to a limited number of case reports, suggest that IGF-I production *in utero* is largely GH-independent, and is critical for normal neurological development.

GH insensitivity resulting from IGF-I insensitivity (resistance)

The critical role of the IGF system in mammalian growth is not limited to the postnatal phase of life. A series of studies involving targeted disruption of genes for various components of the IGF axis indicated that knockout of the genes for either IGF-I or IGF-II resulted in a 40% reduction in fetal growth, while knockouts of both genes or the genes for the IGF-I receptor (IGFR) led to a 55% decrease in fetal size and, generally, demise of the animal shortly after death (24). The possibility that abnormalities of IGFR could result in some combination of intrauterine and postnatal growth failure was supported by observations in patients with deletions of chromosome 15q and consequent haploinsufficiency for *IGFR* (25). Interpretation of the phenotypes of such patients is complicated, however, by the loss of contiguous genes which might contribute to the growth characteristics. Recently, nevertheless, Abuzzahab *et al.* (26) have reported that one of 42 children studied with intrauterine growth retardation and subsequent postnatal growth failure had compound heterozygosity for point mutations of *IGFR*; a second

study in Europe of children with growth failure in the face of elevated serum concentrations of IGF-I yielded one child with a history of both prenatal and postnatal growth retardation and heterozygosity for a nonsense mutation of *IGFR*.

To date, no patients have been identified with GH resistance from defects of IGFR signal transduction, although it is to be anticipated that such a situation, paralleling the recently reported STAT5b defect in the GH signaling cascade, is likely to be found. Given that approximately 75% of children identified as 'idiopathic short stature' have apparently normal serum concentrations of IGFs, one may infer that the apparent GH resistance (i.e. poor growth in the face of normal GH secretion) actually results from IGF insensitivity, either at the receptor or postreceptor level. The possible role of abnormalities of IGFBPs in patients with IGF resistance has been a subject of speculation, but our understanding of the relative physiological roles of bound and 'free' IGFs in stimulating skeletal growth remains incomplete at this time, and no molecular defect of genes for the IGFBPs have been identified in the context of growth failure to date. In the one report of a patient with a complete absence of circulating acid-labile subunit, essential for stabilization of the IGF-IGFBP complex in a ternary form in the circulation, minimal slowing of linear growth was observed (27).

Finally, the combination of GH and IGF resistance can result from a downstream defect, involving end organ unresponsiveness to IGFs. Our understanding of proteins that are active at the epiphyseal growth plate and interact with growth factors, such as IGFs, to mediate normal skeletal growth, is woefully incomplete. *Shox* is one recently identified gene, located on the sex chromosomes, that seems to fit that role; defects in *Shox* are characteristic, for example, of Turner syndrome, and have been reported in some cases of idiopathic short stature without obvious accompanying skeletal dysplasia (28).

Conclusion

It has been almost 40 years since the initial description of GH insensitivity, but it has only been in recent years that we have begun to realize fully the range of molecular disorders that may contribute to a clinical phenotype of GH resistance. It is now apparent that defects anywhere along the pathway from GH binding to its receptor to IGF action at the growth plate may contribute to postnatal, and, under certain circumstances, prenatal growth failure. Indeed, it is likely that many cases which have been lumped indiscriminately into the categories of idiopathic short stature and/or intrauterine growth retardation actually will prove to have underlying GH resistance (29). The unmasking of the molecular bases for these defects will contribute greatly to our future understanding of both normal and aberrant growth.

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