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Human centrosomal epitope is shared specifically with human lactate dehydrogenase-B isozyme

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A rabbit serum (0013) used to identify pericentriolar proteins from isolated centrosomes (Gosti-Testu, F., Marty, M.C., Berges, J., Maunoury, R. and Bornens, M. (1986) EMBO J. 5, 2545–2550) was shown also to react through the same epitope with several non-centrosomal proteins including a major 36 kDa cytosolic antigen. This protein was identified to be human lactate dehydrogenase and the co-distribution of 0013 epitope on the centrosomal protein and on lactate dehydrogenase (LDH) was shown to be specific for human cells (Gosti, F., Marty, M.C., Courvalin, J.C., Maunoury, R. and Bornens, M. (1987) Proc. Natl. Acad. Sci. USA 84, 1000–1004). Human hepatic cells constitute, so far, the only exception to this co-distribution rule. By using this cell type which expresses only the LDH-A4 isozyme, we demonstrate that 0013 epitope is specific for the human LDH-B subunit, making serum 0013 the strongest anti-LDH-B available so far. The evolutionary and physiological significance of this situation is discussed.

LDH-B; Centrosome; Human hepatic cell

1. INTRODUCTION

Non-immune sera have proved to be useful cytological tools to probe cellular structures (for a review, see [3]). Serum 0013, originally reported for its strong spontaneous anti-human centrosome specificity [4] has been utilised as such to identify, in KE37 lymphoblastic cells, high molecular weight pericentriolar antigen involved in microtubule nucleation [1]. However, serum 0013 was complex as evidenced by its reaction with non-centrosomal cytological structures and their corresponding proteins. Using several independant cell fractionation procedures and affinity purification of immunoglobulins on antigens, serum 0013 was demonstrated to contain a major family of immunoglobulins reacting with an epitope present on the high molecular weight centrosomal antigen, a 80 kDa nucleolar protein [5], and surprisingly, an abundant cytosolic protein identified as the human lactate dehydrogenase (LDH) [2]. In mammals, the five isozymes of the tetrameric LDH are found in various proportions among different somatic tissues and are produced in vivo by the combination of A (muscle) and B (heart) subunits, whereas the homotetrameric LDH-C4 is present only in mature testis and spermatozoa [6]. We report here that the 0013 epitope common to the centrosomal antigen and to LDH is found only on the LDH-B_4 isozyme.

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2. MATERIALS AND METHODS

2.1. *Material*

Purified human lactate dehydrogenase (LDH) isoforms A4 (ref: L3632) and B4 (ref: 6508) were purchased from Sigma. The 0013 serum was obtained as described previously [1]. Human monolayer cell cultures used were Hela, D-17, SA4, SA39, SA45, SA101, SA52, SA72, SA87 [7,8], HepG2 [9] and KE 37 [10].

2.2. *Immunofluorescence microscopy*

After a washing step in PBS (150 mM NaCl/10 mM sodium phosphate, pH 7.4), cells were fixed directly in methanol for 6 min at −20°C. The 0013 antibody was diluted in PBS containing 3% (v/v) bovine serum albumin. All the washing steps were performed in PBS containing 0.1% Tween 20.

2.3. *Gel electrophoresis and immunoblots*

Gel electrophoresis analysis was performed according to Laemmli [11]. After electrotransferring to nitrocellulose filters [12,13], antigenic proteins were visualised with a 1:500 or a 1:100 dilution of 0013 or anti-LDH-A sera, respectively, using alkaline phosphatase coupled with anti-rabbit immunoglobulins (Promega). LDH isozymes were separated on agarose gels in non-denaturing buffer (pH 8.2) and activities were visualised by Nitro blue tetrazolium reduction to formazan (Paragon electrophoresis system and lactate dehydrogenase isozyme kit, Beckman Instruments) according to the manufacturer's recommendations. When needed, isoelectrophoresed LDH isozymes were transferred to nitrocellulose filters by diffusion.

2.4. *RNA analysis*

Total RNA from Hela, KE37 and HepG2 cells was extracted according to Auffray and Rougeon [14]. Poly(A)$^+$ RNA, isolated by selection on oligo d(T)cellulose, was then fractionated by electrophoresis through a 1% agarose gel containing formaldehyde, and transferred to nylon filter. Probes were either a 1.7 kb *Xho*I fragment containing the full-length cDNA sequence coding for human LDH-A

