The Endocrine Function of the Placenta: Human Placental Growth Hormone Variant

Frank Talamantes

Sinsheimer Laboratories, University of California, Santa Cruz, California 95064, USA

The placenta of mammals is a very adaptable organ that accomplishes many critical functions necessary for the well-being of the host and developing fetus. For example, the placenta serves as an attachment site to secure the developing fetus to the uterus, and it transports nutrients from the maternal to the fetal compartment. During the evolution of viviparity, the placenta has assumed various morphologies and a diverse set of endocrine functions. As an endocrine organ, it is very versatile. Its versatility arises from its ability to produce a wide array of protein and steroid hormones. The protein hormones that are elaborated by the placenta share structural and functional overlap with those produced by the hypothalamus and the anterior pituitary gland. The structural and biologic features of the various protein hormones produced by the placenta are very species-dependent. For example, in humans the placenta produces human chorionic gonadotrophin (hCG), a hormone that shares structural and functional properties with pituitary luteinizing hormone; decidual prolactin and chorionic somatomammotrophin (hCS) (also referred to as human placental lactogen), which share structural and functional overlap with pituitary growth hormone (hGH) and prolactin; and human placental growth hormone (human growth hormone variant, hGH-V), which shares structural and functional overlap with pituitary growth hormone (reviewed in ref. 1). In this review, I will limit my discussion to the knowledge that is available on the structure and function of hGH-V.

The human growth hormone locus (Fig. 1) contains five structurally related genes spanning 47 kB (2,3) and located on chromosome 17q22-q24 (4). The five growth hormone genes are all organized in the same transcriptional orientation and are each composed of five exons. From 5' to 3' these genes are as follows: growth hormone (hGH-N), chorionic somatomammotrophin-like (hCS-L), chorionic somatomammotrophin-A (hCS-A), growth hormone variant (hGH-V), and chorionic somatomammotrophin-B (hCS-B) (5). Interestingly, despite linkage and homology, the growth hormone genes are expressed in two mutually exclusive tissue-specific patterns. The hGH-N gene is expressed solely in the somatotrophs and lactosomatotrophs of the anterior pituitary, whereas hCS-L, hCS-A, hGH-V, and hCS-B are specifically expressed in the syncytiotrophoblastic layer of the placenta (reviewed in ref. 6). Each
The presence of hGH-V in the blood and placenta was originally suggested by the observation that when two different monoclonal antibodies were used to measure the concentration of GH-like immunoreactivity in the serum, the resulting gestational profiles were very similar until about week 25, but differed considerably thereafter (8,9). After week 25, increasing concentrations of GH-like immunoreactivity were detected with one of the antibodies, whereas declining concentrations of GH-like activity were detected with the other. GH-like activity was also detected in term placental extracts with the former antibody, but not the latter, which suggested that the placenta contains GH-like activity that is not related to the presence of pituitary hGH (9). Further evidence to substantiate the presence of a placental growth hormone included the binding of an hGH-V probe to placental RNA by dot-blot analysis (10), and the cloning of a complementary DNA (cDNA) for placental growth hormone from a placental cDNA library (11). Placental growth hormone was then purified (12), and sequence analysis of its cDNA and the purified protein showed that it was the product of the hGH-V gene.

GENE STRUCTURE

The hGH-V gene was discovered when the entire hGH cluster was sequenced (3). Originally, it was speculated that it was a pseudogene. The primary transcript of the hGH-V gene undergoes alternative splicing to yield hGH-V and hGH-V2 messenger RNAs. Messenger RNA for hGH-V conforms to the predominant splicing pattern of other members of the gene family and contains the five complete exons of the gene and no intron sequences. On the other hand, the hGH-V2 messenger RNA contains five exons and all of intron 4 (13). Both messenger RNAs are present in the placenta, with hGH-V2 messenger RNA accounting for about 5% and 15% of hGH-V gene transcripts in the first and third trimesters, respectively (14). Unlike the primary transcript of the hGH-N gene, which undergoes alternative splicing in exon 3 to yield a messenger RNA in which the first 15 codons of exon 3 are deleted, the primary transcript of the hGH-V does not appear to undergo alternative splicing at this site (15). The nucleotide differences between the hGH-N and hGH-V genes
that are responsible for this difference in splicing patterns have been identified (16). The transcription start site of the hGH-V gene is 30 nucleotides downstream of a TATAAA sequence (16). The 5'-flanking region of the hGH-V gene contains the distal, but not the proximal, binding site for GHF-1 (17,18).

