

Title	<ORIGINAL REPORT>IMMUNOHISTOCHEMICAL STUDIES ON LACTATE DEHYDROGENASE SUBUNITS IN LUNG CANCER CELLS
Author(s)	ITO, Motohiko; FUKUDA, Haruo; BABA, Mitsuo; HARADA, Takayuki; MORIKAWA, Shigeru
Citation	京都大学結核胸部疾患研究所紀要 (1974), 8(1): 11-18
Issue Date	1974-12-30
URL	http://hdl.handle.net/2433/52248
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

IMMUNOHISTOCHEMICAL STUDIES ON LACTATE DEHYDROGENASE SUBUNITS IN LUNG CANCER CELLS

**Motohiko ITO¹⁾, Haruo FUKUDA¹⁾, Mitsuo BABA²⁾,
Takayuki HARADA²⁾ and Shigeru MORIKAWA²⁾**

*Department of Thoracic Surgery¹⁾, and Department of Pathology²⁾,
Chest Disease Research Institute, Kyoto University*

(Received for publication on September 21, 1974)

INTRODUCTION

It is well established that lactate dehydrogenase is a tetramer composed of H and M subunit which combine randomly to make five isozymes,^{1,2,3)} and that the isozyme pattern of tumor tissue is quite different from that of normal tissue in which tumor is originated.^{4,5,6)}

We have already reported the difference of immunohistochemical distribution of LDH subunits in normal bovine tissue,⁷⁾ and in normal porcine liver⁸⁾ using fluorescent antibody technique.

In this study we examined the immunological cross reactivity between porcine LDH and human LDH against anti-porcine LDH antisera, and the immunohistochemical distribution of LDH subunits in human lung cancer cells in histological sections and in cytological smears of pulmonary adenocarcinoma.

MATERIALS AND METHODS

Preparation of antisera

Lactate dehydrogenase from porcine heart(LDH-4H) and lactate dehydrogenase from porcine muscle(LDH-4M) obtained from Boehringer and Sohne GmbH(Mannheim, Germany) were used as antigens without further purification.

Antisera were prepared in male albino rabbits as reported in a previous paper.⁷⁾

Specificity of antisera was examined by double gel diffusion method of Ouchterlony and by immunoelectrophoresis, procedures of which were fully described in our previous papers.^{7,8)}

Immunological cross reactivity between porcine LDH and human LDH against anti-porcine-LDH antisera was also examined by double gel diffusion method of Ouchterlony.

This study was supported by a Grant-in Aid for Scientific Research from the Ministry of Education.

Preparation of fluorescein conjugates

Globulin fraction of antisera was conjugated with FITC (Baltimore Biological Lab., Baltimore). Following separation from free fluorescent dyes by gel filtration on Sephadex G-50, the conjugate were purified by fractionation on DEAE-cellulose columns.

These procedures are fully described in our previous papers.^{7,9)}

Preparation of specimen

Cells and tissues from resected cases of pulmonary adenocarcinoma were used in this study.

Touch smears from resected adenocarcinoma were dried for 15–30 minutes at room temperature, fixed in ether-alcohol-acetone mixture for 15 minutes, washed with phosphate buffered saline (PBS) and then stained with FITC-conjugates.

Small piece of fresh cancer tissue was put in a test tube and frozen at -70°C immersing the test tube in an acetone-dryice mixture.

Frozen specimen were then cut into $4-5\ \mu$ sections in a cryostat at -20°C , placed on a glass, dried for 15–30 minutes at room temperature, and then fixed with ether-alcohol-acetone mixture for 15 minutes. Fixed specimen were then washed with PBS and stained with FITC labelled antisera.

Staining with FITC conjugate and microscopy

After washing with PBS, specimen were stained with FITC labelled conjugates in a moist chamber at room temperature overnight.

These slides were then washed with PBS, mounted in glycerol containing 10% PBS and examined under a Carl-Zeiss fluorescence microscope.

RESULTS

Specificity of antisera

The antisera were tested for the specificity by immunodiffusion and immunoelectrophoresis as shown in Fig. 1 and Fig. 2. No contamination was observed between antigen and antisera in our immune system.

