Human lymphocytes express hGH-N gene transcripts of 22 kDa, 20 kDa and minor forms of GH, but not hGH-V gene

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Abstract
Expression of human growth hormone (hGH) in lymphocytes was examined by reverse transcription polymerase chain reaction (RT-PCR) in five normal subjects. Transcripts of hGH-N gene, but not hGH-V gene, were detected. Sequence analysis revealed four kinds of transcripts: 22 kDa GH, 20 kDa GH and two other forms of variant GH. The 20 kDa GH transcript was generated by alternative splicing within exon 3, resulting in a 45 bp deletion. One of the variant GH transcripts was also generated by alternative splicing within exon 3, but at a different site, resulting in a 73 bp deletion. Because of a frameshift, this variant GH transcript may encode a 6.6 kDa protein (truncated GH) that structurally differs from that of 22 kDa GH after residue 31. In the other variant GH mRNA, exons 3 and 4 were completely skipped. The proportions of expression of 22 kDa GH, 20 kDa GH and the truncated GH were 60.9 ± 6.13% (S.D.), 32.7 ± 14.1% and 6.4 ± 1.1% (n=5), respectively, by comparative RT-PCR.

We conclude that human lymphocytes, like the pituitary gland, express hGH-N gene transcripts of mainly 22 kDa GH, but also 20 kDa GH and minor variant forms of GH.
Reverse transcription-polymerase chain reaction (RT-PCR)

GH primers (GH-N-S1 and GH-N-AS1) flanking the last 73 nucleotides of exon 2, full exons 3 and 4 and the first 99 nucleotides of exon 5 were designed on the basis of the sequence of the hGH-N gene (15) (Table 1). The amplified fragment of the 22 kDa GH cDNA was 457 bp in length. Because nucleotide sequences of hGH-N and hGH-V cDNAs are very similar (16), hGH-V-specific primers were carefully selected so that as many different nucleotides, especially at the 3' end of the primers, were included (Table 1). The length of amplified hGH-V cDNA was 265 bp for primer pairs of hGH-V-S1 and hGH-V-AS1, and 127 bp for those of hGH-V-S1 and hGH-V-AS2. RNA samples were treated with DNase I (Gibco BRL, Rockville, MD, USA) to remove trace amounts of RNA. Thirty-eight cycles of PCR were carried out in a Gene Amp PCR System 9600 (Perkin-Elmer Co., Foster City, CA, USA) under the following conditions: denaturing at 94 °C for 40 s, annealing at 64 °C for 40 s and extension at 72 °C for 40 s.

Cloning and sequencing

The PCR products were electrophoresed in 1% agarose gel containing ethidium bromide, the bands excised from the gel, and the cDNA fragments cloned using the TA cloning kit (Invitrogen Co., Carlsbad, CA, USA). Sequencing of the cDNA was performed with a Thermo Sequence dye terminator cycle sequencing pre-mix kit (Amersham Life Sci. Inc., Cleveland, OH, USA) in a Gene Amp PCR System 9600.

Quantitative RT-PCR

To separate the bands of PCR products more accurately, we designed another set of primers for hGH-N gene transcripts (hGH-N-S1 and hGH-N-AS2) to produce shorter PCR products, and analyzed them by polyacrylamide gel electrophoresis and fluorescence detection (Table 1). The predicted length of the 22 kDa GH cDNA fragment was 168 bp. RT was performed as described above and 27–38 cycles of PCR were carried out at intervals of 1–2 cycles. The PCR products were electrophoresed on 10% polyacrylamide gel and the bands of PCR fragments were stained with Vistra green (Amersham, Buckinghamshire, UK) for 20 min. The fluorescence intensity of each band was measured by a Fluor Imager 595 (Molecular Dynamics, Sunnyvale, CA, USA), which is an optical scanner that detects light emitted from fluorescent samples and produces a digital image. The wavelengths of excitation and emission were 488 and 530 nm respectively. The data were then analyzed by Image Quant, a software program from Molecular Dynamics. To estimate the amount of the template, regression equations of the form: 

\[ y = a \times b^n \]

where \( y \) is the fluorescence intensity and \( n \) is the number of cycles, were fitted to the data in the linear portion of the semilogarithmic graphs. Constants \( a \) and \( b \) of the equation reflected the amount of the original template and the efficiency of amplification every cycle respectively (17). Because the fluorescence intensity is proportional to the size of cDNA, the ratio of fluorescence intensity of each band was corrected by the molecular weight to yield the molar ratio. The data are presented as the mean ± S.D.

