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Hideo Nobuto* Takao Kawashima[†] Nobuto Shimatani[‡]
Kisaburo Takeuchi^{**} Akemi Mihara^{††}
Takashi Seito^{‡‡} Takuro Ogata[§]

*Okayama University,

[†]Okayama University,

[‡]Okayama University,

^{**}Okayama University,

^{††}Okayama University,

^{‡‡}Okayama University,

[§]Okayama University,

A histochemical study on hydrolytic and oxidative enzymes in human sarcomas*

Hideo Nobuto, Takao Kawashima, Nobuto Shimatani, Kisaburo Takeuchi, Akemi Mihara, Takashi Seito, and Takuro Ogata

Abstract

Histochemical evaluations of human sarcomas such as reticulum cell sarcoma, fibrosarcoma, lymphosarcoma and neurofibrosarcoma, were carried out with five hydrolytic enzymes and eight oxidative enzymes. The activities of acid phosphatase and beta-glucuronidase were slightly positive in the neoplastic cells observed. Beta-esterase activity was also positive but varied according to the kind of sarcomas. Alkaline phosphatase activity was faint or negative in sarcoma cells, though positive in capillary walls. Leucine aminopeptidase activity was negative giving not any appreciable coloration of the cell as far as the method employed is concerned. Among the activities of dehydrogenases, the most intense activity was observed in lactic dehydrogenase. The activities of succinic and beta-hydroxybutyric dehydrogenases were slight. The activities of alpha-glycerophosphate, glutamic and beta-hydroxybutyric dehydrogenases were faint or slight. The activities of NADPlinked dehydrogenases, glucose-6-phosphate and isocitric dehydrogenase were all faint or slight in these sarcoma cells.

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MEDICAL SCHOOL

A HISTOCHEMICAL STUDY ON HYDROLYTIC AND OXIDATIVE ENZYMES IN HUMAN SARCOMAS

Hideo NOBUTO, Takao KAWASHIMA, Nobuto SHIMATANI,
Kisaburo TAKEUCHI, Akemi MIHARA, Takashi SEITO,
and Takuro OGATA

*Department of Surgery, Okayama University Medical School, Okayama
Japan (Director: Prof. S. Tanaka)*

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Biochemical investigations occasionally fall in great errors on the estimation of chemical substance or enzyme activity of neoplastic tissues because of the unavoidable inclusion of non-neoplastic tissues during homogenating process, while histochemical investigations have great advantage to analyse the localization of enzyme activity in the unit of cellular or tissue structure. In this decade, there appeared many histochemical observations on tumor cells and several observations on human sarcomas such as those reported by MONIS *et al.*^{2,9,11,12}, SELIGMAN *et al.*^{3,7,10}, MORI *et al.*¹³ and KABAT *et al.*¹. They observed various tumors histochemically but only a few of human tumors, and only a poor information on the enzyme activities of human sarcomas. Therefore, the authors observed the enzyme activities of human sarcoma cells by histochemical methods for the purpose to disclose some possible difference in enzyme activity between normal and tumor cells. The present paper deals with the enzyme activities of nine cases of human sarcomas. The enzyme activities observed were those of hydrolytic and oxidative ones, such as alkaline phosphatase, acid phosphatase, beta-estrerase, leucine aminopeptidase, β -glucuronidase, succinic dehydrogenase, lactic dehydrogenase, malic dehydrogenase, α -glycerophosphate dehydrogenase, glutamic dehydrogenase, β -hydroxybutyric dehydrogenase, glucose-6-phosphate dehydrogenase and iso-citric dehydrogenase.

MATERIALS AND METHODS

Nine cases of sarcomas were studied. They were 5 cases of reticulosarcoma, 2 cases of fibrosarcoma, 1 case of lymphosarcoma and 1 case of neurofibrosarcoma. The blocks for the experiment were obtained exclusively from surgical specimens and frozen in the deep freezer at -30°C . Frozen blocks were sliced 20μ thick in a -20°C cryostat, with a sliding microtome. Parallel sections were stained with hematoxylin and eosin for morphological orientation.

The sliced sections were processed for the histochemical procedures. For the histochemical demonstration of hydrolytic enzymes, the sections were fixed in 10 per cent neutral formalin for 10 minutes.

