Growth Hormone & IGF Research 42–43 (2018) 58–65

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

journal homepage: www.elsevier.com/locate/ghir

Review article

A useful model to compare human and mouse growth hormone gene chromosomal structure, expression and regulation, and immune tolerance of human growth hormone analogues

Peter A. Cattini[a,*], Margaret E. Bock[a], Yan Jin[a], James A. Zanghi[b], Hana Vakili[c]

[a] Department of Physiology & Pathophysiology, University of Manitoba, Manitoba, Canada
[b] Elixera, Inc., Los Altos, California, USA
[c] Department of Pathology, University of Texas Southwestern Medical Center, TX, USA

ARTICLE INFO

Keywords:
hGH
Chromosomal looping
Circadian control
Hyperinsulinemia
Somatopause
GH analogue immune tolerance

ABSTRACT

Human (h) pituitary growth hormone (GH) is both physiologically and clinically important. GH reaches its highest circulatory levels in puberty, where it contributes to energy homeostasis and somatogenic growth. GH also helps to maintain tissues and organs and, thus, health and homeostasis. A reduction in the rate of hGH production begins in middle age but if GH insufficiency occurs this may result in tissue degenerative and metabolic diseases. As a consequence, hGH is prescribed under conditions of GH deficiency and, because of its lipolytic activity, stimulation of hGH release has also been used to treat obesity. However, studies of normal GH production and particularly synthesis *versus* secretion are not feasible in humans as they require sampling normal pituitaries from living subjects. Furthermore, human (or primate) GH structure and, as such, regulation and potential function, is distinct from non-primate rodent GH. As a result, most information about hGH regulation comes from measurements of secreted levels of GH in humans. Thus, partially humanized hGH transgenic mice, generated containing fragments of human chromosome 17 that include the intact hGH gene locus and many thousands of flanking base pairs as well as the endogenous mouse (m) GH gene provide a potentially useful model. Here we review this mouse model in terms of its ability to allow comparison of hGH *versus* mGH gene expression, and specifically: (i) GH locus structure as well as regulated and rhythmic expression; (ii) their ability to model a clinical assessment of hGH production in response to overeating and hyperinsulinemia as well as a possible effect of exercise, and (iii) their hGH-related immune tolerance and thus potential for testing hGH-related analogue immunogenicity.

1. Where and when is growth hormone produced?

Growth hormone (GH), also referred to as somatotropin, is the product of the somatotrophs of the anterior pituitary gland. It is the most abundant hormone produced by the anterior pituitary gland [66]. GH is a 22-k Dalton (kDa) polypeptide hormone composed of 191 amino acid residues forming a single chain with four helical regions and two disulfide bridges [17]. Pituitary GH is generally regarded to be an essential factor for regulating somatic growth in vertebrates from fishes to mammals [34,57]. Human (h) growth hormone (GH), like other structurally related cytokines, plays an important role controlling health and disease processes [8]. GH is produced preferentially by somatotrophs in the anterior pituitary gland, and exerts its effects directly through its own receptor or indirectly through the stimulation of insulin-like growth factor 1 (IGF1) [60]. GH reaches its highest circulatory levels in puberty, where it contributes to energy homeostasis and somatogenic growth [45]. GH also helps to maintain tissues and organs, including bone and muscle mass [5], metabolic, central nervous system and physical adaptations [59] that together maintain health and homeostasis [76]. Thus, while a decline in the rate of hGH production from middle age (somatopause) is normal, GH insufficiency, if it occurs, has been linked to osteoporosis, sarcopenia, cognitive dysfunction as well as cardiac and metabolic diseases [3,50,62,63].

