

# Human Ferritins Present in the Sera of Nude Mice Transplanted with Human Neuroblastoma or Hepatocellular Carcinoma<sup>1</sup>

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## ABSTRACT

Fifteen nude mice were inoculated with a human neuroblastoma cell line and 14 with a human primary hepatocellular carcinoma cell line. Human ferritins were detected in the sera of the mice which developed tumors. Of 14 mice bearing human neuroblastoma, 12 had human liver-type ferritin (8 to 52 ng/ml) in their sera, and three of these also had HeLa-type ferritin (acidic ferritin) (29 to 40 ng/ml). Of 10 nude mice bearing human primary hepatocellular carcinoma, eight had human liver-type ferritin (10 to 820 ng/ml), and one of these had HeLa-type ferritin at a level of 43 ng/ml.

Since the ferritins in the sera of these mice were produced by the human tumor cells, these observations support the hypothesis that the elevated ferritins often found in the serum of patients with cancer are, in part, derived from their tumors.

## INTRODUCTION

Elevated serum ferritin levels are frequently observed in patients with cancers such as Hodgkin's disease, breast cancer, PHC,<sup>3</sup> neuroblastoma, and others (4-6, 11, 12, 15, 16, 20). The levels of serum ferritin often correlate with tumor activity (11). Increased amounts of serum ferritin in cancer patients could be due to tissue damage caused by tumor invasion and/or to an increase of unused iron associated with the chronic anemia seen frequently in cancer. However, a possible explanation is that the elevated ferritin detected in the patients' serum is derived from the tumor itself.

We tested the latter hypothesis by transplanting human neuroblastoma or PHC cells grown in tissue culture into nude mice. Human ferritins were identified in the sera of almost all the mice receiving transplants, thereby demonstrating the tumor origin of the ferritin.

## MATERIALS AND METHODS

### Cell Lines

Human neuroblastoma cell line (CHP-100) (22) and human PHC cell line (PLC/PRF/5) (1) were used for transplantation. CHP-100, a known neuroblastoma cell line, was established by Schlesinger *et al.* (22) in 1976 and originated from a 12-year-old girl with Stage IV neuroblastoma. PLC/PRF/5 is a liver cancer cell line established in 1976 by Alexander *et al.* (1) from a patient in South Africa with PHC. The cells were cultured

in roller bottles in RPMI 1640 containing 10% fetal bovine serum supplemented with penicillin (100 µg/ml) and streptomycin (50 µg/ml) as modified by the methods of Schlesinger *et al.* (22) and Alexander *et al.* (1).

### Transplantation of Tumor Cells in Nude Mice

Thirty nude mice (BALB/c-*nu/nu*) were maintained under specific-pathogen-free conditions in clam shell incubators (Clean Room Products, Bay Shore, NY). Each of 15 nude mice was inoculated s.c. in the dorsum with  $1 \times 10^7$  cells of CHP-100 in 0.5 ml of medium with a 20-gauge needle using the method of Helson *et al.* (14). Each of 14 nude mice (one died before transplantation) was inoculated s.c. in the dorsum with  $7 \times 10^6$  cells of PLC/PRF/5 in 0.5 ml of medium with a 20-gauge needle according to the method of Shouval *et al.* (23).

### Isolation and Purification of Ferritins

Ferritins were extracted and purified from HeLa cells, normal mouse liver, PHC, and neuroblastoma tumors grown in nude mice, using the method described by Arosio *et al.* (3). Ferritin protein was estimated using the method of Lowry *et al.* (17).

### RIA for Human Ferritins

Blood samples were obtained from mice by orbital sinus bleeding before transplantation and weekly after the tumor became palpable.

Because cancer cells produce ferritins with different immunological specificities (8, 12, 13, 15), assays for both liver-type and HeLa-type ferritins were used. The RIA techniques for human liver and HeLa ferritin assays were basically similar except for the incubation times with ferritins (labeled and unlabeled), antiferritin antibody, and secondary antibody.

**Human Liver Ferritin Assay.** RIA for human liver ferritin was conducted using anti-human liver ferritin (rabbit) and human liver ferritin as standards (RIANEN Kits; New England Nuclear, Boston, MA). Incubation time of cold ferritin (unknown samples or ferritin standards) with labeled liver ferritin and anti-liver ferritin antibody was 60 min at 37°, and incubation with the secondary antibody (anti-rabbit  $\gamma$ -globulin) was 10 min at room temperature before centrifugation.

