Short Communication

Complex alternative splicing of the GH-V gene in the human testis

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Abstract

The human growth hormone variant (GH-V) gene is expressed during pregnancy in the syncytiotrophoblast and, as shown recently, in the normal human testis. In addition to the classical transcript encoding for the 22 K major form, intron D-retaining processed mRNAs (GH-V2) have also been described in both tissues. In the present study we analyzed testicular GH-V RNA alternative splicing patterns, a major source of GH variability. We observed three types of GH-V-derived mRNAs by reverse transcription-polymerase chain reaction amplification of GH/placental lactogen mRNA, subsequent cloning into appropriate vectors, vector amplification, restriction-endonuclease map-analysis and double-strand sequencing of GH-V clones. Apart from the conventional splice product encoding classical hGH-V (22 K, 191 amino acids (aa)) and intron D-retaining mRNA GH-V2 (230 aa), we detected an additional GH-V mRNA variant, GH-VΔ4, utilizing a competitive splice-donor site 4 bp 5’ of the conventional exon 4/intron D splice-donor site, but retaining the genuine intron D/exon 5 splice-acceptor site. This mRNA encodes a putative 25 K protein of 219 amino acids in length, having the first 124 amino acids and, thus, two and a half structural alpha-helices in common with hGH-V. hGH-VΔ4 has lost the N-glycosylation site at Asn 140 of hGH-V, but acquires a novel site at position 148 as well as a cystein-rich domain in the 65 carboxyl-terminal amino acids, potentially involved in multiple disulfide-bridge formation. Tissue specificity and possible functions for testicular physiology remain to be investigated.

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Introduction

The human (h) growth hormone/placental lactogen (GH/PL) gene cluster, located on chromosome 17 (bands q22–24), consists of five highly related genes, GH-N, PL-L, PL-A, GH-V and PL-B. These genes share more than 90% nucleotide sequence identity in their coding and flanking regions (1).

GH/PL gene transcription is controlled in an organ-specific manner during human ontogenesis: GH-V, PL-A, PL-B and PL-L genes are transcriptionally active in the placenta (2), whereas after birth pituitary-derived hGH-N becomes the predominant endocrine-active GH. In human testes transcripts of all five genes can be observed (3) and in human ovaries PL-A/B as well as GH-N transcripts are present (4).

In contrast to hGH-N, placental hGH-V is synthesized during the first months of human life, but its functions for growth, development and metabolism are still not clear. Among the gestational polypeptide hormones, hGH-V and hPL secreted by the placenta, only hGH-V secretion is modulated by glucose, suggesting a metabolic key role for this hormone during pregnancy (5). The majority of total placental GH/PL mRNAs is derived from the PL-A and PL-B genes (95–99%) and only 1–4.2% encode GH-V gene products (1, 2, 6).

Two alternatively processed mRNAs, omitting or including intron D of the GH-V gene, are expressed by the syncytiotrophoblast resulting in either secreted (22 K, 191 amino acids (aa)) or presumably membrane-associated (26 K, 230 aa) proteins (7). Secreted hGH-V, alternatively termed placental hGH, a potent somatogen, is detectable in the serum of pregnant women and becomes the predominant serum hGH by the third trimester of gestation (8–11).

In contrast to hGH-N and hGH-V2, hGH-V contains a consensus sequence for N-glycosylation at Asn 140. Glycosylated hGH-V variants have been demonstrated by inhibition of glycosylation with tunicamycin and endoglycosidase treatment. In the placenta a 25 K glycosylated form has been observed (11), whereas GH-V transfected fibroblasts produce two hGH-V isoforms of

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24 K and 26 K, presumably differing in their oligosaccharide structures (12).

Alternative splicing which is a major source of GH/PL variability, has been especially well investigated for placental expression of PL-L (13). In the present study we aimed to elucidate the testicular splicing pattern of GH-V RNA and to delineate the amino acid sequence of potentially resulting proteins.

Patients and methods

Testicular tissues (n = 3) from previously untreated prostate cancer patients undergoing subcapsular orchietomy (age range 65–78 years) were snap-frozen in liquid nitrogen and subsequently used for mRNA analysis. cDNA from extracted pooled testicular total RNA was generated by reverse transcription (RT) as described elsewhere (3). GH/PL cDNAs were amplified by polymerase chain reaction (PCR) using a set of primers located at the beginning of exon 1, 5'-CACTCAGGTCTCGTGGGACAG-3' and at the end of exon 5, 5'-ACAGAGCGGCACTGCACGATG-3'. High fidelity, proof-reading Pfu-Polymerase (Stratagene, La Jolla, CA, USA) was used under the following conditions: 63 °C annealing temperature, 1.5 mmol/l MgCl2 and 35 cycles of amplification. cDNA products were purified from primers and nucleotides (Gene-Clean, Bio 101 Inc., Vista, CA, USA), cloned into PCR-Script vectors (Stratagene) and the plasmids amplified in Epicurian-Coli XL1 (Stratagene). GH-V clones were discriminated from co-amplified GH-N and PL-A/B cDNAs by restriction endonuclease map analysis of the plasmids with XbaI, BglII, Rsal, BamHI, BstEI and Stul (Promega, Madison, WI, USA) and subjected to subsequent double-strand sequencing using the Sequinase version 2.0 Kit (US Biochemical Corp., Cleveland, OH, USA).

