Ultrastructural Survey of Primary Liver Cell Carcinomas From Uganda[1,2,3]

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SUMMARY — Seventeen surgical biopsy specimens from Ugandan patients with liver cell carcinoma were studied by electron microscopy. The ultrastructural features observed in this series are similar to those previously reported by others. A narrow range in the degree of differentiation of tumor cells is noted, but in most cases they are easily recognizable as of hepatic origin. No morphologic correlation with a-feto-protein in the serum or with Australia antigen was found and no virus particles were seen in the material examined. Despite the absence of significant new findings in this survey, it is suggested that electron microscopic studies in liver cancer should be pursued, but more specifically directed toward the investigation of in vitro cultures and the search for virus therein. Application of special techniques for localization of fetal protein production might also provide new information on the relationship of this antigen to other functions of the hepatic cell during tumorigenesis and following therapy.—J Nat Cancer Inst 48: 587–603, 1972.

'LTRASTRUCTURAL MORPHOLOGY has ıade selected but rather limited contributions to ıe study of human neoplasms. The expectation ıat high magnification would permit recognition morphologic features characteristic of malig-ancy has not been realized. With few exceptions, ectron microscopy has likewise not greatly dvanced our knowledge as to the histogenesis of ecial tumor types, such as Ewing's sarcoma, nor

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can we always identify with certainty those specific organelles that might indicate the origin of many undifferentiated tumors. The negative tone of this introduction reflects partly the absence of any significant new findings in our preliminary survey of liver cancers from Uganda. More importantly, however, we wish to emphasize the need to identify objectives and to pose specific questions when subsequent ultrastructural studies are pursued.

Although there is adequate literature pertaining to the fine structure of induced liver cancer in experimental animals (*1–4*), published papers describing ultrastructural characteristics of human liver cell cancer are few and include only about 18 cases (*5–11*). Most reports describe only a single case. Theron from Pretoria, however, studied 9 cases in the South African Bantu, and in 1 of these he found virus-like particles ***(22).*** The studies described herein were undertaken as part of a multidisciplinary investigation of liver cell carcinoma by the Uganda Cancer Institute. Correlation with light microscopy and serologic studies was specifically sought.

MATERIALS AND METHODS

Table 1 lists 67 glutaraldehyde-fixed specimens received from Kampala. On receipt, these were postfixed in osmium tetroxide and embedded in Epon 812: 1-μ pilot sections were stained with azure B and basic fuchsin for light microscopic survey. Representative areas were selected and thin sections were cut, stained with uranyl acetate and lead hydroxide, and examined in a Siemens 1A microscope. It was clear from the onset of specimen collection that postmortem material was generally unsatisfactory. Even in some fresh biopsies obtained at surgery there were severe degenerative changes that disallowed adequate study. Often these were the result of sampling and reflected changes in a rapidly growing tumor with inadequate blood supply. In addition, some specimens that had been stored in buffer before shipment to Bethesda were contaminated with fungus.

Of the 27 surgical biopsy specimens of liver cancer, 17 were ultimately studied. By light microscopy of pilot sections as well as by electron microscopy, in this group there were a range of differentiation and a variation in morphologic features suggesting that these specimens were a reasonable representation of liver cell cancer, at least as seen in Uganda, Selected specimens of normal liver were also examined.

TABLE 1.—Liver biopsy specimens from U

	1969	1970	197
Surgical			
Cancer	6	19*	2
Noncancer	3	15	2
Postmortem			
Cancer	3	4	—
Noncancer	5	4	—
Metastatic liver cancer (postmortem)	3	1	—

*One case of cholangiocarcinoma.

RESULTS

With only minor differences, previous descriptions of the fine structural features of hepatomas are similar and agree with ours. While doubling the total number of cases studied, we extended and confirmed the findings of others and suggest that there are no essential differences in the ultrastructural morphology of hepatoma in the African and the Caucasian.

Table 2 lists the principal morphologic features of liver cell cancer as seen by electron microscopy, and these are illustrated in figures 1–12.

TABLE 2.—Ultrastructural characteristics of liver cell cancer

Nucleus	Large, irregular, pseudoinclusions
Nucleolus	Large, irregular, multiple
Rough endoplasmic reticulum	Disorganized Associated with mitochondria Cisternal dilatation
Mitochondria	Pleomorphism Irregularities of cristae
Smooth endoplasmic reticulum	Increased
Golgi	Increased, prominent
Lysosomes	Primary ↓, cytolysosomes Lipofuscin ↑, microbodies
Fat and glycogen	Variable

The nucleus in hepatic cell cancer is larger than normal and the nuclear cytoplasmic ratio is in-

creased (figs. 3–5, 7). These are features common to many cancers, but the frequency of nuclear pseudoinclusions, resulting from cytoplasmic invagination and a function of nuclear irregularity and lobulation, is perhaps remarkable in liver cell cancer and often not readily appreciated by light microscopy (fig. 3). Again, as in other tumors, the nucleoli are enlarged and multiple, and nucleolonema organization is often prominent and irregular (figs. 4–6).

The most important general characteristic of the liver cancer cell is a loss of orderly arrangement and organization of the cytoplasmic components as compared with the normal hepatic cell. The number and type of specific organelles vary in the cells of different tumors, but usually there is more uniformity between cells of the same tumor. Mitochondria showed considerable pleomorphism and irregularities of their cristae (figs. 6, 9–11). Crystalline inclusions were observed in occasional mitochondria of several cases (figs. 9, 12). These were seen previously in normal livers and once in human hepatic cell cancer *(11, 13)*. Their significance is unknown. Autophagic vacuoles, some of giant sue, were numerous in 1 case and occasional in others (fig. 7). Fat and glycogen varied. Glycogen, when present, was usually seen as rosettes but in 1 instance (fig. 8) many cells were filled or distended by a monoparticulate form that stained poorly. By light microscopy these were seen as groups of "clear cells."

