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HSP70RY: FURTHER CHARACTERIZATION OF A NOVEL MEMBER OF THE HSP70 PROTEIN FAMILY

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Hsp70RY was identified as a member of the hsp70 protein family on the basis of cDNA sequence homology (Fathallah, et. al. (1993) J. Immunol. 151, 810-813). We have shown that mRNA encoding hsp70RY is expressed in a variety of human cell lines and that mRNA expression remains unchanged in human promyelocytic HL-60 cells induced to differentiate with phorbol 12-myristate 13-acetate (PMA). We have also shown that the predicted amino acid sequence of hsp70RY diverges significantly from the other human hsp70 proteins and that it contains a unique glutamate-rich region near its carboxy-terminus. Finally, we have demonstrated the existence of a murine homolog of hsp70RY.

The hsp70 proteins comprise a large family of highly conserved, homologous proteins that function as molecular chaperones (1-5). The hsp70 proteins have roles in numerous intracellular processes, including stabilization and translocation of nascent polypeptides (1, 6-8), clathrin uncoating (9, 10), and targeting proteins to lysosomes for degradation (11, 12). In eukaryotic cells, hsp70 proteins have been found in the cytoplasm, the endoplasmic reticulum, mitochondria, and in the nucleus (see refs. in 1). The amino-terminal portions of hsp70 proteins are the most highly conserved, and share consensus ATP binding domains, while the carboxy-termini contain more variable sequence, and are believed to function as substrate binding domains (1, 13, 14).

Hsp70RY, whose cDNA sequence was reported by Fatallah and colleagues (15) was identified as a member of the hsp70 protein family on the basis of sequence homology. As this unique cDNA sequence was isolated from a B lymphocyte library, we set out to determine whether expression of hsp70RY was unique to B lymphocytes, or whether its pattern of expression was more widespread.

In this report, we describe the expression of hsp70RY in a wide variety of human cell lines. Furthermore, we have found that the expression of hsp70RY in the human promyelocytic

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leukemia HL-60 cell line remains unchanged as the cells differentiate toward the monocyte/macrophage lineage. We also present evidence suggesting that hsp70RY is more distantly related to the other known human hsp70 proteins than the latter are to each other, and demonstrate the existence of a murine homolog for this gene. Finally, we discuss the significance of a unique sequence feature of hsp70RY.

Results and Discussion

As shown in Figure 1, expression of hsp70RY is not limited to EBV-transformed B cells. Messenger RNA encoding hsp70RY was detected in a variety of human hematopoeitic cell lines, including erythroleukemia K-562 (lane 1), promyelocytic leukemia HL-60 (lane 2), and the T lymphocytic leukemia Jurkat (lane 3), as well as in an independent EBV-transformed normal B cell line (lane 4). Messenger RNA encoding hsp70RY was also detected in a malignant melanoma cell line, MeWo (lane 5, (16)) and in the epithelial carcinoma HeLa cell line (lane 6). The MeWo and HeLa cell lines are derived from cells of ectodermal origin, in contrast to the hematopoietic cell lines, which are of mesodermal origin, attesting to the widespread expression of this gene. Interestingly, none of the eukaryotic hsp70 proteins characterized to date have been found to have expression limited to a single cell or cell type.

The Northern blots in Figure 2 demonstrate that the expression of mRNA encoding hsp70RY in the promyelocytic HL-60 cell line was not altered under conditions inducing differentiation toward the macrophage/monocyte lineage (100 nM phorbol myristic acetate) (17).

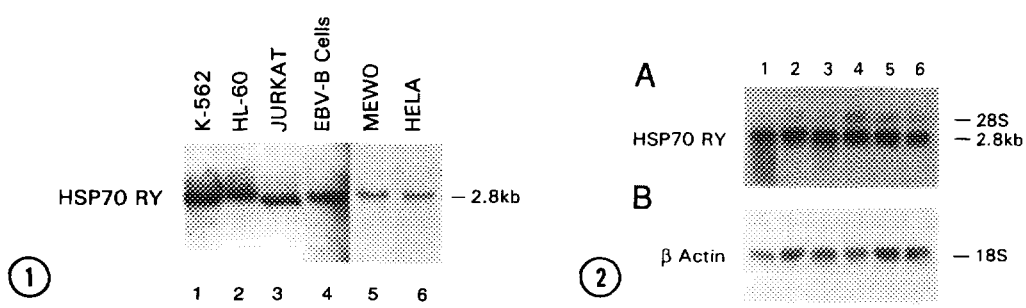

Figure 1. Northern blot probed with the ~1700 bp cDNA encoding hsp70RY. Samples of total RNA (20 μg/lane) were from human cell lines are as indicated above each lane (1-6).