PROTEIN STRUCTURE

The hGH-V protein consists of 191 amino acids. The hormone is glycoslated (5,11,12,19) and contains two disulfide bonds linking cys-53 with cys-165 and cys-182 with cys-189. Interestingly, the amino acid sequence of hGH-V differs from the pituitary-derived growth hormone by 15 amino acids (Fig. 2). Thirteen of the amino acid differences reside in the mature protein and are located throughout the sequence. Two of these amino acid residues in the PRL receptor binding domain of pituitary hGH that have been shown to be involved in coordinating zinc in the hGH-PRL receptor complex (20) are not conserved in hGH-V (positions 18 and 21). The ability to bind zinc in this region appears to be important for binding of hGH and hCS to the extracellular domain of the human PRL receptor but not for the binding of PRL to the same receptor (20,21). Consequently, the binding of hGH-V to the human PRL receptor may occur by a mechanism that is more readily compared with that used by hPRL than with that used by hGH and hCS. When the hGH-V messenger RNAs were cloned from the placenta, it was observed that the hGH-V gene encodes two alternatively spliced messenger RNAs, hGH-V and hGH-V2 (13). Although the actual protein of the hGH-V2 has not been found to be sequenced, its predicted structural sequence and the presence of a hydrophobic domain in the novel C terminus have led to suggestions that hGH-V2 could be an integral membrane protein (13).

![Amino acid sequence of hGH and hGH-V. Numbering for the hGH sequences is shown above the sequence. Helical segments in hGH amino acids are 9-34, 38-47, 64-70, 72-92, 94-100, 106-138, and 155-184.](image-url)
The expression of the hGH-V gene has been sublocalized within the placenta by Northern blot analysis and in situ hybridization. Northern analysis of messenger RNA isolated from four placental layers—amnion, chorion, decidua, and villi—showed specific expression localized only to the villi (13). Expression of the hGH-V was sublocalized within the villi to the syncytiotrophoblast epithelium by histohybridization using a cDNA probe specific for intron 4 of the hGH-V gene (22).

**GESTATIONAL PROFILE**

The gestational profile of hGH-V in maternal serum and its presence in other body compartments (e.g., amniotic fluid) has been determined as the difference in growth hormone values obtained with assays using two monoclonal antibodies, one of which recognizes hGH-V and the other of which does not (9,23). A difference in growth hormone values measured with these two antibodies appears between weeks 21 and 26 of pregnancy, suggesting that hGH-V appears in maternal serum in detectable concentrations during this period. Its concentration increases until about week 36 and then remains relatively constant for the remainder of pregnancy (9,23,24). However, hGH-V may be present in maternal serum much earlier than week 21, as messenger RNAs for hGH-V and hGH-V2 are present in the placenta by week 9 (22). The apparent absence of hGH-V in serum in early pregnancy probably reflects the relative insensitivity of the assay used. At term, hGH-V has not been detected in amniotic fluid or fetal serum (24).

The gestational profile of hGH-V in maternal blood parallels changes in the level of hGH-V messenger RNA in the placenta (14), suggesting that the rate of hormone synthesis could be a major determinant of the hGH-V gestational profile in maternal blood. The retention of intron 4 in the hGH-V2 messenger RNA by alternative splicing is regulated during the development of the placenta, resulting in a threefold increase of hGH-V2 messenger RNA between weeks 10 and 38 of gestation. This regulation of hGH-V intron 4 splicing suggests that synthesis of hGH-V2 might be important for placental function (13).