Cross reactivity between anti-porcine LDH antisera and human LDH

The results of Ouchterlony's immunodiffusion between the anti-porcine LDH antisera and human LDH is shown in Fig. 3. Cross reactivity is seen between the antisera and human lactate dehydrogenase.

Immunohistochemical distribution of LDH subunits in lung cancer cells

Generally specific fluorescence of LDH H and LDH M in the cytological smears is concentrated in the perinuclear area in the cytoplasm of cancer cells. Diffuse fine fluorescence of LDH in the cytoplasm is stronger in LDH M than in LDH H. But no remarkable difference of distribution between both subunits can be seen in the cytological smears of adenocarcinoma. These findings are demonstrated in Fig. 4 and Fig. 5.

In cryostat-sectioned specimen, the fluorescence of LDH M seems to be fine and diffuse comparing with that of LDH H in some cases as shown in Fig. 6 and Fig. 7. But in most cases no difference of distribution between both subunits can be seen in the cryostat sectioned specimen.

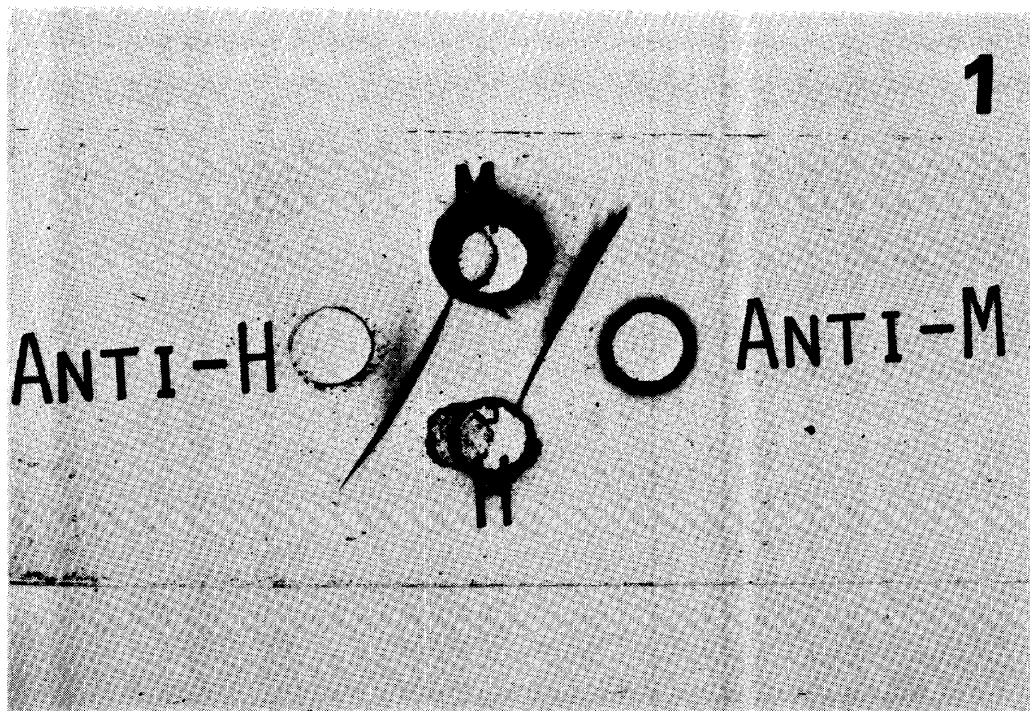


Fig. 1 Double gel diffusion study of LDH subunit and antisera. A precipitation band was observed between LDH 4H and anti-LDH H, and between LDH 4M and anti-LDH M.

DISCUSSION

The difference of the distribution of LDH H and LDH M subunits in normal cells and tissues have been reported from the electrophoretical study¹⁾ and the enzyme histochemical studies.^{10,11)} Recently, the distribution of lactate dehydrogenase subunits were studied immunohistochemically in our laboratory.^{7,8)}

We extended the immunohistochemical study of the subunits in neoplastic tissues, for we have not seen any report on the distribution of both subunits in cancer cells or tissues.

In this paper we tried to demonstrate the distribution of H and M subunits of lactate dehydrogenase in lung cancer cells.

The isozyme pattern of tumor tissue generally shows the predominant M-component of the lactate dehydrogenase comparing with that of normal tissue.^{4,5,6)} So some differences may be expected in the distribution of LDH H and LDH M subunits.

