Fine Structure of Primary Liver Tumors and Tumor-bearing Livers in Man

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SUMMARY

Five hepatomas were studied by electron microscopy. Tumor cells were characterized by scantiness of subcellular organelles. Apart from the apparent deletion of peroxisomes, bile canaliculi, and very-low-density lipoprotein particles in some tumors, ultrastructural alterations were otherwise nonspecific. On the other hand, the hepatocyte origin of four of the tumors was indicated by the occurrence in tumor cells of one or another organelle that characterizes the normal hepatocyte. Eighty-nm virus-like particles were observed in one tumor. The unusual occurrence in some nonneoplastic hepatocytes of numerous microtubular inclusions in the endoplasmic reticulum cisternae is reported.

INTRODUCTION

There have been few studies of the ultrastructure of human primary liver tumors, reflecting perhaps the relative rarity of these tumors in most parts of the world. This study emphasizes the morphological heterogeneity and the uniform subcellular simplicity of human liver tumors. Attention is also directed to the ultrastructural alterations of adjacent nonneoplastic but possibly preneoplastic hepatocytes.

MATERIALS AND METHODS

Five hepatomas were examined. One of the tumors was previously reported in brief (19). Two of the tumors occurred in noncirrhotic livers confirmed by either previous or subsequent laparotomy. Underlying cirrhosis was presumed for the other 3 tumors because the patients had clinical evidence of chronic liver disease before presenting with malignant disease. This study forms part of a larger and more comprehensive ultrastructural study of the human liver cell.

All tissues were obtained by percutaneous needle aspiration. They were fixed for 2 hr in cold 2% OsO4 either in Veronal buffer containing 0.2 M sucrose (4) or in Sorensen’s phosphate buffer (16), immediately dehydrated, and embedded in either Araldite or Epon. One-μm thick sections stained with toluidine blue were used for light microscopic studies and for selecting appropriate areas for thin sectioning. Thin sections for electron microscopy were stained in uranyl acetate (30) followed by lead citrate (23) and examined in a Siemens Elmiskop I electron microscope.

RESULTS

The tumors were all considered hepatocarcinomas of the trabecular type by histological criteria. By electron microscopy, simplicity of intracellular organization and scantiness of cellular organelles characterized the tumor cells.

The hepatomas all differed slightly from one another (summarized in Table 1). The mitochondria differed in size and shape. The most abnormal mitochondria were those of one of the primary hepatomas without underlying cirrhosis (Patient D, Figs. 1 and 2). They were small, measuring 0.18 to 0.35 μm, were without matrix granules, and had lost their spatial relationship to the RER2 characteristic of normal hepatocytes (18). Mitochondria of the other 4 hepatomas were very similar to those of nonneoplastic hepatocytes. Large, spherical, and pale mitochondria, presumably representing swollen forms, were frequently seen in scattered tumor cells (Fig. 3).

RER was present in all hepatoma cells, varying in quantity from scant (Figs. 1 and 2) to moderately plentiful (Figs. 4 to 6). Dilation of the ER cisternae was common, affecting most cells in all 5 tumors. Typical hepatocyte SER occurring as anastomosing tubular and irregular vesicular profiles was present in moderate amounts in 2 tumors that also contained normal glycogen rosettes (Figs. 4 and 5). As in nontumorous hepatocytes, the SER was closely associated with glycogen particles. Small numbers of smooth, roughly spherical vesicles were observed in the other tumors which either had no glycogen (Fig. 1) or had a monoparticulate type of glycogen (Fig. 3). These smooth vesicles may represent SER. Scanty but normal appearing microbodies, presumably peroxisomes, were encountered in 3 of the tumors (Fig. 6).

