SHORT COMMUNICATION

Cloning, Structural Organization Analysis, and Chromosomal Assignment of the Human Gene for the Neurosecretory Protein VGF

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Received April 9, 1997; accepted July 31, 1997

The *Vgf* gene was originally identified as a 2.7-kb cDNA fragment isolated from nerve growth factor-treated PC12 cells by differential display against PC12 cells. It is transcribed solely in subpopulations of neuroendocrine cells *in vivo* and it is induced by neurotrophins in target cells *in vitro*. The single-copy human *VGF* gene was isolated from a genomic library. The gene spans approximately 6 kb and contains two exons. The entire VGF protein is encoded by exon 2, while exon 1 contains only 5′-untranslated sequence. The structural organization of the human gene is similar to that described for the rat *Vgf* gene (S. R. J. Salton *et al.*, 1991, *Mol. Cell. Biol.* 11: 2335–2349) and both the translated and the untranslated regions show a high degree of sequence homology to the rat gene. Northern blot analysis revealed a single transcript of approximately 2.7 kb that was detected only in mRNA preparations from brain. The gene was assigned to chromosome 7q22 by fluorescence *in situ* hybridization.

Vgf is a neurotrophin-responsive gene that is regulated by nerve growth factor in PC12 cells (7) and by brain-derived neurotrophic factor and neurotrophin 3 in primary cultures of cortical or hippocampal neurons (2). It codes for a polypeptide with a predicted molecular weight of 70 kDa that shares similarities with the secretogranin/chromogranin family (11) and that is found in the secretory granules of subsets of neurons and endocrine cells (for review see 5). Its expression is developmentally regulated: *Vgf* mRNA levels peak during a critical period of morphological and functional organization of the cerebellum (12), and it is spatially and temporally modulated during the synaptogenesis of geniculocortical afferents (10). In the adult animal both the mRNA and the protein levels are regulated in different areas of the brain in response to different stimuli (reviewed in 5). It has also recently been shown that transcription of *Vgf* is modulated in gonadotropic cells during the estrus cycle (4).

Tissue-specific proteolytic processing of VGF protein and secretion of the produced peptides have been shown to occur, suggesting that VGF is the precursor for biologically active peptides involved in intercellular communications (13, 9) and possibly in neurite outgrowth and regeneration (12).

Here we describe the cloning, structure, chromosomal localization, and tissue-specific expression of the human *VGF* gene.

Screening of a cDNA library from SK-N-BE neuroblastoma cells with the rat *Vgf* probe led to isolation of few clones. Comparison of the published sequence of the rat *Vgf* cDNA with that of the human cDNA clones showed that even the longest was incomplete at the 5′ end and did not contain the entire sequence. Since rescreening the same library with the human probe did not permit isolation of additional clones, we used the longest cDNA to probe the genomic library. Of 2×10^5 recombinant phages screened, we isolated two identical genomic clones (λDC) containing an 18-kb insert. The coding and the 3′- and 5′-flanking regions were mapped within λDC by Southern blot analysis of the endonuclease-digested DNA using selected fragments of the human cDNA, the rat cDNA, or a rat *Vgf* genomic clone as probes. The sequence of λDC contains 2305 bases of 5′-flanking DNA (EMBL Accession No. Y09938), 723 bases of 5′-untranslated region, 1848 bases of coding sequence, and 457 bases of the 3′-flanking region (EMBL Accession No. Y12661). Strong homology between the human and the rat *Vgf* sequences was observed not only in the 5′-flanking promoter region (3) and in the mature mRNA, but also in the intronic sequence. The first 100 nucleotides of the intron are 80% homologous between human and rat, suggesting a functional role for this region. Comparison between the hu-

Sequence data from this article have been deposited with the EMBL Data Library under Accession Nos. Y09938 and Y12661.

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GENOMICS **45,** 443–446 (1997)
ARTICLE NO. GE974945
0888-7543/97 $25.00

