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ORIGINAL ARTICLE

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Molecular cloning, expression, and mapping of a novel human cDNA, GRP17, highly homologous to human *gadd45* and murine *MyD118*

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Abstract From a human placenta cDNA library, we isolated a novel gene whose predicted product is highly homologous in amino acid sequence to human *gadd45* and murine *MyD118* proteins (about 55% and 52% identity, respectively). The cDNA clone, designated GRP17 (*gadd*-related protein, 17kDa), contained a 477-bp open reading frame encoding 159 amino acids. Northern blot analysis revealed strong expression of a 1.35-kb transcript in heart, placenta, liver, skeletal muscle, prostate, testis, and ovary. A 1.7-kb additional transcript was detected in liver. We assigned the GRP17 gene to chromosome 9q22.1-q22.2 by fluorescence *in situ* hybridization (FISH).

Keywords *gadd45* · *MyD118* · Acidic protein · Growth suppressor · 9q22

Introduction

Growth arrest and apoptosis are important mechanisms for regulating cell numbers within a given tissue. A remarkable overlap has been observed between the "*gadd*" set of growth-arrest and DNA damage-inducible genes (Fornace et al. 1989), and the *MyD* set of myeloid-differentiation primary response genes (Lord et al. 1990). Among these genes, *gadd34/MyD116, gadd45, MyD118, and gadd153* share multiple properties, such as roles in growth control, unusual charge characteristics of their encoded acidic proteins, and similarities in expression and regulation. This group may define a novel gene family encoding acidic proteins that suppress cell growth in a synergistic manner (Zhan et al. 1994). However, the mechanisms involved remain unknown.

Although *gadd45* (Carrier et al. 1994) and *MyD118* (Abdollahi et al. 1991) are very similar in DNA sequences, they are distinct genes. Expression of *gadd45* is inducible by a wide variety of stresses, and is regulated by *p53* (Hollander et al. 1993). Smith et al. (1994) have reported that GADD45 binds to proliferating cell nuclear antigen (PCNA) and stimulates repair of DNA excision. Other studies have indicated that GADD45 interacts with p21, cyclin-dependent kinase inhibitor, and modulates the cell cycle by inhibiting DNA replication (Chen et al. 1995; Kearsey et al. 1995).

Here we report nucleotide and deduced amino-acid sequences of a human cDNA clone whose encoded peptide is highly homologous to human *gadd45* and murine *MyD118*. We also describe its expression pattern in adult human tissues, and document its chromosomal location.

Materials and methods

Cloning and DNA sequencing

As a part of the Human Genome Project, we determined the nucleotide sequences of cDNA clones randomly selected from human fetal brain, adult aorta, and placenta cDNA libraries, and have been comparing them by means of the FASTA program of the UWGCG package (Pearson and Lipman 1988). In the process, we found a 1.1-kb cDNA derived from the placenta cDNA library, termed GEN-554H06, that revealed a significant degree of homology to the human *gadd45* and murine *MyD118* genes. Its nucleotide sequence was determined with an A.L.F. DNA sequencer (Amersham Pharmacia, Uppsala, Sweden) by the dideoxynucleotide chain-termination method.