Results

Figure 1 shows agarose gel electrophoresis of PCR products using hGH-N-S1 and hGH-N-AS1 primers. Although the bands were broad, PCR products with the predicted size (457 bp) were detected in lymphocytes from all five normal volunteers and unexpected shorter bands (172 bp) were observed in two of them. To confirm that the PCR products originated from hGH-N transcripts, we performed sequence analysis. Sequencing of 20 clones obtained from cDNA bands of around 457 bp revealed three kinds of transcripts: 14 clones of 22 kDa GH cDNA, three clones of 20 kDa GH cDNA and three clones of variant GH cDNA. Transcripts of the 20 kDa GH was generated by alternative splicing within exon 3, resulting in a 45 bp deletion, while the variant GH transcript was also produced by alternative splicing within exon 3, but at a different site (Fig. 2). Seventy-three base pairs from the start of exon 3 were deleted in

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**Table 1** Sequences of primers for hGH-N and hGH-V transcripts. Nucleotides underlined in primers for hGH-V transcripts indicate differences from the sequence of hGH-N cDNA.

<table>
<thead>
<tr>
<th>Sense primer</th>
<th>Antisense primer</th>
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<tbody>
<tr>
<td>hGH-N-S1 5'-CAGGCTTTTGACACCGCTATG-3'</td>
<td>hGH-N-AS1 5'-GTTCTTGAGTAGGTCGTCATCGTT-3'</td>
</tr>
<tr>
<td>hGH-V-S1 5'-GTGGAAGAAGCCTATATCC-3'</td>
<td>hGH-V-AS1 5'-CTCTAGGTCTTACGAGGCG-3'</td>
</tr>
<tr>
<td>hGH-V-AS2 5'-GTTAGATTCTGATGCGTTTCCA-3'</td>
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</table>
Because the splicing was between positions 1 and 2 of the original glutamic acid codon (GAG), a frameshift occurred and a stop codon (UAG) appeared within exon 4 in this mRNA. This GH transcript was speculated to encode a 6.6 kDa protein with 48 amino acids, the structure of which differs from 22 kDa and 20 kDa GH after residue 31 (truncated GH). Sequencing of shorter PCR products (172 bp) disclosed another variant GH transcript that completely skipped exons 3 and 4 (Fig. 2).

To determine the ratio of expression of 22 kDa, 20 kDa and truncated GH in human lymphocytes, we performed comparative RT-PCR. Because separation of larger-size PCR products on agarose gel electrophoresis was not adequate, we carried out RT-PCR using another set of primers (hGH-N-S1 and hGH-N-AS2), generating a 168 bp cDNA fragment for 22 kDa GH, 123 bp for the 20 kDa GH and 95 bp for the truncated GH, and separated them by polyacrylamide gel electrophoresis. As shown in Fig. 3a, three bands with the predicted sizes were detected and the fluorescence intensity of each band increased with the increase in cycles. Figure 3b shows the reaction cycle–fluorescence intensity curves of each band. The slopes of the linear portion of the curves (log b) were similar, indicating similar efficiencies of PCR amplification for the three transcripts. The y axis intersection (log a) represents the initial level of transcript. The ratios of expression of the 22 kDa, 20 kDa and truncated GH were determined in five normal subjects. They varied among individuals; the ratio is shown in Table 2.

Figure 4 shows agarose gel electrophoresis of PCR products using two different primer pairs for hGH-V gene transcripts. Although hGH-V gene expression was clearly detected in the placenta with both primer...
pairs, the expression was not observed in human lymphocytes.

Discussion

In this study, we demonstrated that human lymphocytes express hGH-N gene, but not hGH-V gene. Because the nucleotide sequences of the two genes are very similar – that is, 95% of the entire open reading frames are identical – cross-reaction of hGH-V primers with hGH-N transcripts becomes a problem when examining the expression of hGH-V gene by RT-PCR. Therefore, we carefully chose primers that include as many mismatches as possible, especially at the 3' end, so that the cross-reactivity with hGH-N transcripts could be minimized. Our results are in contrast to those presented in a previous report that showed the presence of both hGH-N and hGH-V transcripts by RT-PCR in human peripheral blood mononuclear cells (18). Although the reasons for the discrepancy are unclear, there have been some reports supporting our findings. Kooijman et al. (19) revealed that human neutrophils express hGH-N gene transcript, but not hGH-V gene transcript, using RT-PCR followed by restriction analysis. Palmetshofer et al. (20) showed that lymphoid and myeloid cell lines express hGH-N-derived mRNAs, but not hGH-V mRNAs, using RT-PCR followed by sequencing.