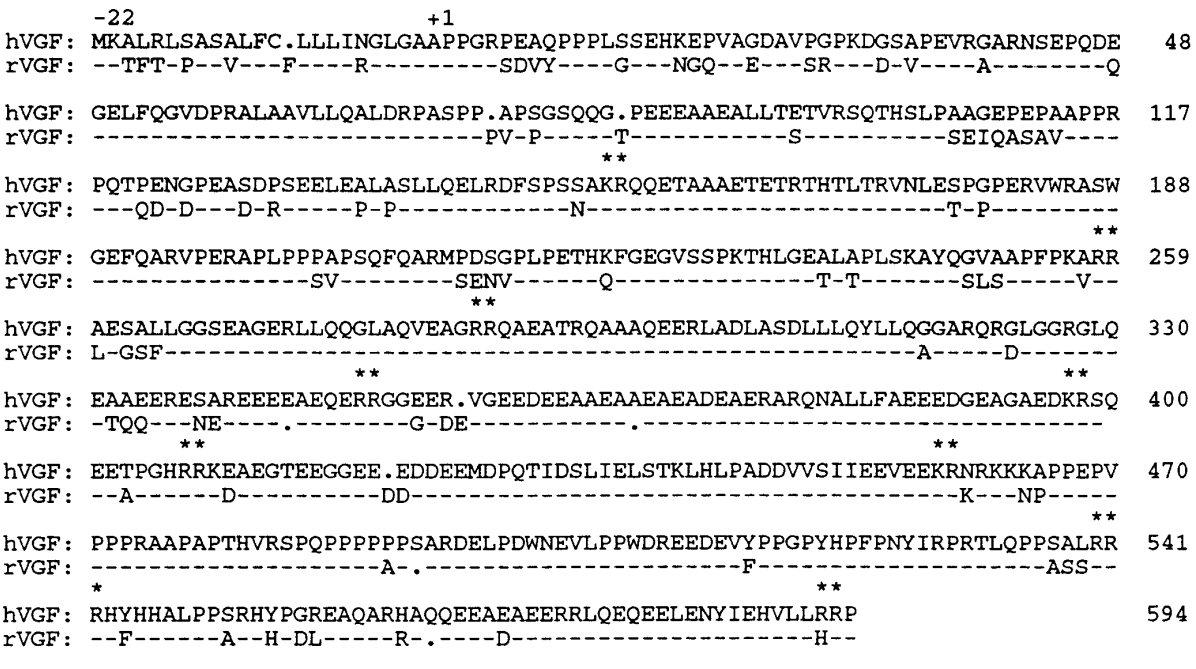

FIG. 1. Comparison of the amino acid sequences of human and rat VGF protein. The rat VGF sequence was derived from Salton *et al.* (12). Gaps introduced for aligning the sequences are indicated by dots. Amino acid residues in human VGF that are identical to the corresponding residues in rat VGF are shown as dashes. Di- or tribasic sites are indicated by double asterisks. Amino acid residue numbers in the mature proteins are given in the right column, −22 indicates the beginning of the signal peptide, and +1 indicates the putative amino-terminal residue after signal peptide cleavage. hVGF, human VGF; rVGF, rat VGF.

man and the rat sequences reveals that the deduced primary structure of VGF protein is highly conserved (Fig. 1). A 22-residue-long hydrophobic sequence follows the first methionine at the N-terminal and is supposedly a signal peptide for the translocation of the nascent protein into the endoplasmic reticulum. This domain is the one with the lowest interspecies conservation (72% identity between rat and human). This is not surprising since signal peptides are important only for intracellular localization and are absent from mature protein. In the residual portion of the protein, 503 of 584 residues are identical (86% identity) and the majority of amino acid substitutions are conservative, involving exchanges among proline, glycine, alanine, serine, and threonine or between alanine and valine, aspartic and glutamic acid, or arginine and histidine. Most of the differences occur in the N-terminal region; interestingly, functional peptides are believed to be derived from the C-terminal portion of the protein (13). Furthermore, all the putative recognition sites for prohormone convertases (1), supposed to be responsible for processing VGF, are conserved between human and rat. It is therefore likely that the mature forms of VGF, endowed with biological activity, are very similar in rat and human.

A human *VGF* cDNA clone was used for probing a Northern blot of human RNA from different tissues. A single transcript of about 2.7 kb was detected only in brain mRNA (Fig. 2A). The intensity of the signal compared to actin mRNA (not shown) suggested that *VGF* is a rare transcript at least 50-fold less abundant than actin. This finding is not surprising since, in rodents, *Vgf* expression peaks before adulthood and decreases afterward; moreover, in adult animals, *Vgf* is detected only in specific neuronal subpopulations in the central nervous system.

In PC12 cells two alternatively spliced forms of *Vgf* mRNA, which differ by a 116-bp-long sequence present in the 5′-untranslated region, were detected (6). The donor and acceptor sites, which give rise to the two alternatively spliced forms, are conserved in the human *VGF* gene sequence. We therefore investigated whether alternative splicing occurs also in human *VGF*-expressing cell lines. mRNAs from human neuroblastoma SK-N-BE and SY-5-Y cell lines and from the dbcAMP-treated human medullary carcinoma TT cell line were retrotranscribed, and PCR was performed using a set of oligonucleotides designed to amplify the two possible transcripts. The PCR products were then resolved on 2% agarose gels, blotted, and hybridized to a terminally labeled oligonucleotide that recognized a sequence of 27 nucleotides common to the two possible transcripts. As shown in Fig. 2B, in all cell lines tested a single transcript was detected that, according to its molecular weight, corresponds to the splicing product derived by joining the donor site at position +199 to the acceptor site located at position +733. Even at longer exposure no product longer than this was observed, and we estimate that if the alternatively spliced mRNA is present, its quantity can not exceed 1% of the shorter species.

As a prelude to gene mapping, we determined the copy number of *VGF* in the human genome. Genomic DNA, digested to completion with selected restriction endonucleases, was subjected to Southern blot analysis with a probe containing most of the coding and 3′-flanking regions of *VGF* (not shown). Only a limited number of fragments for each restriction digestion were detected. Moreover the pattern of the bands was in agreement with the one predicted from the restriction map of the genomic clone. The intensity of the hybridization signal also suggests that *VGF* is a single copy gene in humans as was similarly shown in rats (12). To determine the chromosomal localization of *VGF*, fluorescence *in situ* hybridization (FISH) (8) was carried out using the genomic clone as a probe. A total of 20 metaphase cells were examined and, as shown in Fig. 3, the *VGF* gene was mapped on chromosome 7q22. No hybridization signal was seen in other chromosomes, in agreement with *VGF* being a single-copy gene.