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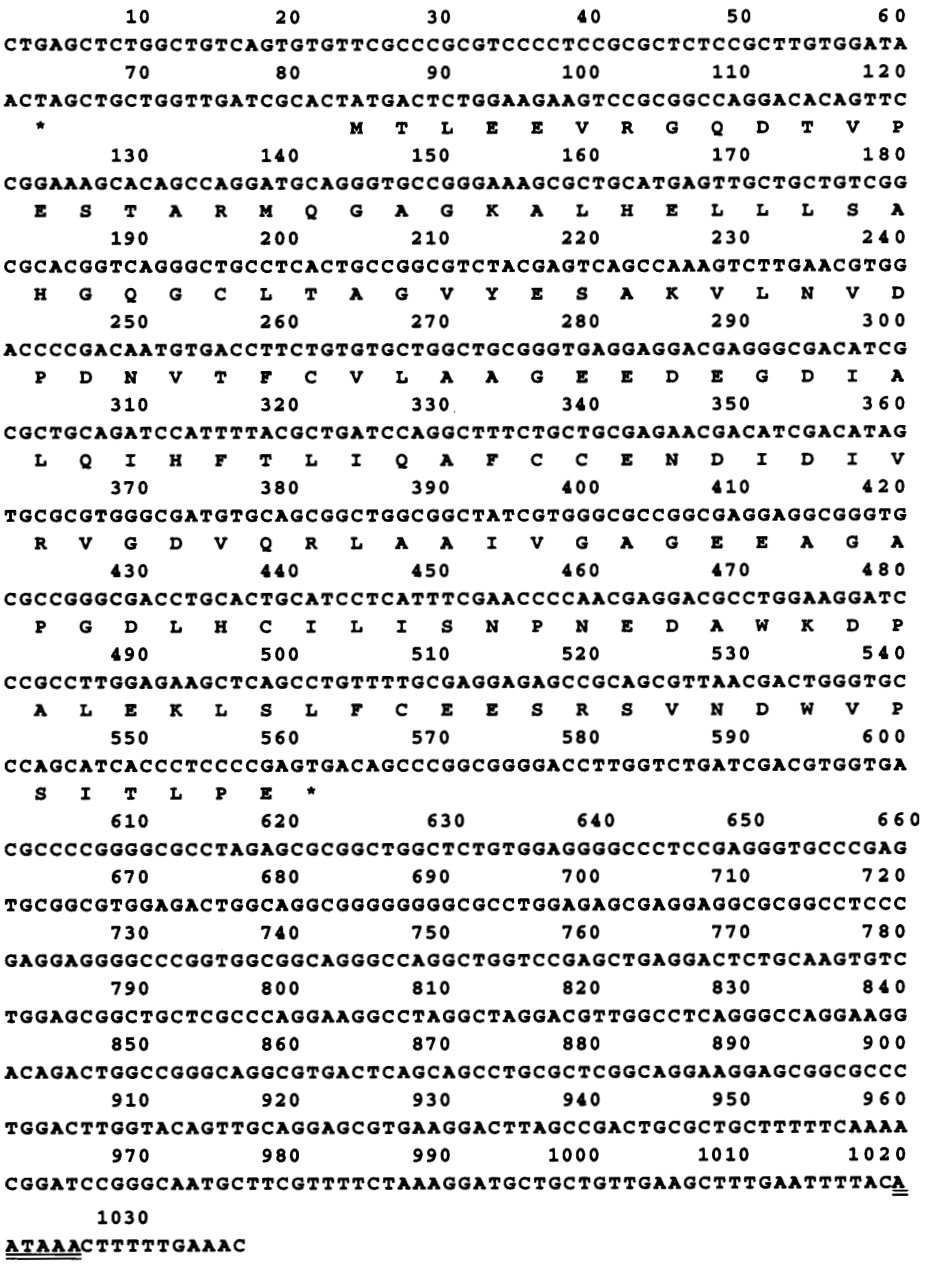

Fig. 1. Nucleotide and deduced amino acid sequences of GRP17. The in-frame termination codons are indicated by *asterisks*, and a polyadenylation signal is *double-underlined*. The nucleotide sequence reported in this paper will appear in the DDBJ, GenBank, and EMBL databases with accession number D83023

Northern blot analysis

Analysis of gene expression was carried out with a Human Multiple Tissue Northern (MTN) blot (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. The entire cDNA sequence was purified, labeled with [^{32}P]-dCTP (random-primed DNA labeling kit, Boehringer Mannheim, Tokyo, Japan), and used as a probe. The blot was prehybridized for 6 h, then hybridized for 17 h at 42°C in a solution containing 50% formamide, 5 × SSC, 10 × Denhardt's solution, 2% SDS, and 0.1 μg/ml denatured salmon sperm DNA. The blot was washed with 2 × SSC, 0.05% SDS for 10 min at room temperature, and then with 0.1 × SSC, 0.1% SDS for 30 min at 65°C. The membrane was autoradiographed at –80°C for 7 h.