Results

When analyzing testicular GH/PL transcripts (n = 38) we observed three different types of GH-V clones (n = 4). Besides classical GH-V and intron D-retaining GH-V2, we detected an additional alternative splicing variant of the GH-V gene (Fig. 1). This splice form uses a splice-donor site 4 bp 5' from the conventional splice-donor site at the beginning of intron D, but retains the splice-acceptor site at the end of intron D (Fig. 1B). This leads to a novel alternatively spliced GH-V mRNA (GH-V-D4) using a different splice-donor site 4 bp 5' of the conventional exon 4/intron D site could be observed in human testes. Grey boxes, amino acids encoding for the signal peptides; circles and triangles, amino acids encoding for the apoprotein; lines beneath the protein sequences symbolize the respective processed mRNAs. (B) Most common sequence motifs of mammalian splice-donor (bold) and splice-acceptor (underlined) sites. At the end of exon 4 only the GH-V, but not the GH-N gene, contains a thymidine located 3bp 5' from the conventional splice-donor site of intron D, thereby creating a novel splice site 4bp 5' from the genuine site and entailing an alteration in the open reading frame of the processed mRNA. (C) Delineated hGH-V-D4 protein sequence (219 aa): the first 124 amino acids of the mature protein are shared with hGH-V (22 K). In contrast, the 95 amino acid carboxy-terminal end is different and contains a cysteine-rich region of 65 amino acids in length (Cys are underlined). These cysteins are presumably involved in multiple disulfide bridge formation and possibly in the formation of a cystine-knot, similar to other growth factors such as NGF, PDGF-B and TGF-β. The Asn at position 148 is a potential site of N-glycosylation (consensus sequence: Asn-X-Ser/Thr). Bold amino acids, different from hGH-N amino acids 1–124 (7).
to a mature mRNA missing 4 bp at the end of exon 4 (GH-VΔ4) and, in consequence, to a frameshift in translation. The open reading frame (ORF) of the entire exon 5 is thereby changed in such a way that the original stop codon is overread and a new stop codon is generated near the polyadenylation signal sequence (Fig. 1A). GH-VΔ4 encodes a putative apoprotein of 219 amino acids, having the first 124 amino acids in common with hGH-V, but differing in the C-terminal 95 amino acids. The novel last 65 amino acids contain 7 Cys residues and make this part of the protein into a Cys-rich region with potential sites for disulfide-bridge formation (Fig. 1C).

Discussion

In the human testes the GH-V gene is expressed and spliced in a more complex way than previously thought. Apart from the main splice variant encoding for GH-V 22 K and the intron D-retaining GH-V2, described for placental tissue and human testes, we observed the additional alternative splicing variant GH-VΔ4. Obviously, the thymidine located 4 bp 5′ from the classical splice-donor site of intron D provides the basis for the observed splicing process by generating a second donor site competing with the original splice-donor site. It is unique to the GH-V gene, is not present in the corresponding other four GH/PL genes (Fig. 1B) and obviously leads to variability in the splicing process of the last intron of the pre-mRNA. As a consequence the original splice-donor site (GH-V) or the competitive splice-donor site (GH-VΔ4) is used alternatively, or the entire last intron is not excised (GH-V2).

This mRNA variability entails several severe changes in the putative amino acid sequences and three-dimensional structures of these GH variants. Due to the incorporation of intron D into the ORF, hGH-V2 has an altered carboxyl-terminal amino acid sequence as compared with hGH-V, and a premature stop codon in the middle of exon 5 (Fig. 1A) (7). Consequently, only the N-terminal 126 amino acids encoding for the first two and a half alpha-helices are in common with genuine hGH-V, which therein differs in 10 amino acids from pituitary-derived hGH. hGH-VΔ2, 230 amino acids in length, has a calculated molecular weight of 26 K, loses the N-glycosylation site at Asn 140 and acquires a hydrophobic carboxyl-terminal end. It is therefore believed to be (cell) membrane-bound rather than secreted (7). Neither its presence in the syncytiotrophoblastic villous layer of the placenta nor its insertion into the membrane and functional relevance could be verified up to now due to the lack of appropriate reagents, e.g. monoclonal antibodies, and its unsolved three-dimensional structure.

The first 124 amino acids corresponding to two and a half structural alpha-helices of the mature hGH-VΔ4 protein are shared with hGH-V (22 K, 191 aa, presumably 4 large and 2 small alpha-helices). In contrast, the carboxyl-terminal 95 amino acids of hGH-VΔ4 are different from hGH-V containing a cyst-rich region of 65 amino acids in length (Fig. 1C), presumably resulting in multiple disulfide bridge formation (4 Cys–Cys bridges). This long ORF, the 7 Cys and the Asn at position 148, which is a potential novel site for N-glycosylation, make it a remarkable protein. Comparison of the carboxyl-terminal amino acid sequence with other proteins in structure databases (EMBL data library/SWISS-PROT) revealed no sequence homologies to other known proteins. On the other hand, one is tempted to speculate that this part of the protein forms a kind of cystine-knot, similar to the ones present in other members of the structural superfamily of cystine-knot growth factors such as nerve growth factor (NGF) platelet-derived growth factor-B (PDGF-B) or transforming growth factor (TGF)-β, resulting in a chimeric cytokine–cystine knot growth factor molecule.

Future studies should provide insights into whether these alternative splicing variants of the GH-V gene, GHVΔ4 and GH-V2, are in fact translated and present as functional proteins in the human testis or even in the placenta. Placental expression would underline the hypothesis of the competitive splice-donor site, based on a thymidine unique to GH-V. Conversely testes-specific factors could be responsible for these splicing events. A major question which remains to be answered concerns the function of the putative novel protein for testicular physiology, which could be involved in the complex process of spermatogenesis.

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