In addition to the search for any unusual ultrastructural features not previously recorded in liver cancer, we examined the material with the following objectives in mind: 1) determination of tumor grade; 2) identification of ultrastructural features that might correlate with presence or absence of a-feto-protein; 3) characterization of nucleolar changes; 4) search for virus or virus-like particles.

The degree of differentiation of liver cancer cells was estimated by the size and regularity of the nucleus, the amount and organization of rough endoplasmic reticulum, the presence of microbodies, the amount of secretory activity exhibited by the Golgi, the regularity and uniformity of the mitochondria, and the polarization of specific cytoplasmic organ-

Obviously, all these are qualitative estimates and are a matter of considerable subjectivity. Although there was some variability in the degree of differentiation as judged by these criteria, all the specimens we surveyed except one were classified as grade 2 or of intermediate differentiation. There was only 1 tumor that we considered to be well differentiated (fig. 2) and none was classified as undifferentiated or highly anaplastic. If grading is of importance in this disease, apparently it would be much more efficiently done at the light microscopic level where histologic as well as cytologic features are better evaluated and where the sampling error can be greatly minimized.

The results of the a-feto-protein test were unknown to us at the time of specimen examination. The most obvious and the only ultrastructural features that we thought might correlate with a-feto-protein production were the amount of rough endoplasmic reticulum and evidence of increased secretory activity or storage. Again, sampling was important. Of the 17 cases studied, 9 showed a significant proliferation of endoplasmic reticulum and cisternal dilatation (figs. 6, 9). In 2 additional instances, focal cells were identified showing cisternal dilatation. When the specimens with a positive serologic test were identified, 11 of the 17 studied, no correlation was found with the morphologic changes described above.

Aflatoxins are among the chemical agents which bind DNA and result in dissociation of the fibrillary and granular components of the nucleolus, with the formation of a capping effect *(14, 15)*. Because mycotoxins are hepatic carcinogens in a number of animal species *(16)* and their possible association with human liver cancer has been considered (*17*), particular attention was given to recognition of this nucleolar change in the specimens studied, but it was not found.

Finally, a search for virus or virus-like particles was made and again the results were negative. In 1 instance a small aggregate of short, branching tubular structures was seen in a single nucleus. This is reminiscent of certain myxoviruses but represents an isolated and inconclusive observation.

DISCUSSION

In the 17 liver cell cancers studied we found a range of cytologic differentiation, but paradoxically this range appeared more limited than that

appreciated by light microscopy. We also noted various abnormalities in the quality and quantity of specific cytoplasmic organelles as well as a lack of their orderly arrangement or organization. In most instances, however, the tumor cells were easily recognizable as of hepatic cell origin. We cannot report any characteristics unique to these cases from Uganda as compared to previous reports from other countries.

This morphologic study was a part of a continuing investigation of etiologic factors and new therapeutic modalities in liver cancer in Uganda. Of particular interest in this respect are the studies of a-feto-protein and Australia antigen which will be reported elsewhere.' No ultrastructual features could be correlated with the presence or absence of a-feto-protein in the serum or with Australia antigen, and no virus particles were found.

It is believed a-feto-protein is a carcino embryonic antigen and that its production results from derepression of a genetically controlled function active in the fetal cell. This suggests that serologic positivity correlates with tumor cell differentiation, and evidence to support this has been claimed by several authors ***(20, 21)***. However, in neoplasia, morphologic and functional characteristics are not necessarily concordant and we were unable, in this or previous studies, to relate tumor grade or histologic subtype to a-feto-protein in the serum (22, ***23)***.

This survey of multiple tumors provides a useful overview and background on which to base more specific investigations. Clearly, as with most neoplasms, ultrastructural morphology alone can contribute little to diagnosis, prognosis, or to our understanding of etiology and pathogenesis. There are 2 or 3 areas of investigation that should be encouraged and in which the electron microscopist can participate. One of these concerns quantitative and qualitative studies of α-feto-protein production with immunologic and radioautographic localizing techniques. Purtilo and Yunis demonstrated a-feto-protein in fetal liver cells and in hepatoma cells by immunofluorescence ***(24)***. Application of ferritin antibody or enzyme conjugation methods in electron microscopy ***(25,*** 26) should provide additional information as to site of production and the differences between producer and nonproducer cells.

The other 2 areas of investigation relate to *in vitro* studies and virus identification. There have only been 2 recorded observations of virus particles in liver cell cancer—1 by Theron *et al*, already mentioned (*12*), and the other by Ma and Blackburn in a New Guinean ***(27)***. Several authors have seen virus-like particles in cases of hepatitis ***(28–30)***. In view of the still uncertain relationship of viral hepatitis to hepatoma, as well as the possibility that an unrelated virus may be important in hepatic neoplasia, it is of interest to continue a systematic screen of human tissue. Related to this objective of virus identification is an h— and sustained effort at *in vitro* culture of normal and neoplastic hepatic epithelial cells. Successful cultivation of liver cancer cells will give the electron microscopist the best opportunity to investigate morphology and function and provide both the microscopist and the virologist the most favorable situation for viral isolation.

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[7] α-Feto-protein studies were performed by Dr. K. R McIntire of the National Cancer Institute using an electroimmunodiffusion method (*18*). Australia antigen was determined with complement fixation tests (*19*) by L. Barker of the Division of Biologics Standards.

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