**HeLa Ferritin Assay.** Purified HeLa ferritin was labeled with <sup>125</sup>I according to the method of Hazard *et al.* (13). The RIA procedure used was that described originally by Marcus and Zinberg (18) and Niitsu *et al.* (19) and modified by Hazard *et al.* (13). Ten µg of HeLa ferritin in 50 µl of phosphate buffer (0.5 M, pH 7.5) were reacted with 1 mCi of <sup>125</sup>I (<5 µl) and 10 µg of chloramine-T at 0° for 60 min. Sodium metabisulfate (100 µg) was added to stop the reaction. Unbound <sup>125</sup>I was separated from protein-bound <sup>125</sup>I by passing the reaction mixture through Sephadex G-200 in a buffer containing 0.05 M Tris-HCl, pH 7.4, and 0.25% gelatin (our experience has been that G-25 does not adequately separate the bound protein from radioactive trichloroacetic acid nonprecipitable entities). Pooled fractions containing ferritin (the first peak eluted from the column) were diluted with 0.05 M Tris-HCl, pH 7.4, containing 1% bovine serum albumin to give a stock solution of <sup>125</sup>I-labeled ferritin and stored at 4°. As a rule, the stock solution of labeled antigen was further diluted 1:2 to 1:5 for assay. Next, the highest dilution of antibody that precipitated 90 to 95% of this dilution of ferritin was chosen as the optimal amount of antibody for assay. This was usually a 1:1000 dilution; 0.2-ml volumes of labeled HeLa ferritin, anti-HeLa ferritin antibody, samples, and unlabeled HeLa ferritin standards were incubated at 4° for

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<sup>3</sup> The abbreviations used are: PHC, primary hepatocellular carcinoma; CHP, Children's Hospital of Philadelphia; RIA, radioimmunoassay; SDS, sodium dodecyl sulfate.

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18 hr; 0.5 ml of Sepharose B-coupled goat anti-rabbit IgG (RIANEN Kits; New England Nuclear) was added and incubated at room temperature for 2 hr. These mixtures were then centrifuged at  $3000 \times g$  for 10 min at  $4^\circ$ . Supernatants were removed by suction, and the precipitates were counted in a  $\gamma$ -counter.

Antisera used in these assays were absorbed with mouse liver ferritin in order to remove any cross-reaction with mouse ferritin. Details are described in the legends of Charts 1 and 2.

**$\alpha$ -Fetoprotein Assays**

Mouse sera were assayed for  $\alpha$ -fetoprotein by enzyme immunoassay using Abbott AFP-EIA Diagnostic Kits (Abbott Laboratories, North Chicago, IL).

**SDS Gel Electrophoresis of Ferritins**

Ferritins extracted from 2 PHC and 2 neuroblastoma tumors grown in nude mice were examined by electrophoresis on SDS gels using the method described by Arosio *et al.* (3). Ferritins extracted from normal human liver and from HeLa cells in culture were used as references.

**Histopathology of Tumors Grown in the Mice**

Tumors were examined by the pathologists at this Institute at the time of postmortem examination.

**RESULTS**

Chart 1 shows a competitive assay of human liver, mouse liver, and HeLa ferritins versus radiolabeled HeLa ferritin against

anti-HeLa ferritin antibody. Immunological differences among these ferritins are seen. There is little or no reaction between human liver or mouse liver ferritin and anti-HeLa ferritin antibody. The standard curve using HeLa ferritin approaches linearity between 3 and 160 ng/ml.

Similar curves are seen in Chart 2 where human liver ferritin concentrates used as standards competed with radioactively labeled human liver ferritin (obtained from New England Nuclear). It can be seen that mouse liver ferritin does not compete whether the anti-human liver ferritin antibody is absorbed with mouse liver ferritin or not.

Among 15 mice receiving transplants of CHP-100 (neuroblastoma cells), 14 developed tumors (Table 1). Tumors became palpable between 2 and 10 weeks (median, 4 weeks) after graft. The largest tumor found was 6.5 x 5.0 x 2.0 cm. Pretransplantation serum specimens of the mice did not contain human ferritin. Human ferritin was first detected at 4 weeks after transplantation. Twelve of the 14 tumor-bearing mice showed human liver ferritin in their sera. The maximum ferritin levels for each mouse measured by liver ferritin assay were 8 to 52 ng/ml (mean,  $22 \pm 14$ ; S.D.). When serum samples of 3 mice with liver ferritin levels of 11, 25.2, and 52 ng/ml were tested by the HeLa ferritin assay, their values were 36, 29, and 40 ng/ml, respectively. Nine remaining mice did not show HeLa-type ferritin in their sera. The amounts of ferritin detected in mice bearing neuroblastoma were relatively small, but they were of human origin.