2. What limits studies of hGH synthesis?

Studies of GH production and particularly synthesis *versus* secretion are not feasible in humans as they require sampling pituitaries from living subjects. Human GH-secreting pituitary adenomas are available but are relatively rare compared to prolactinomas [13]. When assessed

* Corresponding author at: Department of Physiology & Pathophysiology, University of Manitoba, 745 Bannatyne Avenue, Winnipeg, Manitoba R3E 0J9, Canada.
E-mail address: peter.cattini@umanitoba.ca (P.A. Cattini).

https://doi.org/10.1016/j.ghir.2018.09.001
Received 7 July 2018; Received in revised form 15 August 2018; Accepted 6 September 2018
Available online 12 September 2018
1096-6374/

for a response in primary culture, it is not always clear if effects are on hGH secretion and/or cell survival as a result of cell death, lysis and release of intracellular contents, particularly if a marker such as lactate dehydrogenase in the medium is not assayed. In addition to being rare, these tumors are also variable in size and composition making mechanistic assessment of synthetic processes difficult. Some tumors may show no hGH-immunoreactivity, or display variable (control) hGH levels when maintained in culture [13]. These same hGH-secreting cells may also produce prolactin, which is a lactotroph and not a somatotroph product, suggesting abnormal expression and/or the presence of non-somatotrophs [13].

Historically, murine (mouse and rat) systems have been used to model a variety of human diseases because of their anatomical, physiological and genomic similarities to humans. As such, rodents have been the primary source of models for investigations into the physiological regulation of GH, growth and metabolic impact of GH signaling. Although a limited number of non-human primate studies have been reported, this system is not readily accessible. As a result, our knowledge of GH gene control is largely derived from studies done using rodent GH-secreting pituitary cell lines (*e.g.*, GC, GH1, GH3, GH4C1, MtT/S, and MtT/E) and the endogenous rodent GH gene [36,47,49,61]. There are distinct differences in structure between the human (primate) and mouse (non-primate) GH genes, including in both the flanking and coding DNA. As a result, hGH and rodent GH have the potential for different regulatory control and function [32,39,46,71,81,82,84].

In terms of evidence to support differences in primate and non-primate GH gene structure, only 0.3 kilobases (kb) of rat GH upstream DNA is needed to direct efficient pituitary expression of a transgene in mice, whereas the equivalent result requires 14.5 kb of human pituitary GH gene promoter and upstream DNA [32,39]. In terms of protein sequence and based on phylogenic analyses, primate GH diverged, evolutionarily, from the other eutherian (placental mammals) orders approximately 75 million years ago [40,48,80]. This divergence resulted in a 59–63 amino acids difference in the peptide sequence of 190 amino acids, which accounts for a difference of ~33% between primate and rodent GHs [40,48,80]. This is in comparison to most of the other non-primate GHs, which differ from each other by a maximum of four amino acids in their mature peptide [48,80]. Thus, it is reasonable to expect differences between the biological properties of GHs in primates and rodent due to their distinct amino acid sequences and structure. In fact, this is the case and it is clear that specificities in binding of GHs to their respective receptors vary markedly [25,79]. Thus, in terms of coding sequences and function, hGH can bind both the human GH and prolactin receptors while mouse and rat GH bind only the murine GH receptor (and weakly to the hGH receptor) and not prolactin receptors [11,81,82]. As a result, hGH but not murine GHs possess lactogenic in addition to their somatogenic function. Thus, human (or primate) GH structure and, as such, regulation and potential function, is distinct from non-primate rodent GH [32,39,46,71,81,82,84]. As a result, most information about hGH regulation comes from measurements of secreted levels of GH in humans [1,4,27,42,51,78,83]. Thus, the possibility of generating partially humanized hGH transgenic (TG) mice as a model to investigate hGH expression and regulation *in vivo* was pursued by investigators. A prerequisite was a transgene containing the necessary genetic coding and regulatory information to support pituitary-specific and rhythmic expression of hGH, as well as be able to respond appropriately to the metabolic environment, including excess insulin.