Cosmid cloning and chromosomal localization by direct R-banding FISH

To isolate cosmid clones corresponding to the cDNA represented by GEN-554H06, we screened a total of 153 600 cosmid clones by PCR amplification, using primers cos 1 (5′-TAGGCTAGGACGTTGCCTCA-3′) and cos 2 (5′-GCTTCAACAGCAGCATCCTT-3′), with initial denaturation at 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 45 s.

The three independent cosmid clones obtained were used as probes for mapping by direct R-banding fluorescence *in situ* hybridization (FISH), a method based on FISH combined with replicated pro-metaphase R-bands (Takahashi et al. 1990; 1991). For suppression of the repeti-

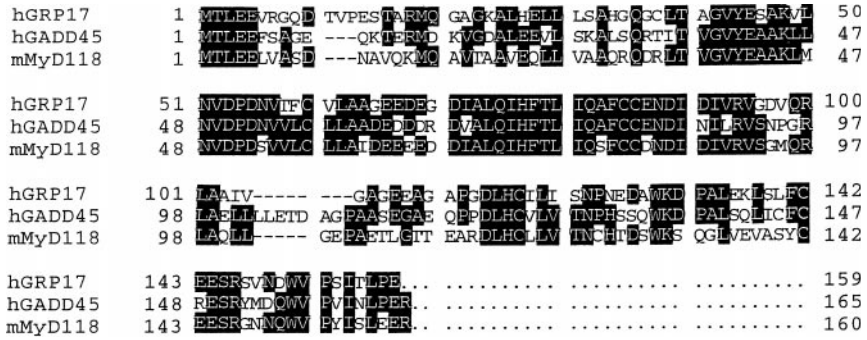

Fig. 2. The predicted amino acid sequence of GRP17 is aligned with the sequences of human GADD45 and murine MyD118. Identical residues are *shaded*

tive sequences contained in the cosmids we used human Cot-1 DNA (Gibco BRL, Rockville, MD, USA) as described by Lichter et al. (1990), except that we used Cot-1 DNA in about 10-fold excess. Labeling, hybridization, rinsing, and detection were performed in a routine manner. Provia 100 film (ISO 100; Fujifilm, Tokyo, Japan) was used for the microphotography (filter combination, Nikon B-2A; Nikon, Tokyo, Japan).

Results

Cloning and DNA sequencing of GRP17

By examining databases we found that a cDNA clone, GEN-554H06, revealed significant homology with the human *gadd45* and murine *MyD118* genes. To confirm its putative open reading frame, we determined the entire coding sequence and the 5′ and 3′ flanking sequences, a total of 1036 nucleotides (Fig. 1). The predicted start codon was at nucleotides 84-86, the first ATG in the cDNA clone. Its sequence context (ACTATGA; nucleotides 81-87) was different from the optimal context, (A/G)CCATGG, for initiation of translation (Kozak 1987). However, its high sequence homology with human *gadd45* at the nucleotide level strongly suggested that the ATG at nucleotides 84-86 was indeed the start codon. An optimal polyadenylation signal was followed by 17 nucleotides before the poly(A) start. The open reading frame consisted of 477 nucleotides, encoding a 159-amino-acid peptide with a calculated molecular weight of 17 030 daltons and a calculated isoelectric point (pI value) of about 4.06, with a high percentage of negatively charged amino acids.

Similarity of the novel gene product to human *gadd45* and murine *MyD118* proteins

Alignment of the new sequence with those of human *gadd45* and murine *MyD118* revealed striking homologies at the amino-acid level (about 55% and 52% identity, respectively; Fig. 2). We therefore designated this molecule GRP17 (for gadd-related protein, 17kDa). Although no known motifs were identified, a region near the N-terminus of the predicted GRP17 peptide (aa 40-95) was conserved very well among the three proteins.