**BIOLOGIC FUNCTION**

In an attempt to understand the biologic function of hGH-V, the activity of the hormone has been analyzed in various bioassays and according to its ability to bind to both PRL and growth hormone receptors. In the rat NB2 lymphoma cells, hGH-V has been shown to be mitogenic, as are hGH and PRLs (25,26). It has also been shown that hGH-V binds to PRL binding sites on the lymphoma cells and in rat liver (27). Its affinity for the rat PRL receptor and its potency in the NB2 cell assay are significantly less than that of pituitary hGH (26,27). This suggests that hGH-V
HUMAN PLACENTAL GROWTH HORMONE VARIANT 79

is not as potent a lactogen as hGH in the rat. Like pituitary growth hormone, hGH-V has been shown to have somatogenic activity in that it stimulates body weight gain in the hypophysectomized rat (26). In addition, it has been shown to stimulate glucose oxidation and lipolysis in rat adipose tissue (28). In both systems, the potency of hGH-V is comparable with that of pituitary hGH. Although the somatogenic biologic activity of hGH-V has not been examined in human tissue, the hormone has been shown to bind to the circulating human growth hormone binding protein with an affinity similar to that of pituitary growth hormone (29). Given that human growth hormone binding protein is structurally similar to the extracellular binding domain of the human growth hormone receptor (30), the affinities of hGH-V and pituitary growth hormone for the growth hormone receptor are probably very similar. Based on the findings described above, it has been hypothesized that the expression of hGH-V during middle to late gestation, coupled with its preference for the somatogenic receptor, may be responsible for the acromegaloïd facial features detected in some women during pregnancy (29). The replacement of hGH-N by hGH-V in maternal serum at midgestation (9) suggests that hGH-V might have a role in mediating the metabolic demands of pregnancy. Examination of the gestational profiles of hGH-V and insulin-like growth factor-1 (IGF-1) in women has revealed a significant correlation between them, suggesting a possible role for hGH-V in regulating IGF-1 production in the second half of pregnancy (23). Additional observations provide evidence that hGH-V may also have a role in preparing the breast for lactation. For example, somatogen receptor messenger RNA has been observed in breast tissue (31,32), growth hormone binding protein has been found in milk (33), and precocious mammary development and lactation have been demonstrated in transgenic mice expressing growth hormone locally in mammary gland tissue. In addition, it has been observed that growth hormone has a potent effect on mammary gland development by acting through the somatogen receptor (34). Thus, given this set of data, one can hypothesize that hGH-V encodes an important gestational hormone whose action might be important for the mother, the developing fetus, or both. Specific binding of hGH-V to human placental membranes (35), expression of hGH receptor messenger RNA (36), and immunoreactivity (37) in human placenta raises the possibility that hGH-V could have an autocrine function in the placenta.

WHAT REMAINS TO BE DISCOVERED

Although a considerable amount of information has accumulated about the structure of the protein and gene for placental growth hormone-V, more effort has to be directed at understanding what regulates the expression of this hormone and what are its relevant functions during pregnancy. To begin to appreciate its function, better discrimination of the circulating forms of hGH is needed through the development of specific methods for measuring hGH-V alone and not hormones like hGH-N. The ability to generate sizable quantities of highly purified hGH-V will permit further detailed studies characterizing its biologic properties. The majority of studies
examining the biologic activity of hGH-V have used heterologous systems. Although much useful information can be generated by cross-species analysis, it is of the utmost importance that attempts be made to sort out the biologic activities of hGH-V on human tissue. To date, very little information exists about the secretagogues that may be important in regulating the expression of hGH-V. One has to be able to verify the hypotheses that have been generated about the role of the hGH-V2 gene product. The maintenance of "normal" pregnancies does not appear to require the presence of hGH-V. This raises the interesting question about the potential compensatory mechanisms that may exist in such cases. Although care has to be used in extrapolating data generated from nonprimate species to the primate species, it may be possible to learn a significant amount about placental growth hormone if one examines whether other species (e.g., mice) produce a placental growth hormone. If this turns out to be the case, it would be easier to design experiments that would shed light on the function and regulation of expression of placental growth hormone.

REFERENCES


DISCUSSION

Dr. Chard: Perhaps I could start off on the functional aspect. I believe that pregnancies with a gene deletion have been described but that fetal growth was effectively normal in those pregnancies.

Dr. Talamantes: That is true, but we don’t know whether it was compensatory mechanisms that had taken over in those particular cases. It’s the same with placental lactogen. It is true that there have been cases in which the genes have not produced the product and we have had a “healthy birth.”