Each reaction mixture was composed as follows

Alkaline phosphatase (ALP) : Sections on slides, fixed in 10 per cent formalin for 10 minutes, were incubated for 1 hour at 20°C in the solution containing 10 mg of sodium α -naphthyl phosphate, 20 mg of Clarks and Lubs' buffer at pH 9.2, and 20 mg of Diazo blue B.

Acid phosphatase (ACP) : The same procedures were taken as those for alkaline phosphatase except that 0.1 M acetate buffer was used at pH 5.8.

β -Esterase (EST) : The formalin fixed sections were incubated for 30 minutes at 20°C in the reaction mixture; 10 mg of β -naphthyl acetate dissolved in 1 ml of acetone, 20 ml of 0.1 M Michaelis buffer at pH 7.2, and 20 mg of Diazo blue B. The stained sections were mounted in glycerin.

β -Glucuronidase (β GL) : The post-azo coupling method was employed. Formalin fixed sections were incubated for 6 hours at 37°C in the following mixture; 15 mg of 6-bromo-2-naphthyl- β -D-glucuronide dissolved in 2.5 ml of methanol, 10 ml phospho-citric buffer at pH 4.95 and 37.5 ml of distilled water. Thereafter these sections were rinsed in water and immersed in 50 ml of 0.02 M phosphate buffer at pH 7.5 containing 50 mg of Diazo blue B.

Leucine Aminopeptidase (LAP) : The sections, fixed in 10 per cent neutral formalin for 10 minutes, were incubated for 30 minutes to one hour at 37°C in the following solution; 8 mg of L-leucyl- β -naphthylamine, 10 ml of 0.1 M acetate buffer at pH 6.5, 8 ml of 0.85 per cent sodium chloride, 1 ml of 0.02 M sodium cyanide, and 10 mg of Diazo blue B. These incubated sections were washed in saline and transferred to a solution of 0.1 M copper sulfate for a few minutes.

On dehydrogenases, sections were incubated at 37°C for 30 to 60 minutes in each mixture and then fixed in 10 per cent formalin solution for 10 minutes.

Succinic dehydrogenase (SDH) : M/5 sodium succinate 5 ml, M/10 phosphate buffer pH 7.6 5 ml, Nitro BT 5 mg/3ml 6 ml, aq. 10 ml.

Lactic dehydrogenase (LDH) : M/2 sodium lactate, 4 ml; M/10 phosphate buffer pH 7.4, 10 ml; Nitro BT 5 mg/3 ml, 3 ml; 100% NAD, 2.5 mg; M/10 KCN, 2 ml; and M/2 HCl, 2 drops.

Malic dehydrogenase (MDH) : 1 M sodium malate, 5 ml; M/10 phosphate buffer pH 7.4, 10 ml; Nitro BT 5 mg/3 ml, 3 ml; 100% NAD, 2.5 mg; M/100 MgCl₂, 2 ml; M/2 HCl, 5 drops.

Glutamic dehydrogenase (GDH) : 1 M sodium glutamate, 4 ml; M/10 phosphate buffer pH 7.6, 11 ml; Nitro BT 5 mg/3 ml, 3 ml; 100% NAD, 2.5

mg; *M*/10 KCN, 2 ml; *M*/2 HCN, 5 drops.

α-Glycerophosphate dehydrogenase (αGDH): 1 *M* sodium *α*-glycero-phosphate, 4 ml; *M*/10 phosphate buffer, 11 ml; Nitro BT 5 mg/3 ml, 3 ml; 100% NAD, 2.5 mg; *M*/10 KCN, 2 ml; *M*/2 HCl, 5 drops.

β-Hydroxybutyric dehydrogenase (βHyDH): 1 *M* sodium *β*-hydroxy-butyrate, 4 ml; *M*/10 phosphate buffer pH 7.9, 11 ml; Nitro BT 5 mg/3 ml; 3 ml; 100% NAD, 2.5 mg; *M*/10 KCN, 2 ml; *M*/2 HCl; 5 drops.

Glucose-6-phosphate dehydrogenase (G6PDH): *M*/200 glucose-6-phosphate, 6 ml; Veronal buffer pH 7.4, 17 ml; Nitro BT 5 mg/3 ml, 4.5 ml; 100% NADP, 7 mg; *M*/100 MgCl₂, 3 ml; *M*/2 MnCl₂, 3 ml.

Isocitric dehydrogenase (ICDH): *M*/10 sodium isocitrate, 4 ml; Veronal buffer pH 7.4, 11 ml; Nitro BT 5 mg/3 ml, 3 ml; 100% NADP, 2.5 mg; *M*/100 MgCl₂, 2 ml; *M*/2 MnCl₂, 2 ml.