Figure 2. Northern blot probed with hsp70RY cDNA (panel A) and then with beta-actin (panel B). Lane 1 contains total RNA (20 μg) from human promyelocytic HL-60 cells; lanes 3-6 contain total RNA (20 μg) from HL-60 cells induced to differentiate with 100 nM phorbol-myristic acetate (PMA) for lane 3, 24 hrs; lane 4, 48 hrs; lane 5, 72 hrs; and lane 6, 96 hrs. Lane 2 contains total RNA from cells grown in the presence of the carrier alone (1:1 DMSO/HBSS). The blot in panel B demonstrates equivalent loading and transfer of all lanes.

Similar results are obtained with the human myelomonocytic cell line U-937 in the presence of PMA (data not shown).

The cDNA and predicted amino acid sequence of hsp70RY suggests its membership in the hsp70 gene family (15). The predicted amino acid sequence of hsp70RY is 33-34% identical to the other human hsp70 proteins that have been characterized (Figure 3). As such, it is a more distant relation, as the other human hsp70 proteins share 63-86% amino acid sequence identity with one another. Most striking is a sequence of approximately 100 amino acids (amino acids 500-600) near the carboxy-terminus of hsp70RY. This is within the region characterized as the variable, substrate binding domain of the hsp70 protein family (1, 13, 14). This sequence insertion is rich in glutamate residues, and is markedly more acidic than the remainder of the protein (calculated pI 4.07, as compared to the calculated pI of amino acids 1-500, at 8.02). The significance of this region is unclear, but it is interesting to note that there are no other members of the hsp70 protein family (human or non-human) that contain this 100 amino acid sequence, and there is no striking homology between this region and any other proteins listed in the Swiss Protein Database.

The Southern blots in Figure 4 contain human (panel A) and murine (panel B) genomic DNA digests probed with an ~1700 bp segment (bp 134-1800) of the coding sequence of hsp70RY (panel C). The results demonstrate the existence of a murine homolog of this gene. In addition, the absence of an ~645 base pair hybridizing band in the DNA cut with PstI (panel A, lane 1) suggests the presence of at least one intron within the coding sequence. Similarly, there are multiple hybridizing bands in the lanes cut with EcoRI and with HindIII, which is also

	hsp70RY	grp78	hsp70	hsc70
hsp70RY	100			
grp78	34.4	100		
hsp70	33.6	63.3	100	
hsc70	33.0	65.7	86	100

Figure 3. Comparison of the degree of sequence identity shared among the members of the human hsp70 protein family. Values represent the degree of sequence identity as calculated by the Bestfit program of the Genetics Computer Group software. Sequences were obtained from references 20-24.