Antisera prepared from porcine lactate dehydrogenase reported in previous papers^{7,8)} was revealed to cross-react with human lactate dehydrogenase in our immune system as some authors reported.^{12,13)} So our immunofluorescent conjugates might be able to locate the distribution of LDH subunits in human cancer cells.

The tissue from the case of squamous cell carcinoma contain many necrotic masses, and there appear many non-specific fluorescence. Small cell undifferentiated carcinoma cells have poor cytoplasm. Both of them are not adequate for studying the intracytoplasmic distribution of lactate dehydrogenase subunits.

So we examined the distribution of lactate dehydrogenase subunits in the cytological smears

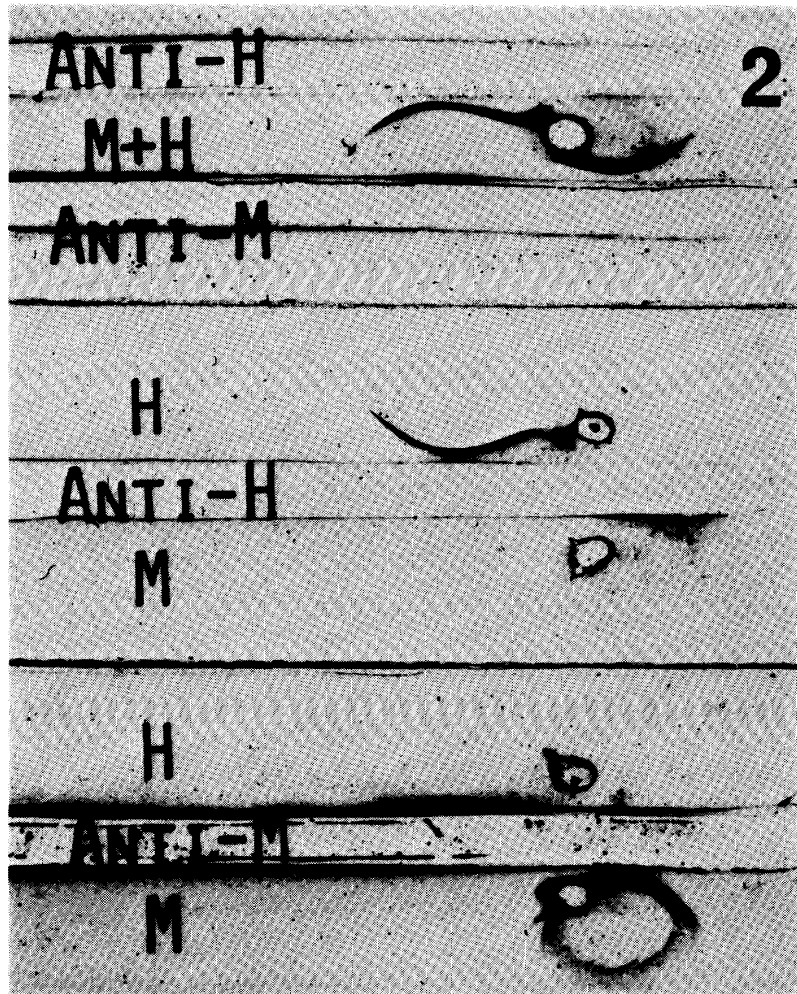


Fig. 2 Immunoelectrophoretic study of LDH subunit.