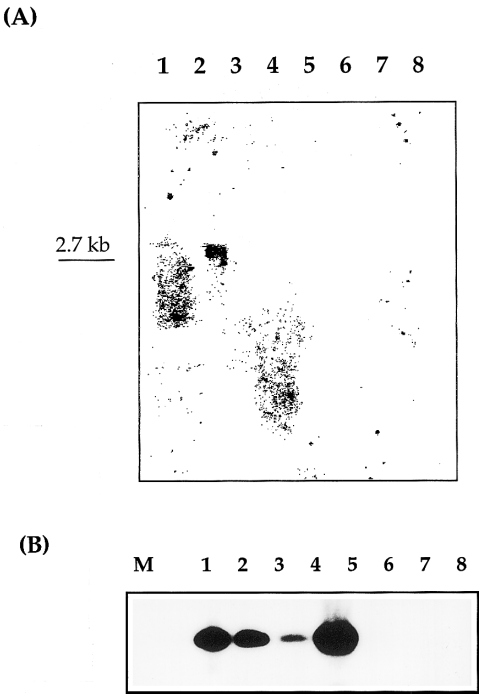

FIG. 2. (**A**) Tissue-specific expression of human *VGF*. Northern blot analysis of mRNA extracted from different tissues of adult humans was performed with a ^{32}P-labeled human *VGF* cDNA probe. Each lane contains approximately 2 μg of mRNA isolated from the following human tissues: lane **1,** heart; lane **2,** brain; lane **3,** placenta; lane **4,** lung; lane **5,** liver; lane **6,** skeletal muscle; lane **7,** kidney; and lane **8,** pancreas. (**B**) Detection of a single splice variant of *VGF* mRNA in human cell lines. Retrotranscribed mRNAs from SK-N-BE cells (lane **1**), SY-5Y cells (lane **2**), TT cells (lane **3**), and cAMP-treated TT cells (lane **4**) were amplified by PCR and hybridized with an oligonucleotide capable of recognizing both putative splice variants. Lanes **5** to **8** represent controls of RT-PCR on the same mRNAs done with the omission of reverse transcriptase. The following primers were used for the PCR: 5′-TGCTGAAGCCGGAGCGAG-3′ (from +7 to +24) and 5′-GCCGACAATCTGAGGGCT-3′ (reverse complement of bases +729 to +745). The amplified products were hybridized with the ^{32}P-labeled oligonucleotide 5′-AGCTGGTGTCACGACGCGAGAGGT-3′ complementary to nucleotides +174 to +191.

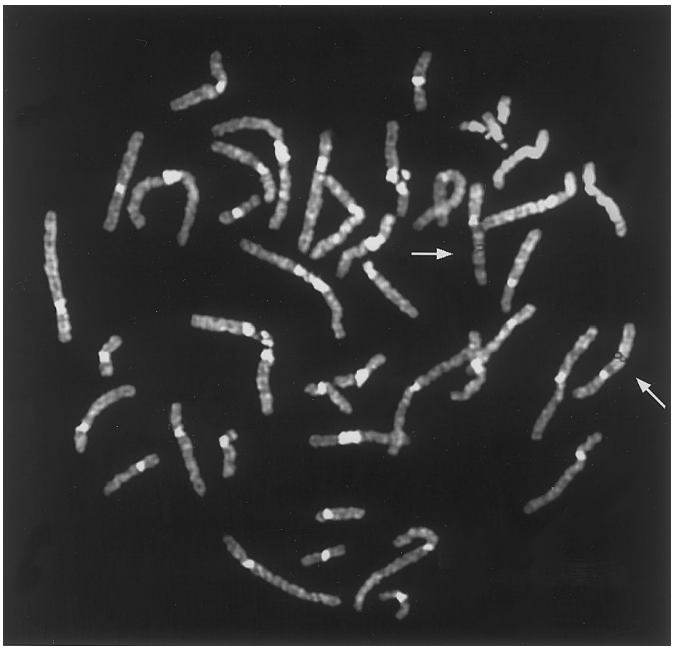

FIG. 3. Chromosomal mapping of the human *VGF* gene by FISH on metaphase chromosome spreads. Digital images were obtained using Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Cy3 and DAPI fluorescence signals, detected using specific filters, were recorded separately as gray-scale images. Pseudocoloring and merging of images were performed using the Adobe Photoshop software. This is a spread showing signals (arrows) only on 7q22.

ACKNOWLEDGMENTS

This study was supported by grants from C.N.R. (P. F. ACRO P. F. invecchiamento) and from MURST to A.L. and by grants from Telethon and AIRC to M.R. The following colleagues are warmly acknowledged for helpful discussions: M. Pescatore, D. Mercanti, R. Butler, E. Trani, and A. M. Rinaldi.

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