ALTERATIONS OF CYTOPLASMIC PROTEINS IN RAT HEPATOMAS DURING THE ADMINISTRATION OF CHEMICAL CARCINOGEN

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ABSTRACT

The cytoplasmic proteins of rat liver following exposure to the hepato-carcinogen, 3'-methyl-4-dimethylaminoazobenzen (3'-MDAB) were separated by a two-dimensional gel system using isoelectric focusing in the first dimension and sodium dodecyl sulfate (SDS) gel electrophoresis in the second dimension. After commencement of administration of the carcinogen, the rats were sacrificed at 14 weeks (hepatoma A) just after the occurrence of hepatomas, 16 weeks (hepatoma B), and 22 weeks (hepatoma C), respectively. All three hepatomas have six protein spots that have not been found in normal liver; i.e., 35/7.8 and 20/7.3 (molecular weight x 10^-3/isoelectric point) as representative proteins. Some proteins 72/6.8, 72/6.3, 65/7.3, and 44/7.8 which were present in hepatoma, were found in large amounts in accordance with the tumor growth. The patterns of spots of cytoplasmic proteins in liver showed alterations during the course of carcinogenesis, and finally those of hepatoma C were very different from those of normal liver. These changes in composition of cytoplasmic proteins reflect earlier changes in the rate of synthesis of individual protein species.

INTRODUCTION

Based upon the finding that the non-histone chromosomal proteins may have an important regulatory function in transcription of the eucaryotic genome because of involvement in the specific control of gene expression, some differences in those proteins have been observed at various stages of neoplastic transformation and carcinogenesis. Although conventional electrophoresis and chromatography have been employed to elucidate this problem, these systems
could not provide good separation of proteins.\textsuperscript{5,6,7} Recent improvement of the two-dimensional gel electrophoresis by O'Farrell \textit{et al.}\textsuperscript{8,9} and by Takami \textit{et al.}\textsuperscript{10,11} made it possible to identify highly resolutional cellular proteins. For instance, the previous study using this system demonstrated 483 different nuclear proteins in the hepatoma and 427 in the normal liver.\textsuperscript{10} Also, the earlier reports\textsuperscript{10,11} showed several tumor-related nuclear proteins in comparison of normal liver, slow growing and fast growing Morris hepatomas, Novikoff hepatomas, fetal liver, and regenerating liver. On the other hand, it has been reported that changes in composition of cytoplasmic proteins following exposure to carcinogens may be raised by an altered dynamics of exchange of proteins between the cytoplasmic and nuclear proteins. Therefore, an attempt was made to compare the cytoplasmic proteins at various stages during the induction of hepatomas by 3'-methyl-4-dimethylaminoazobenzene (3'-MDAB).

**MATERIALS AND METHODS**

\textbf{Rats and Dietary Regimens.}

Adult male rats of the Holtzman strain, weighing 200–240 g, were fed 3'-MDAB at a concentration of 0.06\% in a powdered riboflavin deficient synthetic diet. Rats were sacrificed at 14 weeks (hepatoma A), 16 weeks (hepatoma B), and 22 weeks (hepatoma C), respectively, after initial administration of carcinogen diet. Hepatoma A showed well differentiated cell type by 14 weeks, just after appearance of small tumors. In hepatoma B, gross tumors were found. Hepatoma C had metastasized to mesenteric lymph nodes.

\textbf{Preparation of Cytoplasmic Proteins.}

The livers were perfused through the portal vein with 0.13 M NaCl/0.005 M KCl/0.008 M MgCl\textsubscript{2} and removed. The resected tumors or normal liver were squeezed through a tissue press, and homogenized in 0.01 M NaCl/1.5 mM MgCl\textsubscript{2}/0.01 M Tris-HCl (pH 7.6) containing 0.5 mM phenylmethylsulfonyl fluoride and 0.5\% (w/v) Nonidet P40. The homogenate was centrifuged at 27,000 × g for 30 min, and the post-mitochondrial supernatant was further centrifuged at 105,000 × g for 3 hrs to remove microsomes. The middle part of supernatant, avoiding the lipid at its upper part and the glycogen and polyribosome at its lower part, was aspirated carefully. The clear cytosol supernatant was precipitated by the addition of three volumes of 100\% ethanol, and then kept overnight at −20\degree C. The precipitate was washed three times with 66\% ethanol and stored at −20\degree C. Aliquots were vacuum dried before being used for gel electrophoresis.
Two-Dimensional Gel Electrophoresis.