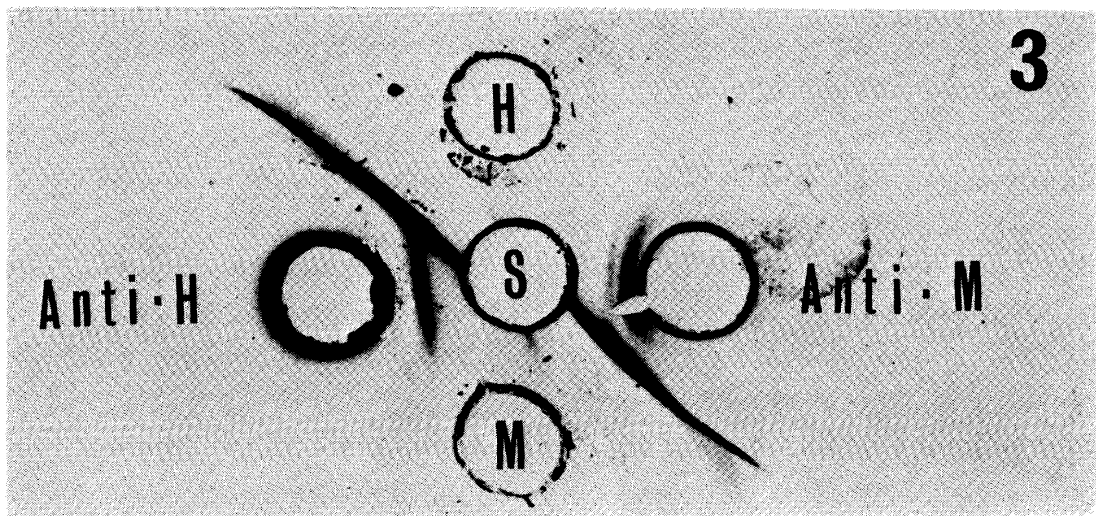


Fig. 3 Double gel diffusion study of human LDH and porcine LDH. (S. :Human serum)



Fig. 4 The immunofluorescence of LDH H in the cytological smear.



Fig. 5 The immunofluorescence of LDH M in the cytological smear.

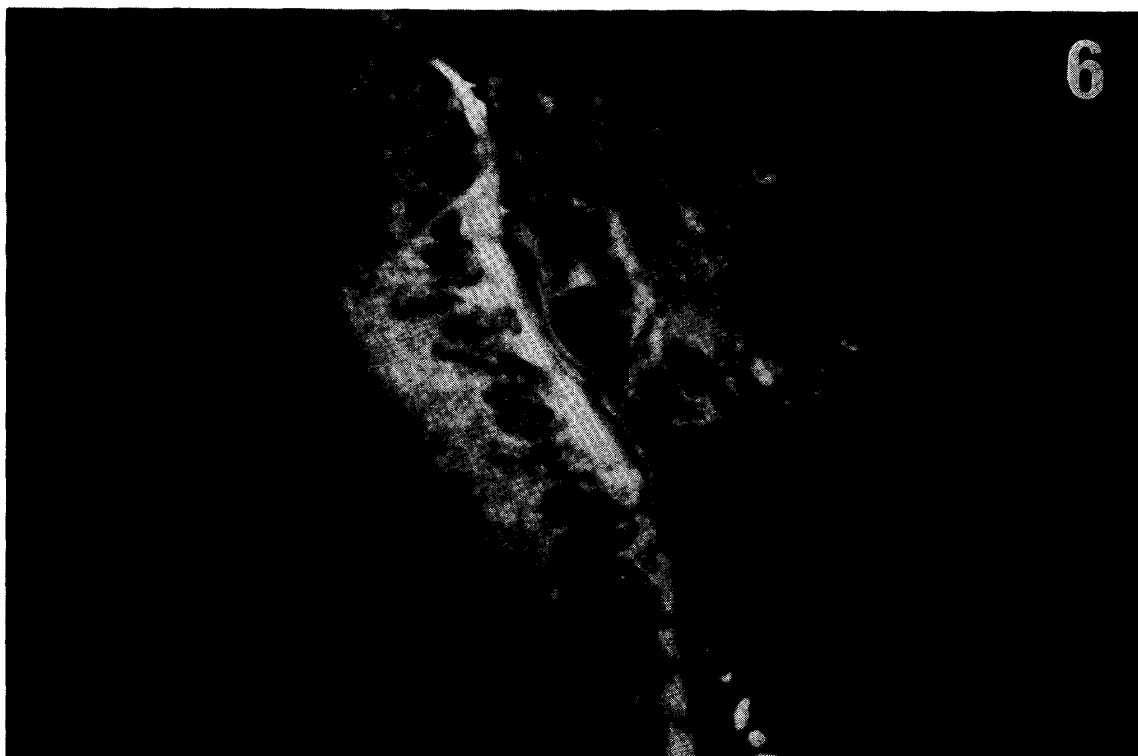


Fig. 6 Immunohistochemical findings of LDH H in the cryostat-sectioned specimen.

