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Atypical defects resulting in growth hormone insensitivity

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ABSTRACT

Besides four well-documented genetic causes of GH insensitivity (GHI) (*GHR*, *STAT5B*, *IGF1*, *IGFALS* defects), several other congenital and acquired conditions are associated with GHI. With respect to its anabolic actions, GH induces transcription of *IGF1*, *IGFBP3* and *IGFALS* through a complex regulatory cascade including GH binding to its receptor (GHR), activation of JAK2 and phosphorylation of STAT5b, which then trafficks to the nucleus. GH also activates the MAPK and PI3K pathways.

The synthesis of GHR can be reduced by estrogen deficiency or corticosteroid excess, and is possibly decreased in African pygmies. An increased degradation of GHRs because of overexpression of cytokine-inducible SH2-containing protein (CIS) was suggested for some children with idiopathic short stature. Effects on several downstream components of GH signaling were observed for FGF21, cytokines, sepsis, fever and chronic renal failure. In Noonan syndrome and other "rasopathies" the activation of the RAS-RAF-MAPK-ERK pathway leads to inhibition of the JAK/STAT pathway. In contrast, fibroblasts from tall patients with Sotos syndrome showed a downregulation of this axis.

Experimental and clinical evidence suggests that the NF-κB pathway plays a role in GH signaling. In a patient with an IκBα mutation presenting with short stature, GHI, severe immune deficiency and other features, NF-κB nuclear transportation and STAT5 and PI3K expression and activity were reduced. A patient with a mosaic de novo duplication of 17q21-25 presented with several congenital anomalies, GHI and mild immunodeficiency. Studies in blood lymphocytes showed disturbed signaling of the CD28 pathway, involving NF-κB and related proteins. Functional studies on skin fibroblasts revealed that NF-κB activation, PI3K activity and STAT5 phosphorylation in response to GH were suppressed, while the sensitivity to GH in terms of MAPK phosphorylation was increased. The expression of one of the duplicated genes, PRKCA, was significantly higher than in control cells, which might be the cause of this clinical syndrome.

1. Introduction

For each child with short stature, the first task of the clinician is to establish the diagnosis, through a multistep process of taking a detailed medical history, collecting all previous growth data, a full physical examination (including an assessment of head circumference and body proportions), a radiologic and biochemical assessment [1] and, in selected cases, genetic assessment. There are many causes of short stature (listed in the ESPE Classification of Pediatric Endocrine Diagnoses) [2], and disorders of the growth hormone (GH) – insulin-like growth factor (IGF) axis represent only a minority. It has been our and others' experience that in approximately 15–20% of short children an established diagnosis can be made; 1–3% children present with an unclassified "syndrome" and in 80% no etiology can be found, and these children are usually labeled as idiopathic short stature (ISS) [3].

An obligatory part of the diagnostic work-up of short stature is the measurement of serum IGF-I and IGFBP-3, and if IGF-I is low or in the lower half of the reference range for age, GH provocation tests are carried out to assess GH secretion. In children with ISS, in whom GH secretion is normal by definition, a serum IGF-I below −2 SDS is found in approximately 30% of short children [4], and also in syndromic children serum IGF-I can be low despite a normal GH secretion. For such children the term "GH insensitivity (GHI)" or "primary IGF deficiency" has been used [2]. In this paper we shall use GHI.

Abbreviations: ALS, acid-labile subunit; CIS, cytokine-inducible SH2-containing protein; E2, estradiol; ER, endoplasmic reticulum; ERK, extracelular regulated kinase; ESPE, European Society for Pediatric Endocrinology; GH, growth hormone; GHBP, growth hormone binding protein; GHI, growth hromone insensitivity; GHR, growth hormone receptor; GHSR, growth hormone secretagogue receptor; GM-CSF Ab, granulocyte macrophage colony stimulating factor autoantibodies; GR, glucocorticoid receptor; HNF, Hepatocyte nuclear factor; IBD, Infammatory Bowel Disease; IGF, insulin-like growth factor; IGFALS, the gene encoding acid-labile subunit; IGFBP-3, insulin-like growth factor binding protein-3; ISS, idiopathic short stature; JAK2, janus kinase 2; MAPK, mitogen activated protein kinase; PBMCs, peripheral blood mononuclear cells; PI3K, phosphatidylinositol 3′-kinase; PDTC, pyrrolidine dithiocarbamate; SHP-2, SH2 domain-containing protein-tyrosine phosphatase-2; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription.