The Golgi apparatus was identified in all tumors, being in close proximity to the bile canaliculi where such were present (Figs. 5 and 6). It was very well developed in 2 tumors where, because of the small size of tumor cells, the Golgi apparatus occupied a relatively much larger area of the cytoplasm than is seen in normal cells (Fig. 4). Moreover, in the same 2 tumors,
Ultrastructure of Human Hepatoma

Table 1
Summary of morphological findings

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<td>Age (yr)</td>
<td>48</td>
<td>42</td>
<td>54</td>
<td>36</td>
<td>24</td>
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<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Survival (Mo.)</td>
<td>3</td>
<td>Lost to follow-up</td>
<td>Lost to follow-up</td>
<td>42</td>
<td>4</td>
</tr>
<tr>
<td>Underlying cirrhosis</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Hepatocyte-like</td>
<td>Hepatocyte-like</td>
<td>Hepatocyte-like</td>
<td>Small size and long cristae</td>
<td>Hepatocyte-like</td>
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<tr>
<td>Matrix granules present</td>
<td>Matrix granules present</td>
<td>Matrix granules present</td>
<td>Matrix granules present</td>
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</tr>
<tr>
<td>Relation to RER maintained</td>
<td>Relation to RER maintained</td>
<td>Many enlarged forms</td>
<td>Relation to RER maintained</td>
<td>Many enlarged forms</td>
<td>Relation to RER maintained</td>
</tr>
<tr>
<td>RER</td>
<td>Moderate to plentiful Dilated cisternae</td>
<td>Plentiful</td>
<td>Moderate amounts Dilated cisternae</td>
<td>Scanty amounts Dilated and vesiculated cisternae</td>
<td>Moderate to plentiful Dilated cisternae</td>
</tr>
<tr>
<td>SER</td>
<td>Moderate amounts Anastomosing tubular and vesicular profiles</td>
<td>Scanty amounts Anastomosing tubular and vesicular profiles</td>
<td>Very scanty Vesicular profiles</td>
<td>Very scanty Vesicular profiles</td>
<td>Moderate amounts Anastomosing tubular and vesicular profiles</td>
</tr>
<tr>
<td>Glycogen</td>
<td>As rosettes</td>
<td>Absent</td>
<td>As monoparticulate forms</td>
<td>Absent</td>
<td>As rosettes</td>
</tr>
<tr>
<td>Microbodies</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Golgi apparatus</td>
<td>Well developed; VLDL particles present</td>
<td>Well developed; VLDL particles present</td>
<td>Poorly developed, no visible contents</td>
<td>Variable development, no visible contents</td>
<td>Variable development; VLDL particles present</td>
</tr>
<tr>
<td>Lipofuscin granules</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Multivesicular bodies</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Bile canaliculi</td>
<td>Frequent</td>
<td>Frequent</td>
<td>Absent</td>
<td>Rare</td>
<td>Frequent; cholestatic features present</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Highly differentiated</td>
<td>Highly differentiated</td>
<td>Poorly differentiated</td>
<td>Poorly differentiated</td>
<td>Highly differentiated</td>
</tr>
</tbody>
</table>

*Very-low-density lipoprotein.*

electron-dense particles presumed to be very-low-density lipoproteins (10, 15, 20) were observed within Golgi saccules and in adjacent vacuoles (Figs. 5 and 6).

The most commonly encountered lysosomes were multivesicular bodies. Usually 3 to 4 were present in each section of a tumor cell (Fig. 5). Autophagic vacuoles with enclosed cell organelles in various stages of degradation were also present but much less commonly. Lipofuscin granules were not seen. In the tumor that had led to obstruction of the common hepatic duct, many membrane-bound inclusions identical to those seen in hepatocytes in cholestasis were present in the tumor cells.

In 1 tumor, microvilli were present along those cell surfaces adjacent to blood capillaries (Fig. 4). The plasma membranes were otherwise simple, lacking in surface modifications. Bile canaliculi were identified in 4 of the tumors (Fig. 5). The
chanal sideriules were generally irregular in outline and possessed few microvilli which were often stunted. Desmosomes were much less common than in nontumorous tissues. The nuclei were large relative to the cytoplasmic volume. Nucleoli varied in prominence and the heterochromatin were evenly dispersed. In 1 tumor, extensive continuities between the nuclear envelope and RER were frequently observed (Fig. 3).

Numerous virus-like particles were present in some 90% of neoplastic cells of 1 tumor (Patient D). The particles were scattered free in the cytoplasm. None was detected in nuclei or attached to cytoplasmic organelles. They were round or oval, measuring approximately 70 to 120 nm (average 80 nm) in diameter (Figs. 1 and 2). The particles had a central electron-dense nucleoid surrounded by a zone of electronlucent material and covered by a single smooth outer membrane. An inner membrane-like material surrounding the nucleoid was discernible in favorably oriented sections. The particles were absent in all nontumorous cells examined.