RESULTS

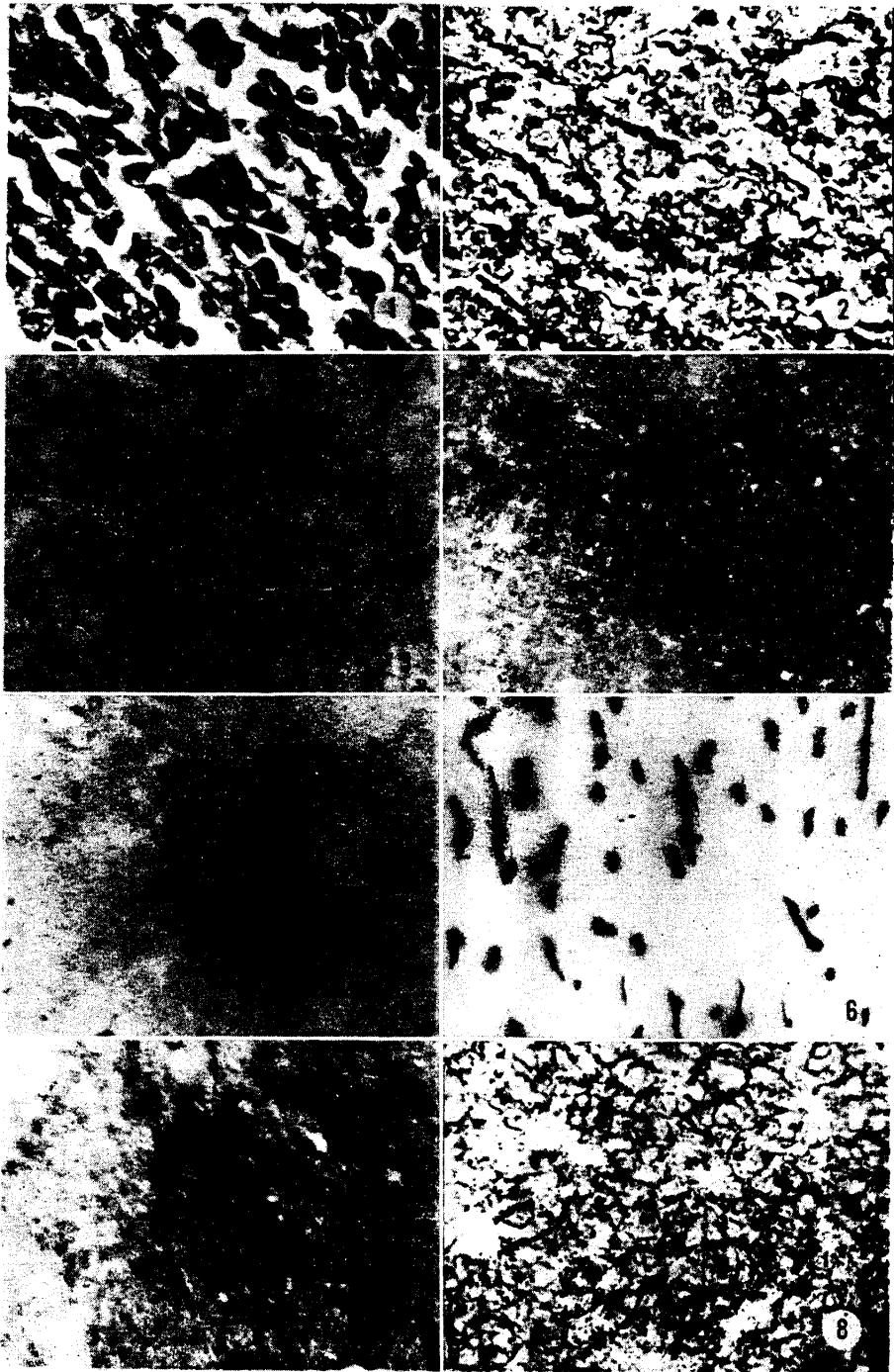
ALP: Enzymatic reaction was faint or negative in all the sarcomas examined in the present study. Only capillary wall showed a strong enzyme activity.

