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 (Hollandet 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.
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 $[^{32}\text{P}]$-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 $\times$ SSC, 10 $\times$ Denhardt’s solution, 2% SDS, and 0.1 $\mu$g/ml denatured salmon sperm DNA. The blot was washed with 2 $\times$ SSC, 0.05% SDS for 10 min at room temperature, and then with 0.1 $\times$ 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′-GCTTCAACAGCACGCTTCA-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-