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At present, there are several known genetic causes of (apparent) GHI. Two of these, bioinactive GH syndrome and *GHSR* mutations, indeed present as GHI at first sight, but correspond strictly speaking to a form of secondary IGF deficiency due to a decreased secretion of GH (in patients with *GHSR* mutations) or due to the secretion of an abnormal GH1 molecule; both can present with normal of high GH peaks in response to GH stimulation tests. "Real" subclasses of GHI include mutations in *GHR*, *STAT5B*, *IGF1*, and *IGFALS*. Several algorithms have been proposed about which patients should undergo genetic testing [5–8]. However, when we tested children with apparent GHI for such genes, the diagnostic yield was low [9].

In this minireview, we shall first discuss that GH signaling is dependent on cell type, and within one cell type the GH receptor (GHR) can have a spatial and temporal expression pattern. We shall then review pathophysiological mechanisms that could lead to GHI, including: 1) conditions influencing synthesis, complex-formation, or recycling of GH receptors; 2) Acquired conditions influencing the main growth promoting signaling pathway (STAT5B); 3) Interaction between the STAT5B and MAPK pathways; 4) abnormalities in well-established signaling pathways modifying the main growth promoting signaling pathway; and 5) abnormalities in the NF-κB pathway.

2. Cell type-specific GH signaling

The expression of the gene encoding for the GHR (*GHR*) is cell type-specific, and there is also a large variation in the individual GH signaling pathways between cell types, depending on the relative expression of the component parts. Furthermore, the expression of target genes of the GH signaling pathway can differ between cell types. An example of the latter phenomenon is that experimental data in the rat liver have shown that *Igfbp3* mRNA is selectively concentrated in portal venous and sinusoidal endothelium (not in Kupfer cells), while *Igf1* and *Igfals* mRNAs are localized in hepatocytes [10]. This would imply that ternary complex formation takes place in the small venules of the portal system.

Based on this experimental information one could speculate that there might be growth disorders in which only IGF-I and acid-labile subunit (ALS) secretion are affected, and others where only IGFBP-3 is deficient. Indeed, in clinical practice, some short children show a low serum IGF-I, in contrast to a normal serum IGFBP-3, and one could predict that in such children a hepatocyte-specific disruption of GH signaling may occur that only affects IGF-I and ALS secretion.

Even within the same cell-type, GHR expression may show a spatial and temporal expression pattern. For the rat, we showed such pattern for epiphyseal growth plate chondrocytes using immunohistochemistry [11]. In 1-, 4- and 7-week old normal male rat growth plates, GHR staining was present in cells of the resting zone, proliferative cells, and early hypertrophic chondrocytes, while in 12-week-old rats staining was mainly seen in early hypertrophic chondrocytes in the transition zone between the proliferative and hypertrophic cell layer.

3. Pathophysiological mechanisms that could lead to GHI

With respect to its anabolic actions, GH induces *IGF1* transcription (as well as transcription of several other genes, including genes encoding for IGFBP-3 and ALS) through a complex, regulatory cascade that is initiated when GH binds to the GHR on the cell surface. This interaction causes the GHR to activate janus kinase 2 (JAK2), which in turn phosphorylates members of the STAT family. STAT5B is considered most important for the GH-IGF-I axis. The phosphorylated form of STAT5B subsequently translocates to the nucleus and activates the expression of various target genes. GH also activates the MAPK and PI3K pathways, and several proteins in these three pathways interact with each other and with proteins involved in other ligand signaling pathways. Therefore, in theory, there are multiple potential mechanisms that could lead to GHI.