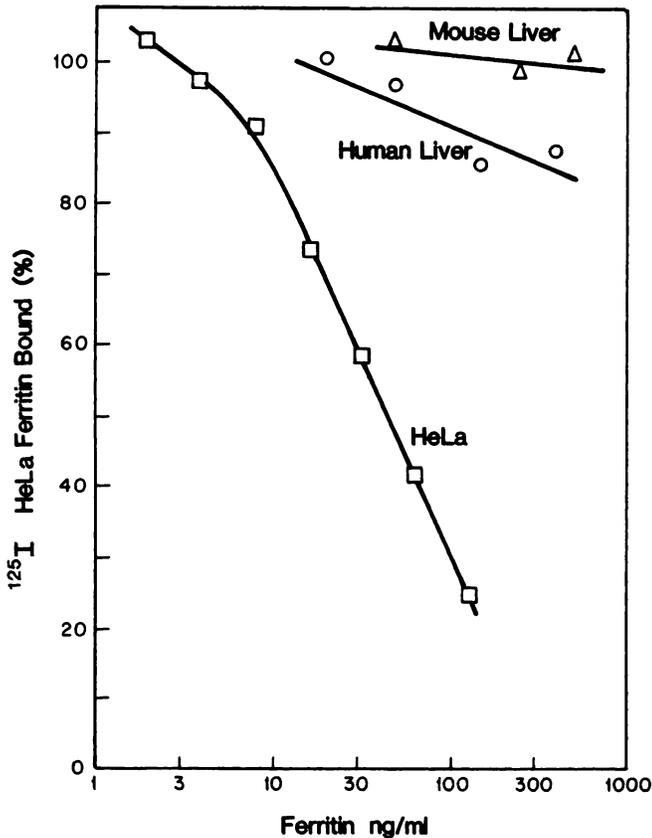


Chart 1. RIA standard curves using rabbit anti-HeLa ferritin antiserum absorbed with mouse liver ferritin (2.5 mg of mouse ferritin added to 1 ml of undiluted anti-HeLa ferritin antiserum and then diluted 1:1000 with phosphate-buffered saline) plus  $^{125}$ I-labeled HeLa ferritin and known amount of human liver, mouse liver, and HeLa ferritin.

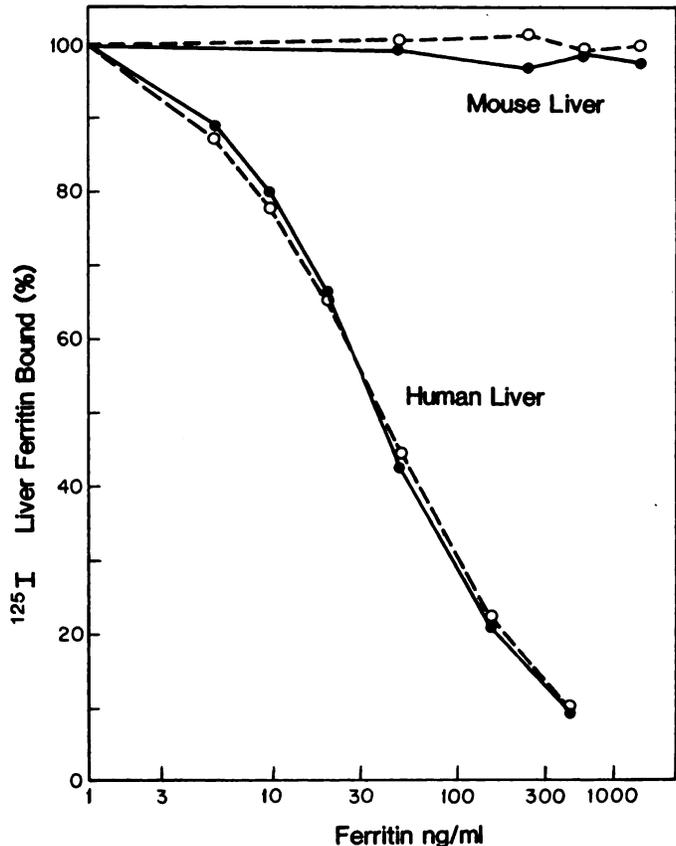


Chart 2. RIA standard curves using  $1:1.2 \times 10^6$  dilution of rabbit anti-human liver ferritin antiserum either absorbed with 50 ng of mouse liver ferritin or unabsorbed, plus  $^{125}$ I-labeled liver ferritin and known amounts of human liver and mouse liver ferritin. O, absorbed; ●, unabsorbed.