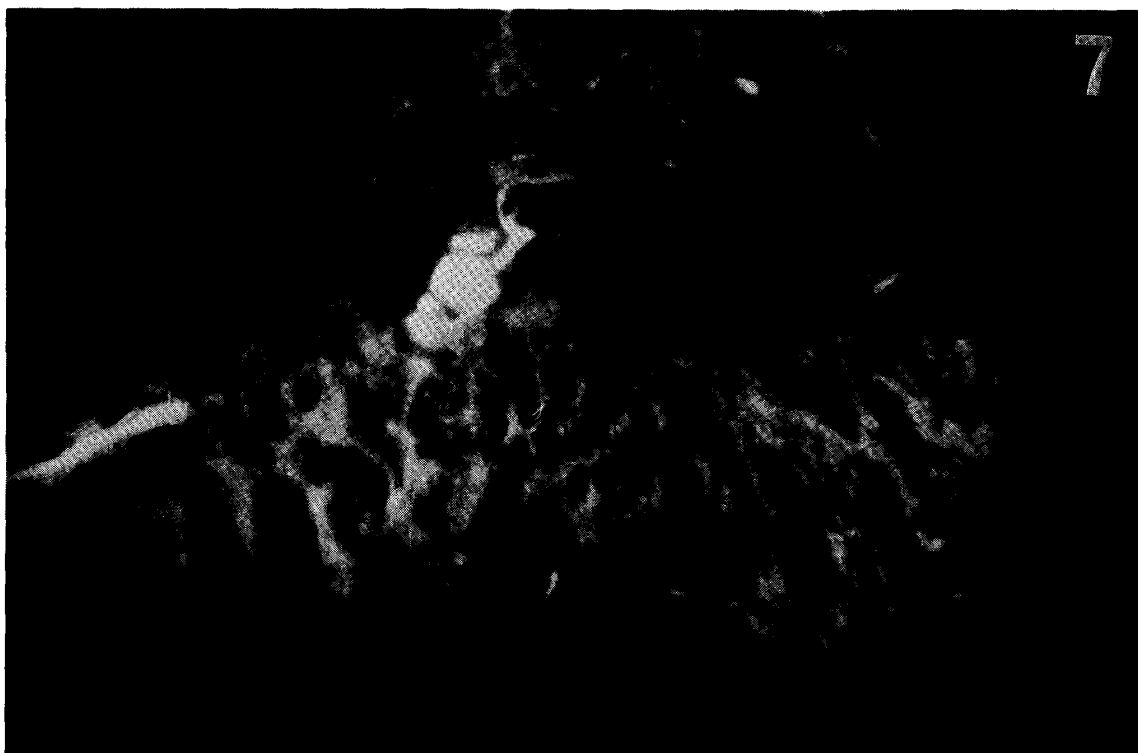


Fig. 7 Immunohistochemical findings of LDH M in the cryostat-sectioned specimen.

or cryostatt-sectioned specimen from the case of pulmonary adenocarcinoma.

Both in cytological smears and in cryostatt-sectioned specimen, intracytoplasmic distribution of LDH subunits are almost same. But in some cases specific fluorescence of LDH-M was rather stronger and diffuser comparing with the fluorescence of LDH-H which was somewhat localized and granular in the cytoplasm.

Diffuse distribution of LDH-M fluorescence may be due to the fact that M-subunit is mainly contained in soluble fraction by cytochemical analysis. On the other hand, H-subunit is observed to be combined with subcellular organellas.

In order to clarify these problems, ultramicroscopical immunohistochemistry must be performed in future.

SUMMARY

Immunohistochemical distribution of lactate dehydrogenase(LDH) subunits in human lung cancer cells were studied using fluorescent antibody technique.

Both in cytological and histological specimen, specific fluorescence of LDH-H and LDH-M were seen in the cytoplasm of cancer cells, and in most cases no remarkable difference could be demonstrated between the distribution of H and M subunit.

But in some cases specific fluorescence of M-subunit was stronger than that of H-subunit. The fluorescence of M-subunit was demonstrated as fine granules diffusely in the cytoplasm. On the other hand the fluorescence of H-subunit was rather localized and demonstrated as rather coarse granules.

And this observation was discussed.

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