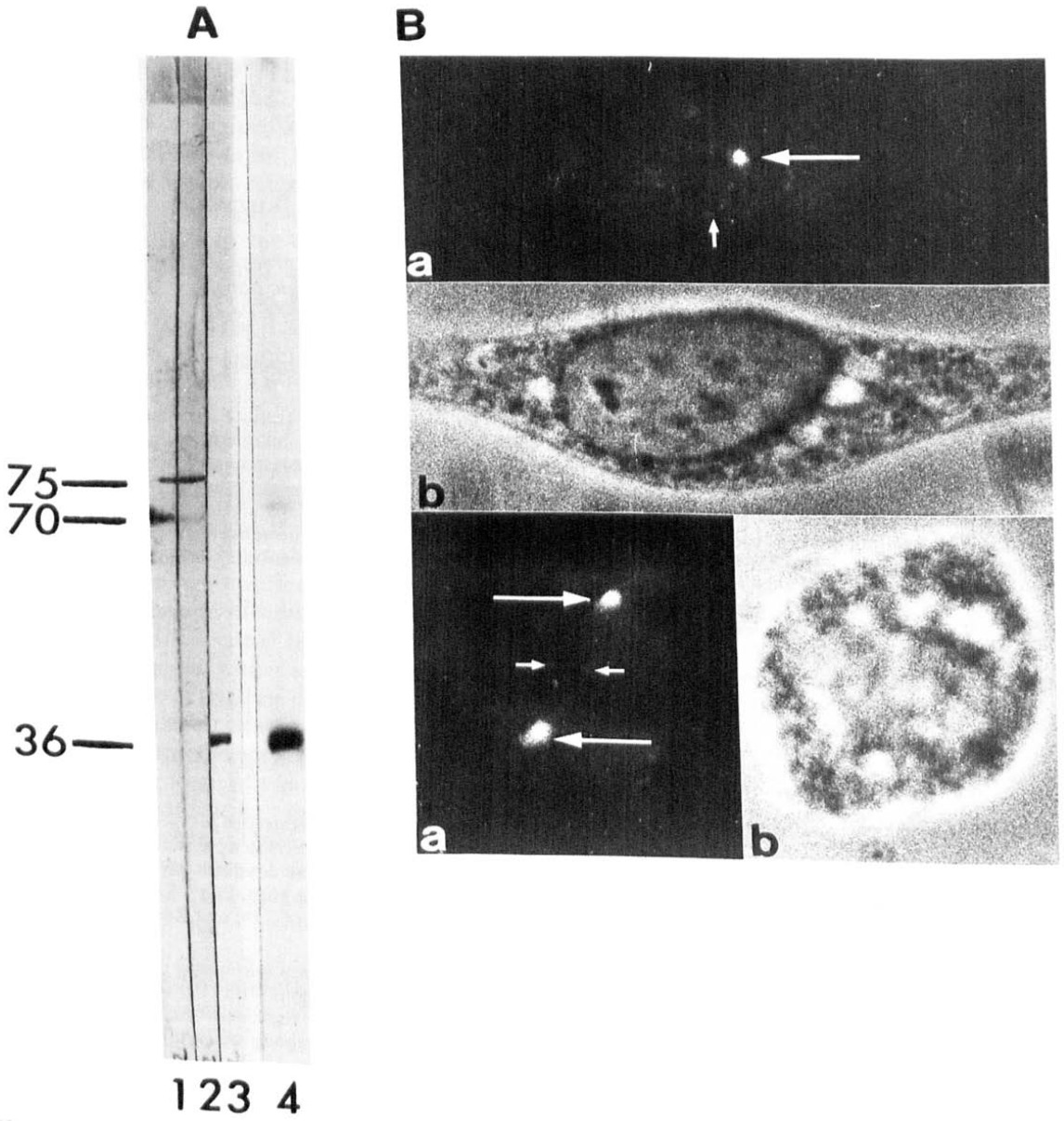

Fig. 1. Identification of 0013 antigens in human hepatic cells. (A) Comparison of the antigens revealed by 0013 serum in hepatic and HeLa cells. Western blot of total cellular proteins from a human liver biopsy with serum 0013-33 (lane 1), serum 0013-34 (lane 2) and with serum anti-human LDH-A (lane 3). (Lane 4) Western blot of a detergent-soluble protein fraction from HeLa cells with serum 0013-34. Note the absence of LDH labelling in lanes 1 and 2. A similar result is obtained with cultured HepG2 cells. Molecular weights are indicated in kDa. (B) Immunofluorescence of HepG2 cells extracted with 1% Triton X-100 before fixation with methanol: a, immunofluorescence staining with serum 0013-34; b, phase contrast. Note the labelling of the centrosome (⟶) and the NoR (→) in interphase as well as in mitosis.

(from clone pCD380; [15]) or a 1.3 kb *Eco*RI fragment containing the full length cDNA coding for human LDH-B (from clone B619.3; [16]). They were labelled by nick-translation [17]. Hybridization and high-stringency washing steps were performed according to Church and Gilbert [18].

