Studies on Nucleoli and Cytoplasmic Fibrillar Bodies of Human Hepatocellular Carcinomas

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SUMMARY

The ultrastructure of neoplastic cells of hepatocellular carcinomas in 3 untreated male patients of similar ages was studied and compared with liver cells of 2 persons with normal histology of the liver. As in rapidly growing experimental hepatomas and the Walker tumor of rats, nucleoli in a rapidly growing human hepatocellular carcinoma were characterized by a decrease of the relative percentages of total nucleolar areas containing fibrillar components when compared with slowly growing tumors or normal liver. The nucleolus-associated chromat in the rapidly growing human hepatocellular carcinoma contained some “giant” microspherules with diameters up to 1400 Å. The cytoplasm of these hepatocellular carcinoma cells contained fibrillar inclusion bodies and another type of fibrillar body.

INTRODUCTION

Although several studies have been made on the ultrastructure of hepatocellular carcinomas (8, 11, 15, 23, 29–32), there is little information on the ultrastructure of their nucleoli and cytoplasmic fibrillar bodies. Inasmuch as many studies have been made on both the ultrastructure and biochemistry of nucleoli of hepatomas of experimental animals (4, 34), it was of interest to compare the results of such studies with those on similar human tumors. However, one of the problems of such studies is the difficulty in obtaining appropriate surgical or biopsy specimens from untreated adult patients and control biopsy specimens from other patients.

In this study, the ultrastructure of hepatocellular carcinomas was investigated in tumors from 3 untreated male patients of similar ages. Since 1 of the 3 hepatocellular carcinomas grew rapidly and the other 2 grew at slower rates, it was possible to make some comparisons of the nucleolar ultrastructural characteristics in a manner similar to those previously reported for slowly and rapidly growing Morris and Novikoff hepatomas (4, 34).

The present results indicate that nucleoli of neoplastic cells of rapidly growing human hepatocellular carcinomas, like those of rapidly growing experimental hepatomas (4, 34), are characterized by the relatively smaller fibrillar areas as compared with slowly growing hepatomas or normal liver. In addition, the nucleolus-associated chromat in neoplastic cells of rapidly growing human hepatomas occasionally contained unusual “giant” microspherules. In some sections, inclusion bodies and fibrillar inclusions were found in the cytoplasm of the human hepatocellular carcinoma cells.

MATERIALS AND METHODS

The samples for the electron microscopy were taken from 3 patients, ages 49, 49, and 47, respectively, who had hepatocellular carcinomas, had not received any anticancer therapy, and had no history of alcoholism. The 1st case was characterized by very rapid growth of the tumor, and this patient died 2 weeks after the liver biopsy. The progress of the malignant disease in the 2nd and 3rd patients was slower than that in the 1st case. The 2nd patient died 2 years after the samples used for the present study were taken for light and electron microscopy. The 3rd patient died from pneumonia 4 months after the samples were taken; metastases were found only in portal lymph nodes.

When the samples were taken for electron microscopy, the physical examination of the 1st patient showed hepatomegaly, and after laparotomy tumors were found in the right lobe. The laboratory findings were “normal” except for slight elevation of the alkaline phosphatase (95 milliunits/ml, compared to the normal range of 30 to 85 milliunits/ml), slight elevation of serum glutamic-pyruvic transaminase² (95 Reitman-Frankel units/ml, compared to the normal maximum of 40 units/ml), and positive results for the Australia antigen and α₁-fetoprotein. Light microscopy demonstrated a hepatocellular carcinoma with a trabecular pattern (9, 10); frequent mitotic figures; acidophilic bodies (2, 13); small, basophilic, cytoplasmic inclusions (Fig. 1); and occasionally Feulgen-positive bodies (Fig. 2). Postnecrotic cirrhosis was found in the adjacent nontumor liver tissue.

The physical examination of the 2nd patient showed hepatomegaly, and the laboratory analyses were positive for the Australia antigen and slightly positive for α₁-fetoprotein.

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²The area containing fibrillar components of the nucleolus is defined as an area that contains characteristic fibrillar components but no defined granular components. In some areas of the nucleolus, the fibrillar components were not clearly demarcated from granular ones. These fibrillargranular areas previously found in experimental tumors were not included in the areas containing fibrillar components (28).
The alkaline phosphatase was 110 milliunits/ml, and the serum glutamic-pyruvic transaminase was 84 units/ml. Light microscopic examination indicated a hepatocellular carcinoma with a trabecular pattern (9, 10), vascular invasion, and postnecrotic cirrhosis in another fragment of the liver tissue.