Table 1  
Human ferritin levels detected in serum of nude mice bearing human neuroblastoma and PHC

Human tumor transplanted	No. of nude mice	No. of mice with tumor growth	No. of mice with circulating human ferritin	Ferritin levels <sup>a</sup> (ng/ml)	
				Human liver-type ferritin	HeLa-type ferritin
Neuroblastoma <sup>b</sup>	15	14	12	22 ± 14 <sup>c</sup> (8-52) <sup>d</sup>	29, 36, 40, respectively, in 3 mice; 0 in 9 mice
PHC <sup>e</sup>	14	10	8	231 ± 270 <sup>f</sup> (10-820)	43 in 1 mouse 0 in 7 mice

<sup>a</sup> Ferritin levels of the maximum value for each mouse.

<sup>b</sup> Mice with neuroblastoma usually showed low levels of serum ferritin. Therefore, the magnitude of change of ferritin levels was not as impressive as in mice bearing PHC.

<sup>c</sup> Mean ± S.D. of 12 mice.

<sup>d</sup> Numbers in parentheses, range.

<sup>e</sup> Four mice showed gradual increase of ferritin levels from 6.7 to 340 ng/ml during the period of 2 to 16 weeks after transplantation while tumors increased in size. One mouse reached a peak level of ferritin of 820 ng/ml at 12 weeks posttransplantation, but the levels declined to 520 and 280 ng/ml at 14 and 18 weeks, respectively, while the tumor continued to grow. Histological examination showed a tumor with extensive necrosis. One mouse showed a gradual decrease of ferritin from 16 to 0 ng/ml when the 0.8 x 0.8-cm tumor regressed during the period of 3 to 21 weeks postimplantation. One mouse showed fluctuations of ferritin levels (44, 0, 13.6, and 30.4 ng/ml) along with changes of tumor size (0.95 x 0.6 cm → 0 → 3.5 x 3.5 cm) during the period of 3 weeks to 4.5 months. One mouse was tested for ferritin only once.

<sup>f</sup> Mean ± S.D. of 8 mice.

Among the 14 mice transplanted with human PHC cells, 10 developed tumors (Table 1). Tumors first became palpable between one and 3 weeks (except one, 8 weeks). The largest tumor measured 5 x 4 x 2 cm. Human ferritin was first detected 2 weeks after implanting the graft. Of the 10 mice bearing human PHC, 8 were found to have human ferritin in their sera. The maximum ferritin levels ranged from 10 to 820 ng/ml (231 ± 270) by liver ferritin assay. When serum samples were tested by HeLa ferritin assay, one (with a liver ferritin level of 280 ng/ml) showed HeLa-type ferritin (43 ng/ml), and the remaining 7 did not.  $\alpha$ -Fetoprotein was measured in single samples of 5 mice bearing PHC, and the levels ranged from 2,180 to 480,000 ng/ml (median 298,000). All 5 contained human liver ferritins (30.4 to 320 ng/ml), and one of these also contained HeLa ferritin (43 ng/ml).

Ferritins extracted from 2 neuroblastoma and 2 PHC tumors grown in nude mice were characterized by subunit analysis on SDS gel. The subunit composition of 2 neuroblastoma tumor ferritins was 50% heavy and 50% light. This was in agreement with our previous finding with the profile of ferritin extracted from neuroblastoma cells of CHP-100 in tissue culture (11). The profile of 2 PHC tumor ferritins was 40% heavy and 60% light. Our SDS analysis of human liver ferritin showed 10% heavy and 90% light, and ferritin extracts of HeLa cells showed 90% heavy and 10% light.

Histopathological examination of neuroblastoma and PHC tumors grown in nude mice confirmed human neuroblastoma and PHC with varying degrees of mitosis and necrosis. In mice with PHC, 6 had localized tumors at the injection site. Among the remaining 4, one showed direct invasion of kidneys and adjacent lymph nodes, one had both lung and kidney involvement, and 2 had lung metastases; none had liver involvement. All mice bearing neuroblastoma had localized tumors at the site of implantation without metastasis.