Nontumorous liver tissues were available for examination in 3 cases. In 1 noncirrhotic liver, bearing the tumor containing virus-like particles, the only detectable deviation from the normal was a striking increase in the SER in 10 to 15% of the hepatocytes. In another, also noncirrhotic liver, which had caused obstruction in the common hepatic duct, the hepatocyte changes were those of cholestasis. In a 3rd, a cirrhotic patient (Patient A), the most notable abnormality involved the ER. The abnormality consists of slender tubular inclusions within the ER cisternae (Figs. 7 to 9). In longitudinal sections, these tubules appeared as long (up to 3 μm) filamentous structures whereas in cross-sections, they appeared as "hollow spheres measuring 15 to 20 nm. The tubules were present in the ER of 30 to 80% of cells examined, depending on the areas selected for ultramicrotomy. Almost all of the SER but only part of the RER, that which was adjacent to the SER, contained these inclusions (Fig. 8). No continuity between the tubules and the membranes of the ER was apparent.

**DISCUSSION**

The ultrastructural diversity of human hepatomas observed in this study may be appreciated from the reports of previous investigators (5, 8, 22, 24, 27, 28, 31, 32). Nevertheless, some basic similarities between the tumor cells and hepatocytes were often apparent.

The histological diagnosis of primary liver tumors is not difficult in most cases. The routine use of electron microscopy for this purpose is therefore superfluous. Occasionally, in poorly differentiated tumors where acinous formations are present, the differentiation between hepatocarcinomas and cholangiocarcinomas must be somewhat arbitrary and rest on uncertain grounds. Electron microscopy may help to settle this problem. In experimental animals the differentiation between hepatomas and cholangiocarcinomas are readily achieved (26).

In this study, the hepatocyte-like nature of tumor cells in 4 cases was indicated by the occurrence of one or another specific hepatocyte subcellular components: glycogen rosettes, microbodies (presumably peroxisomes), very-low-density lipo-protein particles in Golgi saccules and vacuoles, and bile canaliculi. Although the 5th tumor lacked specific hepatocyte organelles, its subcellular organization and the appearance of its mitochondria were much more like those of hepatocytes than like bile ductular cells.

Simplicity of subcellular organization and increased nuclear cytoplasmic ratios as observed in hepatoma cells in this study appear to be characteristic of most tumor cells. Thus, there is generally a decrease in the number of mitochondria and in "organized ergastoplas" (21). In regenerating liver cells too, there is a decrease in mitochondrial number (1). In rats, the fastest growing transplantable hepatomas have a simple organization of the cytoplasm whereas the slow-growing hepatomas have better developed and more organized cytoplasmic membrane systems (12). The presence or absence of microbodies apparently bears no relationship to the rate of growth of tumors. In this study, the patient bearing the least ultrastructurally differentiated tumor survived 3 years, the longest survival in the group. However, it is difficult to relate tumor growth rate to survival in humans; many factors such as the presence or absence of underlying cirrhosis may modify survival time.

The only tumors with a normal hepatocyte type of glycogen also had plentiful, normal-appearing SER. It is generally considered that the SER is active in glycogen metabolism, and there is some evidence for the role of the SER in glycogen synthesis as well as in glycogenolysis (14). The presence of normal SER only in tumor cells that also have normal glycogen rosettes further supports the role of SER in glycogen metabolism.

In the absence of supporting evidence, the 80-nm particles present in the cytoplasm of 1 tumor can only be considered virus-like. It is possible but unlikely that they are liposomes, secretory granules, or even pinocytotic vesicles. Liposomes such as those in rat liver are electron-dense homogeneous lipid material present within SER. In human hepatocytes fixed in OsO₄, the SER appears as irregularly shaped "vesicular" or anastomosing tubular profiles and lipid material appears as a homogeneous material of very low electron density (17, 18). The particles herein reported are spherical, and they contain an inhomogeneous material (Fig. 1, inset).