The physical examination of the 3rd patient showed slightly icteric scleras and hepatomegaly. The laboratory investigation indicated only slight alteration of liver function tests, i.e., the alkaline phosphatase was normal (71 milliunits/ml), and the serum glutamic-pyruvic transaminase was 62 units/ml. Laboratory tests were positive for the Australia antigen and \( \alpha_1 \)-fetoprotein; the serum pyruvic-glutamic transaminase was elevated. Light microscopy of the tumor demonstrated a hepatocellular carcinoma with a trabecular pattern (9, 10), vascular invasion, and postnecrotic cirrhosis in the nontumor portion of the liver. The presence of acidophilic inclusions of various sizes (2, 13) was also noted in the cytoplasm of the neoplastic cells.

For comparison of the nucleolar ultrastructure, the liver cells were studied in 2 adult patients without hepatic cancer. The liver biopsies of these patients showed a "normal" morphology.

For electron microscopy, the specimens were fixed in glutaraldehyde and postfixed in osmium tetroxide (18, 24). Some of the specimens obtained from the 1st patient were fixed only in glutaraldehyde. After dehydration in ethanol containing uranyl acetate (27), the specimens were embedded in Epon-Araldite (16). Ultrathin sections stained with uranyl acetate and then with lead citrate (35) were observed with a Phillips 200 electron microscope. The ultrathin sections prepared from glutaraldehyde-fixed specimens without postfixation were stained with EDTA or citric acid procedures for the preferential staining of RNA-containing structures.

The percentage of the total nucleolar areas containing fibrillar components was determined on electron micrographs with a planimeter (OTT, Burrell Corporation, Pittsburgh, Pa.) as described previously (4, 28, 34).

## RESULTS

The ultrastructure of nuclei and cytoplasm in "normal" liver cells did not differ from that described in the previous studies on human or rat liver cells (5, 22, 29). The nucleoli (Fig. 3) were composed of nucleolonemas, and occasionally contained "fibrillar centers" (21). The areas containing the fibrillar components were mapped as previously described (4, 28, 34) and composed about 17% of the total nucleolar area (Table 1). The perinucleolar chromatin appears to be a condensed, discontinuous layer around the nucleolar bodies (Fig. 3). The perichromatin granules in the perinucleolar chromatin (Fig. 3) were normal in size and appearance (1).

In general, the ultrastructural morphology of the hepatocellular carcinoma cells was similar to that previously reported (8, 11, 15, 23, 29–32). Fig. 3 is an example of a control nucleolus. An example of the nucleoli of a slowly growing hepatocellular carcinoma is shown in Fig. 4, and a nucleolus of a rapidly growing hepatocellular carcinoma of the 1st patient is shown in Fig. 5. In general, the enlarged nucleoli in the neoplastic cells were more compact and the nucleolonemas were less distinct in comparison to nucleoli of "normal" liver cells. Fibrillar centers were also present in nucleoli of neoplastic cells (Fig. 4). In nucleoli of the cells of the patient with the rapidly growing hepatocellular carcinoma (Fig. 5), the relative size of the nucleolar areas containing fibrillar components was reduced (Table 1) in comparison to those of the normal liver cells (Fig. 3). In some neoplastic cells of the 1st patient, the perinucleolar chromatin contained microspherules (Fig. 5), of which the largest was 1500 Å in diameter.

The microspherules are discrete structures that apparently have properties different from those of other granular structures of nuclei (4, 17, 19, 20, 26, 33). Microspherules appear in cells in which the nucleolar RNA synthesis is either impaired or has spontaneously ceased (4). In the nucleolus in Fig. 5, the nucleolonemal structure is largely obliterated; the granular component has formed a round mass, as is frequently seen following treatment with drugs such as actinomycin D, proflavine, and cordycepin (4). It is possible that the microspherules mark the site of the fibrillar component, which appears to have disintegrated. Although these microspherules appear to be in perinucleolar chromatin, it is possible that this matrix is a remnant of fibrillar nucleolonemal strands. These changes in nucleolar ultrastructure indicate that the nucleolar function of the cell shown in Fig. 5 was severely inhibited.

In addition to various types of lysosomes described previously in the cytoplasm of human malignant hepatoma cells (5, 29, 32), the cytoplasm of some neoplastic cells contained 2 types of fibrillar inclusion bodies (Figs. 6 and 7). The 1st type (Fig. 7) was present frequently in the cytoplasm of neoplastic cells of the 1st patient and occasionally was also found in the neoplastic cells of the 3rd patient. These inclusions were similar to some cytoplasmic bodies found in other cells (6, 12, 14) in that they were not surrounded by a membrane and were composed of fibrils with diameters ranging from 60 to 170 Å (Fig. 8). These fibrils are apparently surrounded by an amorphous matrix and consist of subunits of fine filaments 15 to 35 Å wide (Fig. 8). The light areas in these bodies occasionally contained vesicular structures (Figs. 7 and 8). Some of these bodies (Fig. 9), like chromatin structures (Figs. 10 and 11), did not stain positively with the EDTA technique (1). Such bodies may correspond to the DNA-containing Feulgen-positive bodies seen by light.
microscopy (Fig. 2). Occasionally, such bodies were positively stained, indicating the presence of ribonucleoproteins (Fig. 12).