ACP: Reticulum cell sarcoma had a weak activity, but other sarcomas showed faint or no activity. The azo-dye pigments were deposited in granular

Table 1 Distribution of Enzymatic Activities in Human Sarcoma Cells

Diagnosis	No. of cases	Location	Sex	Age	ALP	ACP	βEST	βGL	LAP	SDH	LDH	MDH	GDH	αGDH	βHyDH	G6PDH	ICDH
Reticulum cell sarcoma	1	Caecum	♀	74	-	+	+	±	-	+	±	+	+	-	+	+	+
Reticulum cell sarcoma	2	Retroperitoneum	♂	62	-	±	±	±	-	+	±	+	+	+	±	+	±
Reticulum cell sarcoma	3	Side of neck	♀	34	+	+	+	±	-	±	±	+	+	+	±	±	+
Reticulum cell sarcoma	4	Side of neck	♀	24	±	+	+	+	-	±	±	±	+	+	±	±	-
Reticulum cell sarcoma	5	Tonsil	♂	17	±	+	-	+	-	+	±	±	+	±	-	-	-
Lymphosarcoma	1	Inguinal lymph node	♀	42	-	-	±	-	±	±	±	±	-	±	±	-	-
Fibrosarcoma	2	Retroperitoneum	♂	12	-	+	+	+	-	±	±	+	+	+	+	+	+
Fibrosarcoma	1	Fibula	♂	38	-	-	+	+	-	±	±	±	+	+	±	+	+
Neurofibrosarcoma	1	Inguinal region	♀	48	-	-	-	±	-	±	±	±	+	+	±	±	+

- : negative, ± : faint, + : slight, ±± : moderate, ±±± : intense



form in the cytoplasm.

LAP : Leucine aminopeptidase activity was absent in all cases of sarcomas.

β -EST : β -Esterase activity was negative or faint in all five reticulum cell sarcomas and a neurofibrosarcoma. In the cytoplasm of fibrosarcoma and lymphosarcoma, the reaction was faint to moderate. Necrotic area of most tumors showed a moderate or intense activity.

β -Gl : A staining reaction for β -glucuronidase was slightly positive in the cytoplasm of all the cases of sarcomas.

SDH : The activity of succinic dehydrogenase in tumor cells was rather slight or weak in lymphosarcoma and reticulum cell sarcomas but rather strong in neurofibrosarcoma. The formazan pigment formed a particularly delicate pattern suggestive of mitochondria.

LDH : As compared with other dehydrogenases, the activity of this enzyme was most intense and accentuated, especially at the periphery of tumor cells. Reticulum cells of reticulum cell sarcomas showed a moderate activity in the cytoplasm and reticulum.

MDH : Although the staining of malic dehydrogenase was somewhat weaker than that of lactic dehydrogenase, patterns of its distribution were similar to those of lactic dehydrogenase, patterns of its distribution were similar to those of lactic dehydrogenase.

Other NAD-linked dehydrogenases : α -Glycerophosphate, glutamic and β -hydroxybutyric dehydrogenases were weak in most cases of sarcomas, especially in lymphosarcoma. Their deposition patterns of formazan granules were similar to those of succinic dehydrogenase.

In both of NADP-linked dehydrogenases such as glucose-6-phosphate and isocitric dehydrogenase their activity was in general faint or slight in sarcoma cells. Occasionally, a weak activity was observed in the small arterial wall.

Fig. 1 Reticulum cell sarcoma. Hematoxylin-Eosin stain. $\times 400$

Fig. 2 Reticulum cell sarcoma. (same specimen as in Fig. 1). Silver stain. $\times 400$

Fig. 3 Reticulum cell sarcoma. (same specimen as in Fig. 1). Acid phosphatase. Enzymatic activity is observed moderately in scattered histocytes or fibrocytes, while there is weak activity in the tumor cells and connective tissue. $\times 40$

Fig. 4 Reticulum cell sarcoma. Lactic dehydrogenase. (same specimen as in Fig. 1). Tumor cells show a moderate or intense activity. $\times 40$

Fig. 5 Reticulum cell sarcoma. β -Hydroxybutyric dehydrogenase. (same specimen as in Fig. 1) Tumor cells show a weak activity. $\times 40$

Fig. 6 Neurofibrosarcoma. Alkaline phosphatase. Small capillary wall shows a strong activity. Tumor cells are not stained. $\times 40$

Fig. 7 Neurofibrosarcoma. Glutamic dehydrogenase. Moderate activity is observed. $\times 40$