3. What sequences are required for pituitary and regulated expression of the hGH gene *in vivo*?

Duplications of the pituitary GH gene in higher primates have given rise to a family of GH-related genes. The human (h) GH-related gene family consists of five members including pituitary growth hormone (*hGH-N* or *hGH1*) as well as placental GH (*hGH-V*), and the chorionic somatomammotropin (CS) genes (*hCS-A*, *hCS-B* and pseudogene *hCS-L*), contained within a single 47 kb locus on chromosome 17 [12]. The family is flanked upstream by the lymphocyte-specific CD79b and skeletal muscle sodium channel α-subunit SCN4A genes, and downstream by the testicular adhesion molecule-1 (TCAM-1) gene [6,7]. Polypeptide products of all members of the GH gene family are highly related based on their structure and biochemical properties [43,44,65,69]. Despite their structural relatedness, these hormones are produced in a tissue-specific manner. Human *GH-N* is expressed predominantly in the somatotrophs of the anterior pituitary gland, whereas the placental members, hCS (also known as placental lactogen) and placental GH (hGH-V), are produced by syncytiotrophoblasts in the placenta during pregnancy [12].

Transfection of rat pituitary tumor cells with *hGH-N* fragments suggested that ~500 base pairs (bp) of *hGH-N* promoter DNA is sufficient to drive pituitary-specific expression, based on the presence of two pituitary-specific transcription factor POU-class homeo-domain Pit-1 sites in the first 150 bp [9,10,35,38]. Mutations of Pit-1 are associated with dwarfism in mice and combined pituitary hormone deficiency in humans [85], but this same ~500 bp promoter was not sufficient to drive consistent or specific *hGH-N* expression in TG mice *in vivo* [21,32]. Thus, a nuclease hypersensitive site (HS) assay was used to detect regulatory regions in the *hGH-N* locus in human anterior pituitary tissue in an unbiased manner [29,32,67]. Five 'open' chromatin domains (HS I-V) were identified ~14–32 kb upstream of *hGH-N* [32]. Inclusion of these sequences conferred site of integration-independent pituitary somatotroph-specific *hGH-N* transgene expression *in vivo*, and defined the hGH locus control region (LCR) [32,67]. HS I/II are pituitary-specific and located ~15 kb upstream of *hGH-N*, HS IV is placenta-specific [32], and HS III and V are found 28–32 kb upstream of *hGH-N* and appear to be 'constitutive' [33]. HS I/II possesses pituitary enhancer activity *in vitro* and *in vivo*, and the pituitary-specific nature of HS I/II is related to the presence of multiple Pit-1 binding sites [31,67]. HS I/II alone can support pituitary expression *in vivo* but levels are variable unless HS III/V are present and confer integration independence by acting as a boundary region [22,32]. In this context, HS V has enhancer blocking activity that is associated with CCCTC-binding factor (CTCF) [30].

4. What is the phenotype and genotype of partially humanized hGH transgenic mice?

Based on the presence of a distant LCR as characterized by five nuclease hypersensitive sites, multiple independent partially humanized hGH-TG mouse lines containing *hGH-N* and the upstream LCR in single continuous ~100–200 kb fragments of human chromosome 17 were generated [29,32]. These fragments include *hGH-N* as well as the lymphocyte CD79B gene and part of the skeletal muscle sodium channel SCN4A gene upstream and placental chorionic somatomammotropin (CS) genes (including *hCS-L* and *hCS-A*) downstream [29,32]. Thus, they contain all the DNA elements required for specific *hGH-N* expression *in vivo* [32]. All the regulatory regions identified using nuclease hypersensitivity as an unbiased assay for screening, specifically HS I-III and V, are features of the *hGH-N* locus in human pituitary somatotrophs. Importantly, HS I-III and V are all re-established in the pituitaries of TG mice [29,32,67].

Two of these mouse lines, the CD-1-derived 171hGH/CS-TG (or F-74) and 141hGH/CS-TG (F-81) have been well described structurally and functionally in terms of the hGH gene. The presence of a single *hGH-N* locus and its chromosomal location in these mice was demonstrated by quantitative PCR and DNA (Southern) blotting [29] and fluorescence *in situ* hybridization (FISH) using cytogenetic preparations of ear fibroblasts (Fig. 1). The hGH gene locus is located on chromosomes 14 at band 14A1 and on chromosome 8 at band 8A1 in 171hGH/CS-TG and 141hGH/CS-TG mice, respectively. Both lines synthesize hGH specifically in the pituitary, and serum hGH as well as mouse (m) GH is detectable by enzyme-linked immunosorbent assay (ELISA)