A 2nd type of fibrillar inclusion (Figs. 6 and 13) was occasionally observed in the cytoplasm of neoplastic cells of all 3 patients investigated. They were composed of anastomosing fibrils with diameters of about 70 Å; these diameters were variable (Fig. 14) than those of fibrils of the bodies discussed above. In addition, these fibrillar bodies were surrounded by rough endoplasmic reticulum (Fig. 6). Endoplasmic reticulum, ribosomes, or other cytoplasmic structures were not found in these bodies (Figs. 6, 13, and 14), in contrast to the findings in “small acidophilic” bodies that may represent focal cytoplasmic degradation (2, 3, 5, 7, 25). The focal cytoplasmic degradation bodies (Fig. 15) occasionally contained “fibrillar bodies” as well as other cytoplasmic components (Fig. 16).

**DISCUSSION**

The present study provides additional information on the ultrastructural morphology of nucleoli and cytoplasmic nucleolus-like bodies of hepatocellular carcinoma cells of untreated patients. As noted earlier for other human tumors and rat hepatomas (4), the ultrastructural organization and composition of nucleoli are variable in human hepatocellular carcinoma cells. The ultrastructural organization and composition of nucleoli in very slowly growing Morris hepatomas did not differ markedly from the nucleoli of normal liver cells (34). The ultrastructural composition of nucleoli of slowly growing hepatocellular carcinomas (Table 1) did not differ, or differed only slightly, from that of normal human liver cells. On the other hand, nucleoli of rapidly growing experimental hepatomas or the Walker tumor of the rat were characterized by a relative decrease of fibrillar areas and by a more compact appearance (4, 28, 34). In the present study, the nucleoli of the neoplastic cells of the rapidly growing hepatocellular carcinoma of the 1st patient (Table 1) also showed a decrease of the relative size of fibrillar areas and a more compact appearance, i.e., less distinct nucleolonemas were apparent in comparison to nucleoli of normal liver cells. The interpretation of these findings is still difficult, although at present it appears that the fibrillar components contain precursors of the granular elements, particularly 45 S RNA (4). It is possible that the relative decrease of the size of the fibrillar areas in nucleoli of rapidly proliferating neoplastic cells reflects a rapid conversion of these components to granular elements, since the half-life of 45 S nucleolar RNA is shorter in rapidly growing tumors containing compact nucleoli than that of liver nucleoli that contain more fibrillar elements (4).

The present study also showed the presence of 2 types of fibrillar bodies in the cytoplasm of neoplastic cells in human hepatocellular carcinomas. The 1st type lacks a limiting membrane and was characterized by a greater variability in the diameter of the component fibrils. The composition as well as the function of such cytoplasmic bodies has not been satisfactorily clarified (14). Some of these bodies, like chromatin structures, did not stain with the EDTA or citric acid procedures (1), which preferentially stain RNA-containing structures. Like the chromatin structures, some of these bodies may contain DNA, since a positive Feulgen reaction was also observed by light microscopy; the origin of the DNA in these bodies is unknown. In contrast, a few such bodies were stained positively for RNA by the EDTA or citric acid procedure, thus suggesting that some cytoplasmic bodies contain components of nucleolar origin (6, 14).

The 2nd type of fibrillar body differed from the 1st inasmuch as it was composed of fibrils about 70 Å in diameter, with less variation and a looser organization, than those in the other fibrillar bodies. These fibrillar bodies were usually surrounded by rough endoplasmic reticulum but did not contain either endoplasmic reticulum or other cytoplasmic structures, in contrast to focal cytoplasmic degradation bodies or some “acidophilic bodies.” At low magnification, this type of fibrillar body resembled alcoholic hyaline bodies (2, 3, 7, 25). According to these ultrastructural studies, the hyaline material in “alcoholic hyaline bodies” appears to consist of membranaceous components. However, no alcoholic history was found in the patients of the present study. This 2nd type of fibrillar body was found in cells that were well preserved in contrast to the 1st type of fibrillar body, which was found in cells that exhibited some degree of degeneration. It is possible that the 1st type of fibrillar body and the 2nd are essentially the same but that the 1st types are condensed forms resulting from cell degeneration.

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1. Figure 9
2. Figure 10
3. Figure 11
4. Figure 12

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