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Review article

Lessons learned from studies with the growth hormone receptor

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ABSTRACT

Findings related to GH's biological activities have continued to be a fascinating topic over the past decade. Below, I will review several items related to the actions of GH including the GH/GHR interaction, pegvisomant (a GH receptor antagonist), GHR gene disruptions in mice, and clinical consequences of human GHR gene mutations.

1. Introduction

A growth-promoting activity derived from the pituitary gland was first discovered in 1921 [1]. Since then, human growth hormone (hGH) has been extensively studied. In the late 1970s, hGH complementary DNA (cDNA) was cloned and expressed [2]. Subsequently, several mammalian GH genes and cDNAs were cloned and their nucleotide sequence determined. In 1985, recombinant (r)hGH was generated and approved for clinical use in GH deficient (GHD) patients. Subsequently, several other clinical indications have been approved for hGH including Turner Syndrome, Chronic Renal Insufficiency, Small for Gestational Age or Intrauterine Growth Retardation, Prader–Willi Syndrome, Idiopathic Short Stature, SHOX Deficiency, and Noonan Syndrome.

In the late 1980s, the GH receptor (R) cDNA was cloned and the nucleotide sequence reported. Major scientific findings have been presented related to GH/GHR over the past decade. I do not intend to cover each of them; however, I will focus on four areas in this short review, namely: (1) the mechanism by which GH interacts with the GHR; (2) pegvisomant, a GHR antagonist; (3) mice in which the GHR gene has been ubiquitously or conditionally disrupted; and (4) clinical manifestation of mutations in the human GHR gene.

It is a daunting task to cover GHR related reports over the past decade is a short review, thus, I must apologize to my colleagues where I have omitted their contributions.

2. GH/GHR interaction

The GH molecule has two sites (Site 1 and Site 2) each of which interacts with the extracellular region of the preformed GHR dimer. GH Site 1 first interacts with one of the GHRs and then GH's Site 2 interacts with the other GHR to give a functionally or properly dimerized complex that ultimately induces intracellular signaling. For years, the precise mechanism by which GH binding converts the inactive pre-dimerized GHR to its active conformation was a mystery; however, Waters and his colleagues have presented elegant data over the past 10 years that has become 'dogmatic' in terms of this interaction. They have shown that the interaction of GH with the GHR dimer causes rotation of the two GHRs relative to each other. The resulting repositioning of the intracellular domains of the GHRs brings the catalytic domains of the associated JAK2 molecules in position for trans-phosphorylation to occur. Subsequently to the tyrosine phosphorylation of the JAK2 molecules, several GHR intracellular tyrosines are phosphorylated which provides docking sites or enables binding of adaptor proteins such as STAT-5 [3–5]. This elegant data of the GH/GHR interaction and the animated model of GH induced GHR rotation developed by Waters and colleagues, has greatly influenced and stimulated research on the interaction of GH with GHRs and represents a seminal finding in the GH field over the past decade [3–5].

Additionally, much research has been presented concerning GH induced intracellular signaling systems. A version of the GH induced intracellular signaling pathways is shown in Fig. 1 [4]. Future research that continues to investigate the mechanics of GH induced rotation of the GHR and the relationship of this rotation to individual intracellular signaling pathways will continue to be an active area of study.

3. Pegvisomant: a GHR antagonist

3.1. Development of a GHR antagonist (A) into a drug (pegvisomant)

A novel class of compounds was discovered in the late 1980s/early 1990s that antagonized the action of GH. They were termed GHR antagonists (A). Of the 191 amino acids found in the GH molecule, glycine 119 of mammalian GHs and 120 of human hGH (both found in

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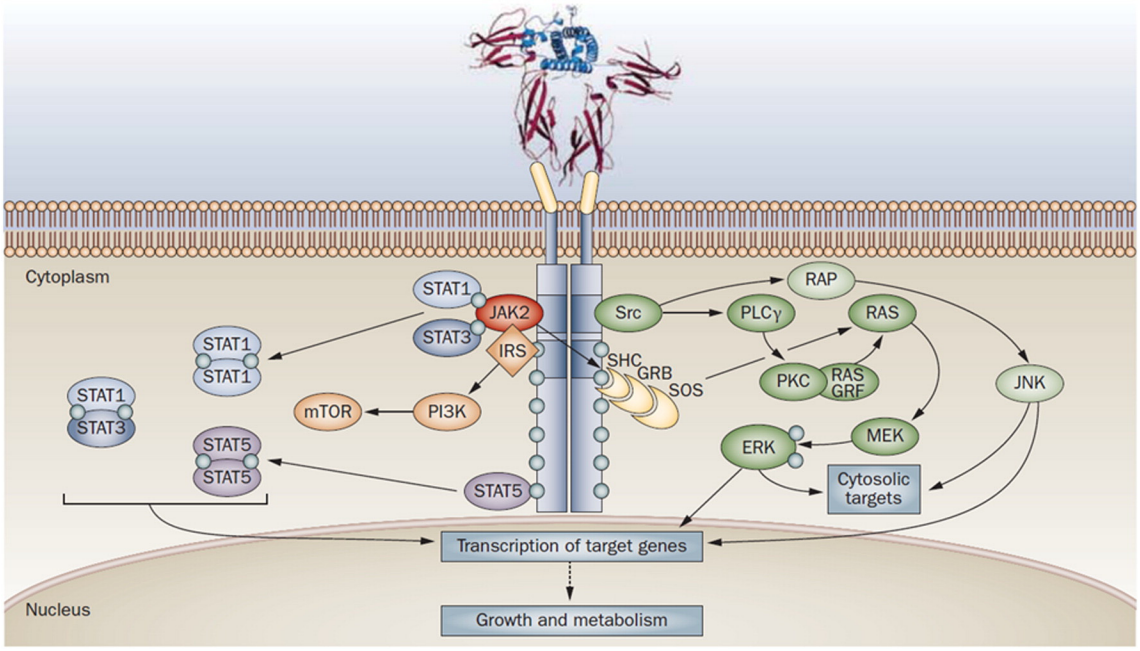