3. RESULTS AND DISCUSSION

3.1. *0013 epitope representation in human cell lines*

Affinity purification of antibodies on immunoreactive bands identified a centrosomal epitope (0013) as present also on an abundant non-centrosomal antigen of 36 kDa, identified as LDH [2]. In nine human cell lines analysed in addition to KE37, the centrosomal labeling was always accompanied by a strong labelling of LDH (data not shown), whereas it was not the case in non-human cell lines (as in rodent, for example, [2]).

Immunostaining of the human hepatoma cell line, HepG2, with serum 0013 shows a labelling of the centrosomal area in interphase as well as a strong staining

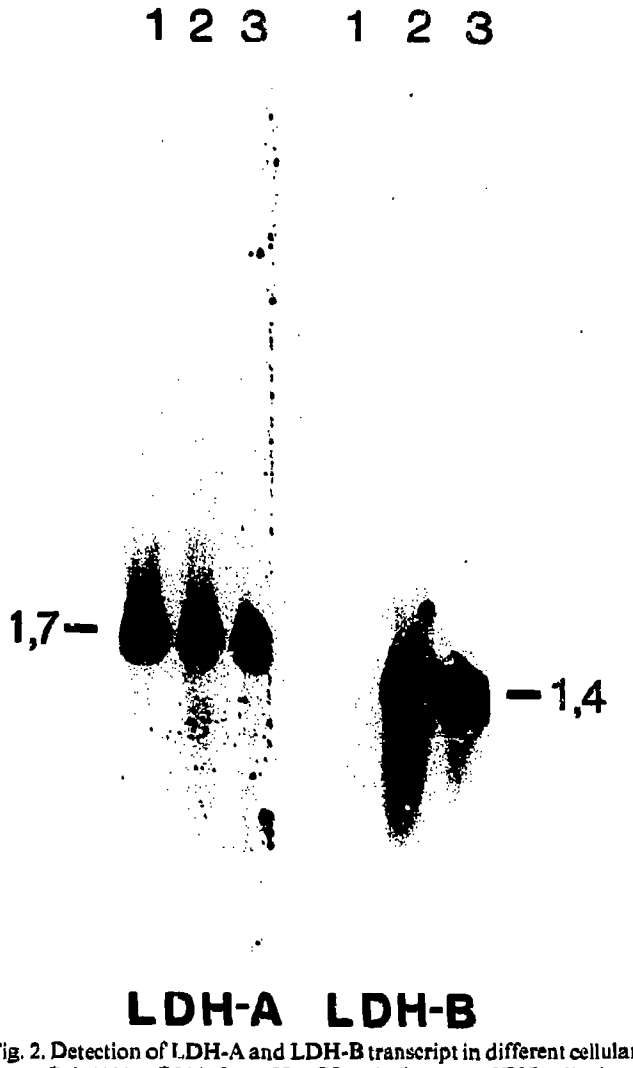

Fig. 2. Detection of LDH-A and LDH-B transcript in different cellular types. Poly(A)$^+$ mRNA from HepG2 cells (lane 1), KE37 cells (lane 2) and HeLa cells (lane 3) were fractionated on a 1.1% agarose gel, transfered to nylon filters and hybridized either with a human LDH-A or LDH-B specific cDNA probe. Note the absence of LDH-B signal in HepG2. Molecular size is indicated in kb.