## DISCUSSION

Evidence that tumors are responsible for the increased amounts of ferritin seen in cancer patients has been presented in the past; leukemic cells synthesize more ferritin than normal

leukocytes (25), peripheral lymphocytes of patients with Hodgkin's disease show increased synthesis of ferritin over that of normal lymphocytes (21), and T-lymphocytes of these patients have been shown to produce preferentially heavy subunits of ferritin (7).

In an earlier study of patients with neuroblastoma (11), we observed that increased levels of serum ferritin were associated with actively growing neuroblastoma and that with clinical remission there was a return to normal levels. This suggested that the elevated serum ferritin was of tumor origin. This correlation was most often seen in advanced Stage IV neuroblastoma (9).

Recently, Watanabe *et al.* (24) transplanted various primary human tumors into nude mice and detected human ferritin in some of the mouse serum samples; one of the 3 nude mice bearing human neuroblastoma showed human ferritin in the serum, and 2 did not; one nude mouse received a transplant of a PHC tumor, and the mouse did not show human ferritin in the serum. Our study confirmed and expanded their observations. Almost all mice receiving implants had human liver-type ferritin in their sera.

Ferritins from human PHC tumors have been shown to be more acidic isoferritins (on isoelectrofocusing) compared with the ferritins from the corresponding normal liver (basic isoferritin) (2, 16, 20). We found by SDS gel analysis that the ferritin from PHC or neuroblastoma grown in nude mice contained a greater proportion of heavy subunit and a lesser proportion of light subunit than liver ferritin, and this composition on SDS gel corresponds to acidic ferritins on isoelectrofocusing (8, 13). However, human ferritin detected in murine serum was almost exclusively liver type except for 4 individual samples. Some unknown selection process seems to determine which isoferritin will be released (or secreted) into the circulation. This relatively small amount of circulating acidic ferritin in the serum of hosts with cancer has been observed by Jones *et al.* (15). Using antibodies to spleen and HeLa cell ferritin, they studied serum ferritin profiles (types) in over 1000 sera from patients with various cancers and those with ferritinemia from other causes. They were unable to show an independent increase of HeLa-type ferritin not accompanied by a corresponding increase of

liver-type ferritin. We have also made a similar observation recently in 25 patients with neuroblastoma. Maximum levels of HeLa-type serum ferritin was 40 ng/ml, whereas liver-type ferritin in the same specimens reached 1460 ng/ml. Serum which contained elevated HeLa-type ferritin also showed an increased amount of liver-type ferritin. HeLa/liver-type ferritin ratios appeared to be nearly constant, at  $\leq 0.1$  (10). Perhaps monoclonal antibody to heavy subunit of the ferritin will measure HeLa-type ferritin far more accurately than the current polyclonal antibody used.

Our study clearly demonstrates that transplanted human neuroblastoma and PHC cells produce human ferritins in the circulation of BALB/c (*nu/nu*) mice. Elevated levels of  $\alpha$ -fetoprotein in mice bearing human PHC further supports this. No  $\alpha$ -fetoprotein was detected in single samples of 4 mice without PHC tumors.

These data are strong evidence in support of the hypothesis that elevated ferritin levels in patients with certain cancers originate, at least in part, from their tumors. When elevations of serum ferritin from other causes are excluded, serum ferritin levels may be a reliable tumor marker and a useful guide in the management of patients with these cancers.