Dr. Anthony: Am I correct in remembering that hGH-V does not get into the fetal circulation?

Dr. Talamantes: Yes, it is not found in the amniotic fluid or in the fetal circulation.

Dr. Milliez: Do you have any data on twin pregnancies, and do you have any data on trophoblastic disease?

Dr. Talamantes: I have not seen anything on twin pregnancies. It does not make a difference if you have a male or a female fetus, and there is only one paper that I know of looking at pathologic pregnancies. I think only in diabetes was there a hint that there might be an alteration. But this is based on only one study. There have not been enough good studies to look at this carefully.

Dr. Ogata: What is the relationship between hGH-V and IGF? You showed plasma concentrations, but is there any information concerning molecular secretion?

Dr. Talamantes: The only data looking at this have examined correlations between the amount of hGH-V and IGF-1. What I neglected to say is that hGH-V has the same affinity for the growth hormone binding protein as does normal growth hormone. In humans and in rodents, there is a circulating binding protein for growth hormone. In the case of the human, the growth hormone binding protein is the hormone binding domain of the receptor once it anchors itself into the cell. The same affinity exists for hGH-V, and it may well be circulating with a binding protein in the pregnancy, but that has not been checked.

Dr. Owens: What is the cross-reactivity of hPL on the somatogenic receptors, given the much higher concentration in the circulation? Is it able to compete effectively?

Dr. Talamantes: No, these assays are really specific. The cross-reactivity was quite low.

Dr. Owens: How do you envisage this growth hormone variant having any real role in control of fetal growth if, for example, it is present in very low concentrations in fetal blood. You have talked about the evidence for a role in mammary development, but do you see it being important in terms of the fetus? Is it simply that it is concerned with maternal adaptation? This would be consistent with its absence not having a great impact on the fetus.

Dr. Talamantes: My feeling is that the hormone need not be in the fetus but could affect transport of all kinds of things across the placenta. It is a unique receptor for growth hormone in the placenta. It is structurally quite different from the growth hormone receptor on the maternal side, in liver or wherever. We don’t know yet know whether growth hormone might not be important in causing transport of important nutrients across the placenta.

Dr. Chard: David Hill published data suggesting that the fetal liver has a specific hPL receptor and is therefore much more sensitive to hPL than adult tissues (1). The same has not been shown for the hGH variant, but it is another hypothesis.

Dr. Boyd: I think when we are looking for purposes for hormones one has to put it in a biologic context. I remember that two to three generations ago epiphyses weren’t fused until the early or middle 20s, and yet people started reproducing in their middle teens. I wonder
whether your human growth hormone variant could be an emergency pelvic growth hormone—whether in the pregnant women with unfused epiphyses it could have an evolutionary drive around that sort of function. You could put it to the test by looking in deprived populations at whether the achieved pelvic growth is bigger in people who have early pregnancies.

Dr. Talamantes: I am not a clinician, so I don’t know. I think that sometimes we may be looking at the wrong thing in relation to the issue of hormones and their function. For example, let’s talk about rodents, which I can deal with in more detail. If one looks at the function of growth hormone (and I am convinced that hGH-V is also very important for the human mammary gland), many years ago Roberto Ceriani at Berkeley took fetal rat skin from day 10 and organ-cultured it to see what kind of hormones were necessary to get the gland to look like that of a 20-day-old rat fetus. And what it needed was growth hormone and prolactin. However, the rat fetal pituitary does not make growth hormone until near term. So it was a question of where that hormone was coming from. That prompted us to look in the amniotic fluid, and the amniotic fluid, at least in mice, contains lots of growth hormone. Obviously, if you make an observation like this in vitro, you need to see what happens in vivo.

Dr. Milliez: I might have missed the point, but can you tell me again how early in pregnancy this hormone is produced by the placenta?

Dr. Talamantes: Circulating hormone begins at about 25 weeks. But if you look at Northern blots, you can see quite a lot of message in the first trimester. The question is whether it is not being expressed at that time. People who work in the field are convinced that at 25 weeks they can see hGH-V from the placenta.

Dr. Milliez: Is it definitely not produced in pre-embryo culture?

Dr. Talamantes: They have not looked at that.

REFERENCE