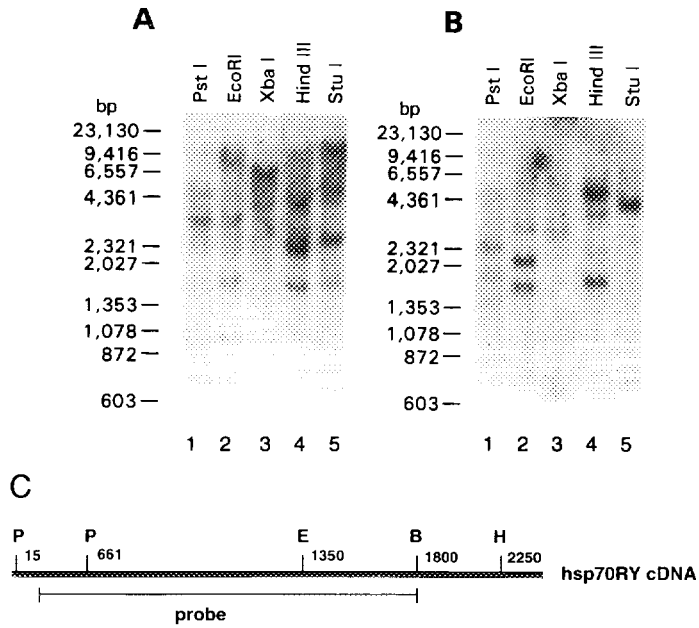

Figure 4. Human (panel A) and mouse (panel B) genomic Southern blots probed with hsp70RY cDNA. Restriction digestion performed with enzymes as indicated above each lane (1-5). Panel C is a schematic of the cDNA sequence of hsp70RY (15) and of the probe used in these studies. The indicated restriction sites include P (Pst I), E (EcoR I), and H (Hind III); the number represents the position of these sites in the hsp70RY cDNA sequence.

consistent with the presence of introns within the coding sequence; however, the existence of additional copies of this or a closely related gene cannot be ruled out.

Methods

Isolation of cDNA for hsp70RY: Total RNA from EBV-transformed normal B cells was reverse transcribed into double-stranded cDNA using an oligo dT primer and RNase H (cDNA synthesis kit, Boehringer Mannheim, Indianapolis, IN). This material was used as a template for polymerase chain reaction (PCR) using primers corresponding to base pairs 134 to 158 (5' to 3') and base pairs 2239 to 2215 (3' to 5') of the original reported sequence of hsp70RY (15). This 2100 base pair PCR product was subcloned into pBluescript (Stratagene, La Jolla, CA) and sequenced to confirm its identity as hsp70RY.

Cell cultures: All cell lines were maintained in active growth in RPMI (Biofluids, Rockville, MD) supplemented with 10% heat-inactivated fetal calf serum (HyClone, Logan, UT), 2mM glutamine (HyClone) and 10 units/ml penicillin, 10 micrograms/ml streptomycin (Biofluids). For induction experiments, HL-60 cells were seeded at 2 x 10^5 /ml in complete medium to which phorbol 12-myristate 13-acetate (PMA, Aldrich Chemical Co., Milwaukee, WI) was added to a final concentration of 100 nM.

Preparation of total RNA: Cells in suspension were harvested by centrifugation (200 g, 5 min at 4°C). Adherent cells were detached by incubation in 0.5 mM ethylene diamine tetraacetic acid (EDTA), pH 8.0 in phosphate buffered saline (PBS) without calcium or magnesium. After washing in PBS, the total cell pellet was either lysed in 4M GT and RNA purified on a cesium

gradient (18), or lysed using RNAzol (TEL-Test, Inc., Friendswood, TX) and processed as per manufacturer's instructions. Purified RNA was quantitated by absorbance at 260 nm in a Bechman DU640 spectrophotometer.

Northern blotting: Total RNA (20 μg/lane) prepared as described above was diluted in sample buffer and subjected to formaldehyde-agarose gel electrophoresis under standard conditions (x). The RNA was then transferred to either a nitrocellulose (Schleicher and Schuell, Keene, NH) or nylon membrane (Amersham, Arlington Heights, IL), and crosslinked using the Stratalinker 1800 (Stratagene). The membranes were prehybridized and hybridized at 42°C under standard conditions (18) with 2 x 10^6 cpm/ml of random-primed (Boehringer Mannheim) alpha [^{32}P]dCTP labelled probe. The probe consisted of the first ~1700 base pairs (134 to 1800 of the original reported sequence) of hsp70RY. The beta-actin probe was a 50 base oligonucleotide (antisense bases 2 to 16 (19)) radiolabelled at the 5' end with gamma [^{32}P]ATP.

Genomic Southern blots: Genomic DNA was prepared from normal human lymphocytes or from mouse 3T3 cells (gift of Dr. Jiliang Gao), digested with the restriction enzymes indicated and electrophoresed on an 0.8% agarose gel. Denaturation, neutralization, transfer and hybridization were all performed as per standard protocols (18) using the hsp70RY probe described above.

Sequence analysis: Searches of GenBank (release 82, 4/94) and Swiss Protein Database (release 28, 2/94) were performed with the assistance of the Genetics Computer Group Software, Version 7 (Madison, WI) on-line at the National Institutes of Health.

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