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#### REFERENCES

- Alexander, J. J., Bey, E. M., Geddes, E. M., and Lecatsas, G. Establishment of a continuously growing cell line from primary carcinoma of the liver. *S. Afr. Med. J.*, 50: 2124-2128, 1976.
- Alpert, E., Coston, R. L., and Drysdale, J. W. Carcino-fetal human liver ferritins. *Nature (Lond.)*, 242: 194-195, 1973.
- Arosio, P., Adelman, T. G., and Drysdale, J. W. On ferritin heterogeneity, further evidence for heteropolymers. *J. Biol. Chem.*, 253: 4451-4453, 1978.
- Aungst, C. W. Ferritin in body fluids. *J. Lab. Clin. Med.*, 71: 517-522, 1968.
- Bieber, C. P., and Bieber, M. M. Detection of ferritin as a circulating tumor associated antigen in Hodgkin's disease. *Natl. Cancer Inst. Monogr.*, 36: 147-157, 1973.
- Coombes, R. C., Powles, T. J., Gazet, J. C., Ford, H. T., Nash, A. G., Sloane, J. P., Hillyard, C. J., Thomas, P., Keyser, J. W., Marcus, D., Zinberg, N., Stimson, W. H., and Munro, N. A. A biochemical approach to the staging of human breast cancer. *Cancer (Phila.)*, 40: 937-944, 1977.
- Dorner, M. H., Silverstone, A., Nishiya, K., de Sostoa, A., Munn, G., and de Sousa, M. Ferritin synthesis by human T lymphocytes. *Science (Wash. DC)*, 209: 1019-1021, 1980.
- Drysdale, J. W. Ferritin phenotypes: structure and metabolism. In: *Iron Metabolism*, Ciba Foundation Symposium 51, pp. 41-67. Amsterdam: Elsevier/North-Holland Biomedical Press, 1977.
- Hann, H. L., Evans, A. E., Cohen, I. J., and Leitmeyer, J. E. Biologic differences between neuroblastoma Stages IV-S and IV. *N. Engl. J. Med.*, 305: 425-429, 1981.
- Hann, H. L., Evans, A. E., Stahlhut, M. W., and Millman, I. Basic and acidic iso-ferritins in the sera of patients with neuroblastoma (NBL) (Abstract C-337). *Proc. Am. Soc. Clin. Oncol.*, 3: 86, 1984.
- Hann, H. L., Levy, H. M., and Evans, A. E. Serum ferritin as a guide to therapy in neuroblastoma. *Cancer Res.*, 40: 1411-1413, 1980.
- Hazard, J. T., and Drysdale, J. W. Ferritinemia in cancer. *Nature (Lond.)*, 265: 755-756, 1977.
- Hazard, J. T., Yokota, M., Arosio, P., and Drysdale, J. W. Immunologic differences in human iso-ferritins: implications for immunologic quantitations of serum ferritin. *Blood*, 49: 139-146, 1977.
- Helson, L., Das, S. K., and Hajdu, S. I. Human neuroblastoma in nude mice. *Cancer Res.*, 35: 2594-2599, 1975.
- Jones, M., Worwood, M., and Jacobs, A. Serum ferritin in patients with cancer: determination with antibodies to HeLa cell and spleen ferritin. *Clin. Chim. Acta*, 106: 203-214, 1980.
- Kew, M. C., Torrance, J. D., Derman, D., Simon, M., Macnab, G. M., Charlton, R. W., and Bothwell, T. H. Serum and tumor ferritins in primary liver cancer. *Gut*, 19: 294-299, 1978.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275, 1951.
- Marcus, D. M., and Zinberg, N. Measurement of serum ferritin by radioimmunoassay results in normal individuals and patients with breast cancer. *J. Natl. Cancer Inst.*, 55: 791-795, 1975.
- Niitsu, Y., Kohgo, Y., Yokota, M., and Urushizaki, I. Radioimmunoassay of serum ferritin in patients with malignancy. *Ann. NY Acad. Sci.*, 259: 450-452, 1975.
- Niitsu, Y., Ohtsuka, S., Kohgo, Y., Watanabe, N., Koseki, J., and Urushizaki, I. Hepatoma ferritin in tissue and serum. *Tumor Res.*, 10: 31-42, 1975.
- Sarcione, E. J., Smalley, J. R., Lema, M. J., and Stutzman, L. Increased ferritin synthesis and release by Hodgkin's disease peripheral blood lymphocytes. *Int. J. Cancer*, 20: 339-346, 1977.
- Schlesinger, H. R., Gerson, J. M., Moorhead, P. S., Maguire, H., and Hummeler, K. Establishment and characterization of human neuroblastoma cell lines. *Cancer Res.*, 36: 3094-3100, 1976.
- Shouval, D., Reid, L. M., Chakraborty, P. R., Ruiz-Opazo, N., Morecki, R., Gerber, M. A., Thung, S. N., and Shafritz, D. A. Tumorigenicity in nude mice of a human hepatoma cell line containing hepatitis B virus DNA. *Cancer Res.*, 41: 1342-1350, 1981.
- Watanabe, N., Niitsu, Y., Koseki, J., Oikawa, J., Yukata, K., Ishii, T., Goto, Y., Onodera, Y., and Urushizaki, I. Ferritinemia in nude mice bearing various human carcinomas. In: *F. G. Lehmann (ed.), Carcinoembryonic Proteins*, Vol. 1, pp. 273-278. Amsterdam: Elsevier/North Holland Biomedical Press, 1979.
- White, G. P., Worwood, M., Parry, D. H., and Jacobs, A. Ferritin synthesis in normal and leukemic leukocytes. *Nature (Lond.)*, 250: 584-585, 1974.

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