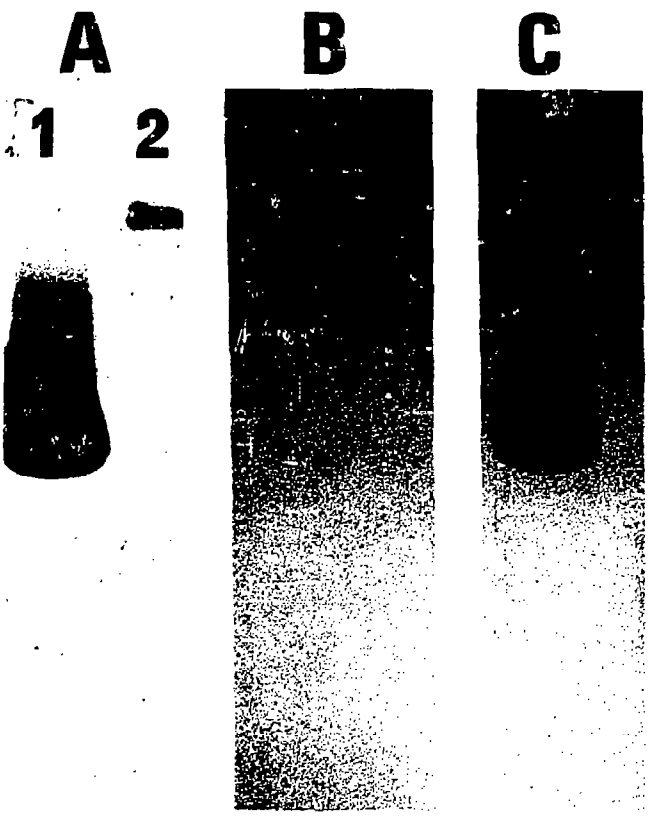

Fig. 3. Serum 0013 is specific for LDH-B isozyme. Purified LDH-A4 (lane 1) and LDH-B4 (lane 2) were separated by isoelectrophoresis and stained for their enzymatic activity (A) or Western blotted either with 0013 anti-centrosome (B) or anti-LDH-A (C) sera. Note that each serum is specific for a different isozyme, 0013 serum detecting specifically LDH-B4.

of mitotic poles (Fig. 1B). However, Western blot experiments with 0013 serum on human liver protein did not show any staining of LDH although this protein is present and perfectly stained by an anti-LDH-A antibody (Fig. 1A). This constitutes the only exception to the centrosomal and LDH co-distribution rule of 0013 epitope distribution in human cells. We noted that in liver, 0013 epitope was also found on a 75 kDa protein, the anture of which has not been explored.

3.2. *0013 epitope is specific for LDH-B*

In mammals, lactate dehydrogenase isozymes are encoded by LDH-A (muscle), -B (heart) and -C (testis) genes, the expression of which is developmentally regulated and tissue-specific [6]. In human liver, only LDH-A_4 isozyme is detected. Northern blot experiments performed with specific probes of each LDH subunit (Fig. 2) demonstrated the presence of LDH-A and LDH-B transcripts in lymphoblastic KE37 and epithelial HeLa cell lines. The sizes of the transcripts (1.7 kb for LDH-A and 1.4 kb for LDH-B) are in agreement with previous results [15,16]. Transcribed mRNA coding for LDH-B isozyme was not detected in the HepG2 cell line suggesting that the absence of LDH labelling by serum 0013 could be due to its specificity for LDH-B subunit. We definitively demonstrated that this was in fact the case by performing Western blot experiments with the two sera: 0013 and anti-LDH-A, on purified LDH isozymes separated by isoelectrophoresis, using 0013 serum and an anti-LDH-A serum (Fig. 3). LDH-A was not stained with 0013 serum, while this antibody recognized the LDH-B subunit.

3.3. *Evolutionary and physiological significance of 0013 epitope distribution in human cell lines*

The antigenicity of proteins is largely due to the topography of a relatively small region of the molecular surface. The nucleotide and amino acid sequences of the human LDH-B coding region show 68 and 75% homologies, respectively, with those of LDH-A, and only 49% of the 322 nucleotide differences resulted in amino

acid substitutions, the largest concentration of changes occurring in amino and carboxyl-terminal arms of the LDH molecule [16].

The detection, by Western blot analysis of the 0013 epitope of the denatured LDH-B, together with the conservation of this epitope on small proteolytic LDH-B peptide products (data not shown), would argue for a linear nature of this antigenic determinant. Identification of this epitope is currently being investigated (testing synthetic peptides for their antigenicity). Indeed, it has been proved experimentally difficult to obtain an anti-LDH-B serum with a high titer. Serum 0013 is thus the best known so far for its strong specificity towards LDH-B (Dr. Li, unpublished observation).

LDH-B is a 'capricious' protein [19]: it is a ubiquitous and important 'housekeeping' enzyme, but at the same time it can be absent in humans without apparent ill-effects [20]. It reaches levels of 5% of total protein in mouse oocytes [21] or even 23% in some avian lenses [22]. Moreover, besides its glycolytic role, it displays unexpected properties such as binding to lipid layers [23], and it has been demonstrated that this enzyme has been recruited (with post-translational modifications) to an extra role as a structural protein in the avian or crocodile lens without gene duplication and sequence divergence [22,24]. Possession of an antigenic determinant in common with centrosomal proteins is an intriguing property to be added to this growing list. In this context we also have to remember that another dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase (GPDH), has been shown to bind to both tubulin and tubulin polymers in a concentration-dependent manner, leading to the formation of microtubule bundles. This effect was shown to be inhibited by ATP [25,26].

The three different genes encoding for LDH-A, LDH-B and LDH-C appear to have originated from an ancestral gene during the course of evolution. They have been mapped on human chromosome 11, 12 and 11, respectively [27]. The sequence of the centrosomal antigen will be necessary to gain more insights onto the extent of homology between LDH-B and the centrosomal protein.

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