Carcinofetal Human Isoferritins in Placenta and HeLa Cells

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SUMMARY

Ferritins from normal liver, placenta, and HeLa cells were resolved into their constituent isoferritins by gel electrofocusing. HeLa cells gave only two isoferritins. These isoferritins were not detected in liver. Placenta and liver each contained about six isoferritins with several in common. In addition, placenta contained two isoferritins that appeared to correspond to the HeLa isoferritins. Both of the carcinofetal forms in placenta and HeLa cells were recognized by antibodies prepared against normal adult liver ferritin.

INTRODUCTION

Ferritin is an iron-storage protein that is found, or may be induced by iron, in most mammalian tissues (17, 23). In many organs, ferritin exists in multiple molecular forms, or isoferritins, of differing structure and metabolism (2, 15, 21, 24, 25). Many of these forms appear to represent hybrid molecules formed from different subunit types (11). In addition to the normal isoferritins, variant forms have been found in human and animal tumor cells (21, 27, 28). These observations and the rapidly accumulating evidence linking certain tumor antigens with counterparts in embryonic development (10, 13, 14, 33, 34) led us to investigate whether isoferritins in human tumors might represent another example of carcinofetal antigens. Analyses of ferritins from liver and primary hepatoma indicated that the tumor contained 2 isoferritins that were not present in normal adult liver. Since these isoferritins appeared to correspond to forms in early fetal but not neonatal liver, they were designated carcinofetal isoferritins (3). This paper provides evidence that similar isoferritins are also present in placenta and in HeLa cells, a cell line derived from a cervical carcinoma.

MATERIALS AND METHODS

HeLa Ferritin. Ferritin was obtained from HeLa cells that were grown in Eagle's minimum essential medium with Earle's salts, modified for suspension culture (Catalog No. 165G; Grand Island Biological Co., Grand Island, N. Y.) and supplemented with calf serum (final concentration, 10%), penicillin (100 units/ml), and streptomycin (100 μg/ml).

Isolation of Ferritin. Ferritin was isolated from liver, placenta, and HeLa cells, essentially by the method of Drysdale and Munro (12) as modified by Powell et al. (26). Purified ferritins were chromatographed on Sepharose 6B columns to remove oligomeric forms. The purity of these preparations was confirmed by gel electrophoresis (12).

Gel Electrofocusing. This was performed in cylinders, 10 x 0.3 cm (inner diameter), of polyacrylamide gel in equipment from Medical Research Apparatus, Boston, Mass., according to the method of Righetti and Drysdale (29, 30). An electrolysis period of 18 hr was used to ensure equilibrium focusing of the large ferritin molecule (M. W. 440,000). Ferritin protein was stained with Coomassie brilliant blue 250 and ferritin iron was stained with Perl's stain for nonheme iron (11). Isoelectric points (pI's) were estimated from gel eluates (29).

Immunofixation. In some experiments, the separated isoferritins were subjected to immunofixation in situ (8) with antibodies prepared in rabbits against normal human liver ferritin (25). Gels were immersed in narrow tubes with 2 ml antiserum diluted 1:3 with 0.1 M Tris-HCl, pH 8.0. Duplicate gels were bathed in nonimmune serum as controls. Nonprecipitated proteins were removed by washing for 48 hr at 30° in repeated changes of phosphate buffer, pH 7.4, 0.10 M, then for a further 24 hr at 0° in water. Proteins precipitated by the antiliver ferritin serum were detected by staining with Coomassie blue.

RESULTS

Chart 1 compares banding patterns of isoferritins from liver, early placenta, and HeLa cells. In this case, we chose as a control the ferritin from the cirrhotic liver of a patient with secondary iron overload, a situation in which the synthesis of all normal isoferritins is markedly elevated. In addition, most of the isoferritins have a high iron content (26). Cirrhotic liver ferritin resolved into 6 iron-containing isoferritins, isoelectric between pH 5.2 and 5.7. This pattern is similar to that given by normal liver ferritin if the isoferritins are detected by protein staining rather than by iron staining. HeLa ferritin resolved into only 2 components, which banded at lower pI's, 4.9 and 5.0, than any of the liver isoferritins. Placenta contained several isoferritins common to liver. In addition, placenta contained 2 more acidic isoferritins that banded in the same position as the HeLa isoferritins.

In order to examine possible immunological relationships of liver and HeLa isoferritins, the various isoferritins were subjected to immunofixation after separation by gel electrofocusing. The results (Chart 2) demonstrate that antibodies to normal adult liver ferritin recognized and precipitated both of the more acidic isoferritins common to placenta and HeLa cells. This was a specific immunoprecipitation, since controls incubated with nonimmune serum gave no banding patterns.