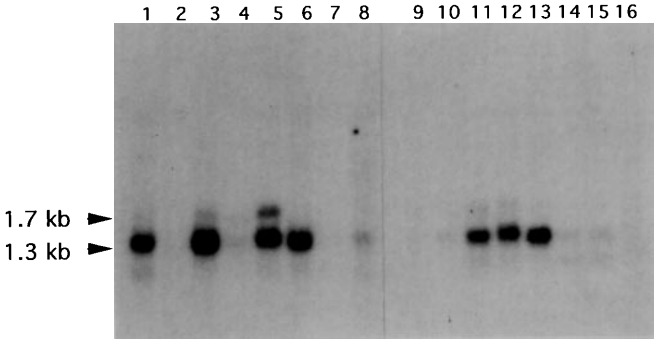

Fig. 3. Expression of GRP17 in human tissues. *Each lane* contains approximately 2μg of poly(A) RNA. Lanes 1-16 contain, in order from left, RNAs from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes

Expression of GRP17 in human tissues

Expression of the GRP17 mRNA in 16 normal adult human tissues was assessed by northern blotting using GEN-554H06 as a probe (Fig. 3). We detected a major transcript of 1.35 kb in heart, placenta, liver, skeletal muscle, prostate, testis, and ovary. A 1.7-kb additional transcript was detected in liver. By careful observation after overexposure (17 h), the 1.35-kb transcript was detectable in brain, lung, pancreas, small intestine, and colon.

Chromosomal localization of GRP17

We isolated three independent cosmid clones corresponding to the GRP17 cDNA, and used these clones as probes for FISH. We performed direct R-banding FISH and observed 100 typical R-banded chromosome plates for each clone. The signals of all three clones were apparent only at band q22.1-q22.2 of chromosome 9 (Fig. 4).

Discussion

Gadd45 and *MyD118*, both of which encode acidic proteins, share unusual charge characteristics and their patterns of

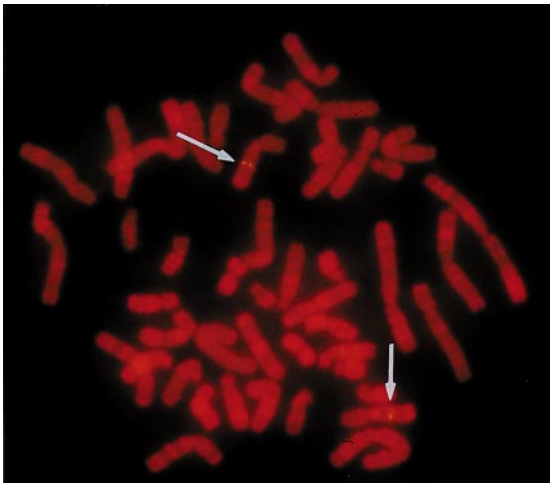

Fig. 4. Localization of GRP17 on R-banded metaphase chromosomes by fluorescence *in situ* hybridization (FISH). *Arrows* indicate the fluorescent signals on 9q22.1-q22.2

induction by a wide variety of stresses are similar. Moreover, both proteins function in a synergistic fashion as growth inhibitors, although the detailed mechanism involved is not yet known (Zhan et al. 1994). The sequence of GRP17 reported here shows significant similarity with *gadd45* and *MyD118*, and it also encodes an acidic protein. These data suggest that GRP17 is a novel member of a family of genes that encode acidic proteins involved in control of cellular growth. By isolating a human homologue of *MyD118*, we confirmed that GRP17 is not the human counterpart of this murine protein (data not shown). Although we have not examined the induction pattern or the function of GRP17, the significant sequence homologies imply that those features may be shared with the two related proteins.

Northern blot analysis revealed tissue-specific expression of GRP17 in normal adult tissues; the additional 1.7-kb transcript in liver could be produced by alternative splicing or derived from some other gene. Of great interest is the abundant expression of GRP17 in muscular tissues, testis, and ovary; that observation may imply an important role for GRP17 in differentiation of those organs.

We localized GRP17 to R-positive chromosomal band 9q22.1-q22.2. Around the region of 9q22, loss of heterozygosity has been reported to be found in bladder cancer (Simoneau et al. 1996). Hereditary sensory neuropathy 1 (HSN1) also has been mapped by linkage analysis in this region (9q22.1-q22.3, Nicholson GA et al. 1996). Detailed analysis at the molecular level will be needed for further understanding of the nature of GRP17 as well as the relation of the above diseases.

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