A two-dimensional gel system was carried out as described previously.\textsuperscript{10,11,12} The first dimension was electrophoresed by isoelectric-focusing in small cylindrical acrylamide gel including 2\% Ampholine (pH 3.5 to 10). After adaptation of the first dimension gel for the second dimension, the first dimension gel was placed directly on the slab SDS acrylamide gel (the second dimension gel) which was polymerized. The proteins in the second dimension were run according to the molecular weight. The slab gel was removed and stained in Coomassie Brilliant Blue R and then destained.

Measurements of pH gradient in the isoelectric-focusing gels were performed by a pH meter after placing the sections of gels into H\textsubscript{2}O. The molecular weights in the SDS gel were determined by comigration of known molecular weight standards.

RESULTS AND DISCUSSION

Comparison of the Spot Patterns for Normal Liver and the Hepatomas.

As might be anticipated, the spot patterns for the normal liver were different from those for all hepatomas. Of the total, 122 protein spots in normal liver (Fig. 1), ten protein spots in normal liver were not found in hepatomas or were present in larger amounts than hepatomas. Particular dense spots were 45/6.7, 45/7.6, 35/6.7, 35/6.4, 22/7.3 (marked as \textbullet). This may be due to the loss of liver functions.

Six protein spots were present in all hepatomas at three different stages (Figs. 2 to 4), but were absent in normal liver. Among those proteins the dense and large proteins were as follows: 35/7.8 and 20/7.3 (\textbullet in Figs. 2 to 4). Such proteins could be related to neoplasia.

Changes of Cytoplasmic Proteins during Chemical Carcinogenesis.

Several proteins found in normal liver were diminished and not detectable in accordance with the development of hepatomas. Although proteins (\textbullet \textbullet), 140/6.5, 57/7.8, 48/7.2, 48/7.7, were found in normal liver (Fig. 1) and hepatoma A (14 weeks) (Fig. 2), these were not detected in hepatoma B (16 weeks) (Fig. 3). Proteins 43/8.2 and 38/6.9 (\textbullet \textbullet) decreased in density and size with the tumor growth, and finally were not found in hepatoma C (22 weeks) (Fig. 4). Although proteins 62/7.7 (\textbullet \textbullet) were visible in all tissues studied, the density and size of protein were decreasing in accordance with development of tumors.

Conversely, some proteins, 72/6.8, 72/6.3, 65/7.3 and 44/7.8, showed in-
Fig. 1 Two-dimensional gel electrophoresis of 105 µg of normal liver cytoplasmic proteins. Proteins were separated according to their pI by isoelectric focusing in the first dimension and according to molecular weight by SDS electrophoresis in the second dimension. Abscissa, pH range; ordinate, molecular weight (× 10⁻³) arrows, proteins present in normal liver and either less in amount or absent from hepatomas. Arrowheads, proteins present in normal liver and hepatoma A (14 weeks) and absent in hepatoma B (16 weeks) and C (22 weeks). Blunt arrowheads, proteins present in normal liver, hepatomas A and B, and absent in hepatoma C (22 weeks). ∇ proteins in all tissues.
Fig. 2 Two-dimensional gel electrophoresis of 95 μg of hepatoma A (14 weeks) cytoplasmic proteins. See legend to Fig. 1 for details except for arrows. Arrows, proteins present in three hepatomas.

Increasing density and size with the tumor growth (V in Fig. 4). These proteins may be related to growth processes of tumor tissues. Of those proteins only 72/6.3 was demonstrated in all hepatomas, showing increasing quantity with growth of tumors. Such a protein was not found in the cytoplasmic proteins of the other normal tissues; brain, spleen, kidney, pancreas and thymus (unpublished data). If further investigations confirm that this protein is not present in any normal tissues, a challenging task would be to find out if the
Fig. 3 Two-dimensional gel electrophoresis of 95 μg of hepatoma B (16 weeks) cytoplasmic proteins. See legend to Fig. 2 for details.

genetic information for protein 72/6.3 is present but suppressed in the normal genome or if it is absent. Such a problem could be approached by using a complementary DNA probe from mRNA of protein 72/6.3. This protein has recently been purified and was subjected to more detailed chemical analyses since it might play a role in the excessive growth of these tumors. The number of protein spots that were not found in normal liver were respectively counted in three hepatomas (Fig. 5). As the tumor was growing during the administration of chemical agents, many proteins which were present in normal liver
decreased while the new proteins which were not found in normal liver appeared. These changes presumably reflect the state of development of tumors involved in increasing synthesis of proteins.

The compact two-dimensional gel system shows highly reproducible results, and needs only a small amount of biopsy material since approximately 100 μg of proteins are all that are necessary for separation on the second dimensional
gel. It therefore seems possible for future studies to compare the cytoplasmic proteins of human tumors.

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REFERENCES