Secretory granules similar to these virus-like particles have not been described in human hepatocyte, a cell from which this tumor has presumably originated. Pinocytotic vesicles too are readily distinguished from these particles. In human hepatocyte, as in the cells of this tumor, pinocytotic vesicles are located predominantly adjacent to the cell surface, are devoid of electron-dense contents, and are often coated (18).

Nevertheless, without further evidence such as negative staining, it is not possible even to attempt to classify these particles on the basis of any known virus thus far described. In a patient with active chronic hepatitis, virus-like structures similar to members of the coronavirus group were identified (33). The particles reported here are also enveloped and are of the same size range as some coronaviruses.

Viruses or virus-like structures have been detected in several human tumors but apart from 2 benign tumors, the molluscum contagiosum and the common wart, a causal relationship between the viruses and tumors has not been established (11).
The tubular inclusions observed in the nonneoplastic hepatocytes of 1 patient are similar to those reported in many diverse cell types and in several different diseases (29) including some benign hepatic tumors in mice and in spontaneous hyperplastic nodules (6) of C3H mice. The preponderance of these tubules in the cisternae of the SER and adjacent RER suggests that they may represent some metabolic products, possibly proteins, which are synthesized in the RER and transported to the SER. Apparently, the tubules are not normal components of the hepatocyte ER since they have not been observed in the normal or in any other condition affecting the liver. Their relationship to the malignant process, if any, is not understood.

Similar microtubular inclusions observed in the kidney of patients with disseminated lupus erythematosus (7, 9, 13) and in subacute inclusion body encephalitis (25) have been interpreted on morphological grounds as nucleoprotein strands of myxovirus. It is considered that they may represent virus responsible for inducing the disease or even viruses or cellular debris phagocytosed from the circulation (9). It is unlikely, however, that phagocytosed material should be contained within the ER as is observed in our patient. Baringer (2, 3) and Uzman et al. (29) on the other hand argue against the viral nature of these inclusions, interpreting them to represent a peculiar cellular response to a wide variety of stimuli.

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REFERENCES

Fig. 1. Hepatoma (Patient D). The mitochondria are small and without matrix granules. Scanty ER occurs as short profiles. A number of virus-like particles are scattered in the cytoplasm. The single limiting membrane and electron-dense “nucleoid” is evident in the inset. Fig. 1, X 35,000; inset, X 95,000.

Fig. 2. Hepatoma (Patient D). Many virus-like particles are present. At arrows, the central nucleoids are better appreciated. The ER is fragmented and the normal spatial relationship between the ER and mitochondria is lacking. X 77,000.

Fig. 3. Hepatoma (Patient C). There is extensive development of dilated RER but little or no recognizable SER. Continuities between RER and nuclear envelope (arrow) are common. Mitochondria are large and pale. Glycogen resembles the monoparticulate variety. The plasma membranes are simple (short arrows). X 38,000.

Fig. 4. Hepatoma (Patient A). The cells contain plentiful RER, SER, and glycogen rosettes (gr). Golgi apparatus (G) is extensive. Lipid droplets (F) are present in 2 nuclei which are large relative to the cells. A capillary is on the right. Tumor cell microvilli are present adjacent to the capillary. X 11,500.

Fig. 5. Hepatoma (Patient A). The resemblance to hepatocytes is striking. Bile canaliculus (BC), RER, SER, glycogen rosettes, Golgi apparatus (G), adjacent vacuoles (V), and multivesicular bodies (mvb) are present. X 22,500.

Fig. 6. Hepatoma (Patient B). Hepatocyte-type mitochondria, peroxisome (P), Golgi apparatus (G), and adjacent vacuoles (V) containing presumed very-low-density-lipoprotein particles. Slender RER profiles characterize this tumor. X 19,000.

Fig. 7. Hepatocyte of tumor-bearing liver. A large misshapened mitochondrion with crystalline inclusions is evident. The SER contains numerous tubular inclusions which are cut on various planes (arrows). X 38,500.

Fig. 8. Cross-sections of ER inclusions show them to be hollow cylinders (arrows). These inclusions are present in the SER and in the adjacent RER. X 71,000.

Fig. 9. The tubular nature of the ER inclusions is illustrated (arrows). An unusual body, presumably a residual body containing “crystalline” lipids (L), is present. X 59,000.
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