3.1. Conditions influencing synthesis, complex-formation, or recycling of GH receptors

The GHR is synthesized as a precursor, provided with 5-high mannose oligosaccharides, and dimerizes in the endoplasmic reticulum (ER). Next, the receptor travels to the Golgi complex, where it becomes complex-glycosylated, and it then translocates to the cell surface. There, the GHR exists as a constitutive dimer, and binding of the hormone realigns the subunits by rotation and closer apposition, resulting in juxtaposition of the catalytic domains of the associated tyrosine-protein kinase JAK2 below the cell membrane [12]. Recently, a Dutch group suggested that GHRs may occur as ≈500-kDa complexes that dimerize into active ≈900-kDa complexes upon GH binding [13]; the dimerized complexes would then act as platforms for transient interaction with JAK2 and ubiquitin ligases. However, these observations need confirmation.

The classical example of a disorder at this level is Laron syndrome, caused by an absence or loss-of-function mutation of the GHR [14]. Depending on the nature and location of the mutation, the phenotype can vary, as reviewed recently [15]. However, it is not unlikely that there may be still uncovered disturbances in the synthesis, dimerization, transport to the Golgi complex, and complex formation that could cause a form of GHI. For example, a decreased synthesis of GHRs was suggested in a study in which RNA was extracted from peripheral blood from 35 pygmies and 14 Bantu subjects. GH1 expression was 1.8 fold reduced and GHR 8-fold reduced in pygmies in comparison to Bantu individuals [16].

At the cell surface, phosphorylation and ubiquitylation regulate the fate of the GHR: two ubiquitin ligases ($SCF^{\beta TrCP2}$ and CHIP) determine the GH responsiveness of cells by controlling its endocytosis, whereas JAK2 initiates the JAK/STAT pathway. $SCF^{\beta TrCP2}$ ubiquitylates the GHR, which is subsequently internalized *via* clathrin-coated pits and degraded in lysosomes [13]. Disorders in this part of the GH signaling pathway have not yet been described.

GHRs are constitutively inactivated by several members of the suppressor of cytokine signaling (SOCS) family, of which SOCS-1 binds JAK2 and inhibits JAK2 kinase activity, SOCS-3 binds phosphorylated GHR and inhibits JAK2 kinase activity, and SOCS-2 and cytokine-inducible SH2-containing protein (CIS) bind phosphorylated GHR and compete with STAT5B for GHR binding sites. Protein tyrosine phosphatases bind to the activated receptor complex and dephosporylate phosphotyrosines on JAK2 or GHR, thus inhibiting GH signaling. The GHRs are then internalized and degraded and ubiquitinated, with these processes being facilitated by CIS and ubiquitin ligase activity associated with SOCS [17]. Under basal conditions, βTrCP binds to the UbE motif and to the constitutively phosphorylated DSGRTS sequence on the GHR. Both motifs contribute equally to the continuous GH-independent turnover of the GHR. After GH stimulation, certain events (such as JAK2 release from the receptor or posttranslational modification on the UbE) may result in an increased βTrCP binding to the UbE motif and, consequently, faster endocytosis of GHR [18].

A disorder of GHR degradation, termed "growth hormone transduction defect", was postulated in two articles from Greece, both based on *in vitro* studies in skin fibroblasts from children with apparent ISS and normal GHR expression. These children had a severe growth impairment (height SDS < −3.5 SDS), normal GH secretion, low IGF-I concentrations but significant increased IGF-I after induction with GH and a significant catch-up growth on GH therapy. In vitro studies on skin fibroblast cultures in the first study showed a reduced ability for activation of STAT3, a cell-cycle arrest at the G0/G1 phase, increased levels of cyclin-dependent kinase inhibitor ($p21^{WAF/CIP1}$) and reduced levels of cyclins [19]. A subsequent study on 10 patients by the same authors showed retarded activation of pJAK2 and pSTAT5 and increased ubiquitinated CIS. GHR was only localized in the cytoplasm, while in controls GHR was also localized at the membrane. The authors speculated that abnormal GH signaling was caused by overexpression of CIS,