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a-fetoprotein (1), colon embryonic antigen (16), and the
This paper provides additional evidence that the iron-storage
biochemical markers of cancer, but also as possible models for
Such carcinoembryonic proteins are useful not only as
steres are not present in normal or cirrhotic human liver but which
results indicate that HeLa cells contain variant isoferritins that
From a comparison of banding patterns and estimated
isoelectric points, it seems likely that the 2 isoferritins
common to placenta and HeLa cells correspond to carcinofetal
variants described previously in human hepatoma and fetal
liver cells. Similar variants have been found in primary

Although the carcinofetal isoferritins were not detected by our analyses in normal or cirrhotic liver, it is still too early to exclude the possibility that they might be synthesized at low levels in these or in other normal tissues. Such a situation is known to occur in other instances of apparent gene derepression as evidenced, for example, in the small but significant synthesis of other fetal proteins such as fetal hemoglobin (35), colon embryonic antigen (9), and a-fetoprotein in normal adults (32).

The apparent correspondence of the carcinofetal isoferritins to those in HeLa cells and the absence of normal isoferritins in HeLa cells are of particular interest. Previous studies with hepatoma and fetal liver and the present studies with placenta indicate that all 3 tissues contain appreciable amounts of isoferritins common to adult liver in normal or nonmalignant disease (3). It was not known whether the

The structural relationships of isoferritins in normal and malignant cells have not yet been elucidated. Much of the present evidence indicates that normal tissue isoferritins are hybrid molecules containing different proportions of at least 2 dissimilar subunits (12). Little is yet known of the subunit composition of the HeLa ferritins. Partial analysis of amino acid composition indicates that the HeLa ferritins differ in primary structure from normal liver ferritin (31). With regard to tissue-specific isoferritins, it should be noted that liver ferritin was used as a reference control in these studies. In some respects, ferritin from normal cervical tissue would have been a more appropriate reference for HeLa ferritin, but it was not available. Such a comparison might be of importance in view of the fact that certain normal tissues such as heart, kidney, and pancreas contain isoferritins of a more acidic nature than those in the liver ferritins (26, 30). It would be most desirable to know whether there is any relationship between these isoferritins and those in cancer and fetal cells.

With regard to the immunological relationships of the carcinofetal and normal tissue isoferritins, Richter (27) and Righetti et al. (31) demonstrated that HeLa ferritins were recognized by antibodies to normal liver ferritin. However, Righetti et al. also noted a large quantitative difference between HeLa and liver ferritin, the HeLa ferritin requiring approximately 10 times as much antiserum for complete precipitation as an equivalent amount of liver ferritin. Similar quantitative immunological differences have been found between hepatoma and liver ferritin in rats (20). Our results indicate that such differences may be ascribed to nonreactive sites on the HeLa ferritins rather than nonreactive HeLa ferritins, since both HeLa isoferritins were recognized by liver

Chart 1. Isoferritin profiles from early placenta, HeLa cells, and cirrhotic liver obtained by gel electrofocusing in the pH 4 to 6 range. Gels are stained for nonheme iron with potassium ferrocyanide.

Chart 2. Immunofixation of isoferritins from normal liver, placenta, and HeLa cells. Samples of liver, placenta and HeLa Cells were subjected to isoelectric focusing in 4% polyacrylamide gels in the pH range, 4 to 6. After focusing, gels were incubated for 18 hr in a diluted antiserum prepared against normal liver ferritin. Nonprecipitated proteins and excess serum proteins were removed by washing for 48 hr and the remaining immunoprecipitates stained with Coomassie blue.

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ferritin antibodies. Considerably more work is required to investigate qualitative and quantitative aspects of this relationship.

Part of our interest in tumor ferritins concerns the possibility of developing serological tests to follow serum levels of ferritin as an indication of cancer. The fact that tumor ferritins are recognized by antibodies to normal liver ferritins presently complicates such attempts. Normally, nearly all of the ferritin is found intracellularly, but small amounts also occur in serum where its level usually reflects the level of body-iron stores (18). However, the level of serum ferritin or β-fetoprotein, as it is has been called, is markedly elevated in many diseased states, often apparently independently of the iron storage levels (4, 5, 7, 18). Buffe and colleagues found high levels of serum ferritin in a variety of malignant diseases, as have Jones et al. (19) and Bieber and Bieber (6) in Hodgkin's disease. In most of these studies, serum ferritin was quantitated with antibodies to normal human liver ferritin. It could not, therefore, be determined whether the serum ferritin in cancer represented tumor ferritin or tissue ferritins released from normal tissue by infiltration of the tumor. Obviously, a more restricted antibody to the tumor ferritin is required before the development of specific serological tests for ferritin in cancer can be considered. The most logical approach would appear to be to produce antibodies against the isolated carcinoembryonic forms. From our results, the HeLa cell would seem to be a most appropriate tissue because of its restricted isoferitin spectrum. However, the finding of similar isoferitins in the more abundant and readily available placenta might allow the isolation of these isoferitins in greater amount for immunological and structural studies.

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