Fig. 8 Lymphosarcoma. Glucose-6-phosphate dehydrogenase. Formazan granules are deposited in the cytoplasm and periphery of tumor cells. $\times 400$

DISCUSSION

Although several studies on sarcomas have been reported, histochemical evaluations of them are not sufficient. With respect to the alkaline phosphatase activity, KABAT *et al.*¹ examined various animal sarcomas induced by virus and methylcholanthrene and found no reaction in these tumors. MONIS *et al.* observed that only fibromyosarcomas of 12 sarcomas showed a weak reaction in the malignant cells. MANHEIMER *et al.*³ demonstrated that only the blood vessels were stained in a fibrosarcoma of the breast and a uterine myoma. FANGER *et al.*⁴ observed the absence of reaction in a fibrosarcoma of the breast and in a leiomyosarcoma of the uterus, but SCHAJOWICZ *et al.*⁵ reported a strikingly strong reaction in a liposarcoma of bone. BIESEL *et al.*⁶ examined tissue cultures from CROCKER mouse sarcoma 180 and observed only a faint nuclear reaction. They also studied rat sarcoma 339 which showed a strong reaction while in RON's sarcoma of the chicken showed only a weak activity.

In the present study likewise, the enzyme activity of alkaline phosphatase in human sarcomas very weak or negative except for capillary walls.

With respect to acid phosphatase activity, REINEH *et al.*⁷ studied fibrosarcoma and polymorph cell sarcoma and found a moderate activity in malignant mesenchymal tumors which showed stronger coloration than benign ones. FANGRE *et al.* revealed a positive reaction in a leiomyosarcoma of the uterus and a fibrosarcomas of the breast. TAKEUCHI *et al.*⁸ observed a positive reaction in YOSHIDA sarcoma. In the present study acid phosphatase activity was found in the cytoplasm of sarcomas with slight activity and in the histocytes with moderate activity.

On aminopeptidase, MONIS *et al.*⁹ found a strong activity in a human fibrosarcoma while entirely negative in a spindle cell sarcoma. In the present study, aminopeptidase activity was negative in all the sarcomas. CHOEN *et al.*¹⁰ could not find esterase activity in sarcomas of soft tissues.

β -Glucuronidase activity in seven sarcomas was faint or none by the study of MONIS *et al.*¹¹. The activity in the present study was slightly positive in the cytoplasm and was increased in the necrotic foci of tumors. The reticulum cells of sarcomas showed a faint or weak activity.

There are very few studies of dehydrogenases on sarcoma. However, it was found that lactic dehydrogenase activity was most intense as compared with the other dehydrogenases studied. MONIS *et al.*¹² observed a malignant lymphoma (lymphocytic), a fibrosarcoma and a spindle cell sarcoma and demonstrated an intense to moderate activity in malic dehydrogenases and a weak activity in the other dehydrogenases such as succinic and β -hydroxybutiric dehydrogenases. In the present study, the similar intensity of coloration was

perceived. MORI *et al.*¹³ reported that reticular cell sarcoma and Hodgkin's sarcoma showed a low activity of iso-citric dehydrogenase, and liposarcoma and lymphosarcoma exhibited varying stainability in glucose-6-phosphate dehydrogenase.

In general, the human sarcomas examined in this study showed a lower activity of hydrolytic and oxidative enzymes than in those of the cancer of stomach, intestines, breast and thyroid glands, as were reported in the previous papers¹⁶⁻¹⁸.

SUMMARY

Histochemical evaluations of human sarcomas such as reticulum cell sarcoma, fibrosarcoma, lymphosarcoma and neurofibrosarcoma, were carried out with five hydrolytic enzymes and eight oxidative enzymes.

The activities of acid phosphatase and beta-glucuronidase were slightly positive in the neoplastic cells observed. Beta-esterase activity was also positive but varied according to the kind of sarcomas. Alkaline phosphatase activity was faint or negative in sarcoma cells, though positive in capillary walls. Leucine aminopeptidase activity was negative giving not any appreciable coloration of the cell as far as the method employed is concerned. Among the activities of dehydrogenases, the most intense activity was observed in lactic dehydrogenase. The activities of succinic and beta-hydroxybutyric dehydrogenases were slight. The activities of alpha-glycerophosphate, glutamic and beta-hydroxybutyric dehydrogenases were faint or slight. The activities of NADP-linked dehydrogenases, glucose-6-phosphate and isocitric dehydrogenase were all faint or slight in these sarcoma cells.

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