Fig. 1. GH/GHR induced intracellular signaling. A model depicting intracellular signaling intermediates induced by binding of GH with the GHR dimer. From Brooks AJ, Waters MJ. The growth hormone receptor: mechanism of activation and clinical implications. Nat Rev Endocrinol. 2010 Sep;6(9):515–25

GH's third alpha helix) were found to be crucial for GH action. Substitution of these glycine residues with any amino acid but alanine resulted in GHRA [6].

In vitro and in vivo studies of GH receptor antagonists (GHRAs) have demonstrated that they can counteract the pathologic conditions of excess GH. Therefore, clinical indications for GHRAs include acromegaly, diabetic nephropathy, diabetic retinopathy, and certain types of cancers. The GHRA (hGH-Gly120Lys), like wild-type GH, has a short half-life [7] which limits its utility in clinical settings. In order to develop the GHRAs into a drug, the half-life of the molecule needed to be increased. To this end, the GHRA was modified by the addition of polyethylene-glycol (PEG). The resulting hGH-Gly120Lys with four to five PEGs and a molecular mass of ~50 kDa has a half-life of approximately 90 h in humans after single intravenous (iv), intraperitoneal (ip), or subcutaneous (sc) injection [8]. When mice (and later humans) received a daily sc single injection of various doses (0.25 to 4 mg/kg) of hGHGly120Lys-PEG or vehicle for 5 days, a significant, dose-dependent suppression of IGF-1 was seen starting at day 3. The maximum suppression (up to 70%) of IGF-1 production was achieved by 1 mg/kg dosing at day 6 after the first injection. These results suggest that exogenous administration of hGHGly120Lys-PEG can dramatically decrease serum IGF-1 levels.

These mouse data led to the development of the first GHRA for the treatment of patients with acromegaly. This GHRA included eight amino acid substitutions at Site 1 of hGH along with the original Gly120Lys substitution at Site 2 and four to five PEG additions (Fig. 2). As stated above, pegylation of the molecule reduces clearance and

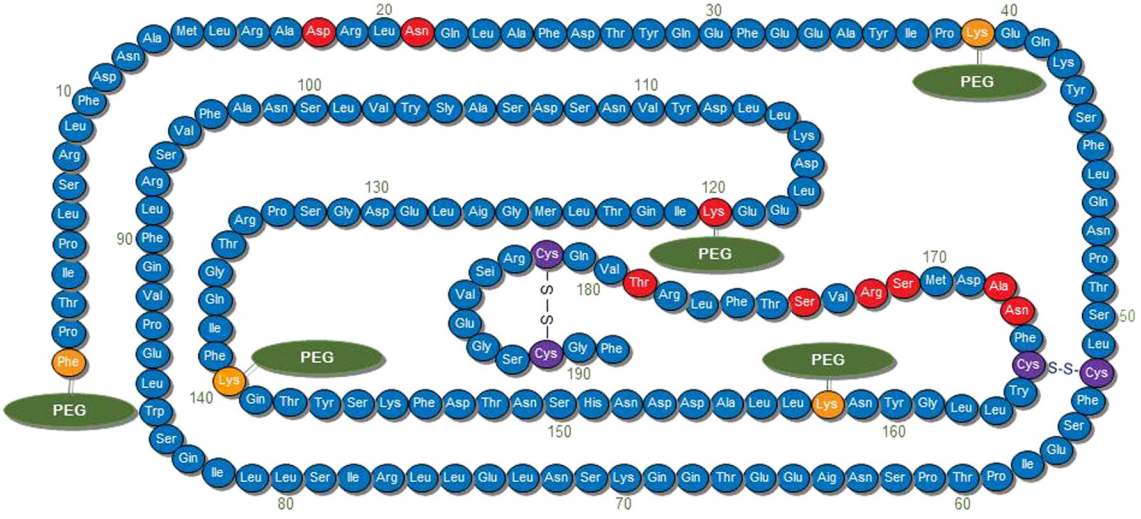

Fig. 2. The amino acid sequence of pegvisomant. The molecule, like hGH, contains 191 amino acids. Five pegylation sites are designated with a green PEG symbol. The related Lys residues are depicted in orange (except Lys at position 120 which is indicated in red). Other amino acid changes found in pegvisomant that differ from wild type hGH are shown in red. Significantly, Lys at position 120 replaces Gly. This amino acid substitution generates the GHRA.
From Kopchick JJ, List EO, Kelder B, Gosney ES, Berryman DE. Evaluation of growth hormone (GH) action in mice: discovery of GH receptor antagonists and clinical indications. Mol Cell Endocrinol., in press

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