In the pituitary gland, the main transcript of the hGH-N gene encodes a protein with a molecular weight of 22 kDa (22 kDa GH). An alternatively spliced mRNA that lacks 45 nucleotides of exon 3 is generated in small amounts (5–10%) (15). Four additional alternative splicing products of the hGH-N gene have been detected in pituitary tumor tissue (20, 21). In three of these mRNAs, exon 3, exons 3 and 4, and exons 2, 3 and 4 are completely skipped. The other mRNA arises from an alternative splicing within exon 3, but at a different site from that for 20 kDa GH. This codes for a truncated GH with 48 amino acids that structurally differs from 22 kDa GH after residue 31 because of a frameshift. In this study, we demonstrated that human lymphocytes also have alternatively spliced mRNAs. One of these mRNA encodes a 20 kDa GH, as in the pituitary gland.

Table 2 Proportions of the expression of 22 kDa GH, 20 kDa GH and a truncated GH in lymphocytes from normal subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>22 kDa GH (%)</th>
<th>20 kDa GH (%)</th>
<th>Truncated GH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>41</td>
<td>76.5</td>
<td>16.9</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>31</td>
<td>63.0</td>
<td>32.1</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>47</td>
<td>37.1</td>
<td>57.1</td>
<td>5.8</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>31</td>
<td>57.3</td>
<td>36.3</td>
<td>6.4</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>33</td>
<td>70.6</td>
<td>21.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td>60.9 ± 13.6</td>
<td>32.7 ± 14.1</td>
<td>6.4 ± 1.1</td>
</tr>
</tbody>
</table>

Figure 3 (a) Representative pattern of polyacrylamide gel electrophoresis of PCR products followed by Vistra green staining. Twenty-seven to 38 cycles of PCR were carried out at intervals of 1–2 cycles using hGH-N-S1 in exon 2 and hGH-N-AS2 in exon 3. Three bands with predicted sizes matching 22 kDa GH, 20 kDa GH and the truncated GH transcripts were detected. The fluorescence intensity of each band was measured by FluorImager. (b) Reaction cycle–fluorescence intensity curves of each band (●, 22 kDa GH; ■, 20kDa GH; ▲, truncated GH) shown in Fig. 3a. The fluorescence intensity reached a plateau in all three transcripts. Regression equations of the form: \( y = a \cdot b^n \), where \( y \) is the fluorescence intensity and \( n \) is the number of cycles, were fitted to the data in the linear portion of the semilogarithmic graphs. Each constant \( a \) and \( b \) represents the amount of the original template and the efficiency of amplification of PCR respectively. Note that the slopes (log \( b \)) of each band (efficiency of PCR) were almost the same for the three transcripts.
performed using hGH-V-S1 and hGH-V-AS1 (a) and hGH-V-S1 and hGH-V-AS2 (b). Lanes 1 and 8: 100 bp DNA markers; lane 2: placenta tissue; lanes 3–7: lymphocytes in volunteers 1–5, respectively. Although hGH-V-gene expression was clearly detected in the placenta with both primer pairs, the expression was not observed in human lymphocytes.

This is in agreement with a previous report that described the presence of a 20 kDa GH transcript of hGH-N gene in lymphoid and myeloid cell lines (20). However, Wu et al. (22) found no hGH-N transcripts other than that of 22 kDa GH. This discrepancy can be explained by the positions of primers for RT-PCR: as Wu et al. designed a sense primer flanking exon 2 and exon 3, 20 kDa GH transcript, which lacks the first 45 nucleotides of exon 3, could not be amplified by RT-PCR using this primer. Variant GH transcript lacking 73 nucleotides of exon 3, could not be amplified by RT-PCR using this primer. Variant GH transcript lacking 73 nucleotides of exon 3 was first demonstrated in the immune cells, and may correspond to the transcript encoding a truncated GH in pituitary tumor tissue (21). The other variant GH transcript that completely skipped exons 3 and 4 was previously reported in the pituitary gland and lymphoid and myeloid cell lines (20). We showed that human lymphocytes from some individuals express the variant GH transcript, but others do not. The reasons for the individual variations and the biological significance are unknown.

In this study, we designed primers sharing 22 kDa, 20 kDa and truncated GH transcripts, to enable a comparative RT-PCR to be performed for the determination of their ratios. Efficiency of PCR depends on many factors, including primers, annealing temperature, Mg$^{2+}$ concentration, DNA polymerase and RNA samples (23). Because we examined the three transcripts in an RNA sample by RT-PCR under the same conditions and using the same primers, the efficiency of PCR amplification was assumed to be equal, enabling comparison of the ratio of each transcript. Indeed, the slopes of the linear portion of reaction cycle–PCR product curves were almost equal among the three transcripts.

Although other minor hGH-N transcripts have also been reported using RT-PCR with different primers in lymphoid and myeloid cell lines (20), GH transcripts expressed in human lymphocytes were hGH-N gene-derived ones of mainly 22 kDa GH, followed by 20 kDa GH and minor variant forms of GH, as in the pituitary gland.

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References

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