Ultrastructural Study of Liver Invasion by Novikoff Hepatoma

Feridoun Babai and Gilles Tremblay

Département de Pathologie, Faculté de Médecine, Université de Montréal, C.P. 6128, Montréal 101, Canada

SUMMARY

The ultrastructural aspect of liver invasion by Novikoff hepatoma transplanted in rat liver was investigated. Invasion of liver parenchyma was seen to begin by the projection of cytoplasmic processes from the tumor cells into the neighboring hepatic cells. This infiltration was, at times, associated with fragmentation of the invaded liver cells. Moreover, the tumor cells showed phagocytosis of hepatic cell debris and erythrocytes.

These findings appear to indicate the active and destructive character of the process of liver invasion by Novikoff hepatoma.

INTRODUCTION

The ability to invade and destroy surrounding normal tissues is one of the basic characteristics of malignant tumors; nevertheless, the precise mechanisms through which this invasion and destruction by cancer cells takes place remain largely unknown. The various theories proposed to explain the process of invasion have been reviewed by Easty (6), Leighton (15), and Weiss (22). Light microscopic studies conducted in vivo and in vitro have provided significant data on the mode of invasion by cancer cells (23, 24). However, little information is available concerning the ultrastructural aspect of the invasive process and the relationship of the surface of malignant cells to that of the invaded host cells.

The purpose of this paper is to describe the ultrastructural features of hepatic invasion by Novikoff hepatoma in rat liver.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180 to 250 g were used. Novikoff ascites hepatoma was serially transferred in rats by i.p. inoculation of 1 ml of ascitic fluid at 7-day intervals. To obtain solid Novikoff hepatoma, 1 ml of ascitic fluid was withdrawn from the peritoneal cavity on the 7th day and inoculated into the anterior femoral muscles of the thigh. Fragments of solid tumor obtained on the 6th or 7th day were used for intrahepatic implantation. Celiotomy was performed under ether anesthesia and a small (1-mm) fragment of solid Novikoff hepatoma was implanted in the left lobe of the liver with a No. 17 trocar. Seven to 10 days later, the animals were killed and perfused via the thoracic aorta for 1 or 2 min with Ringer's solution containing 0.1% procaine and heparin, 5 mg/100 ml (8), and for 15 min with 2.5% glutaraldehyde in phosphate buffer, pH 7.4 (13).

Following perfusion, the liver was sectioned and small fragments containing tumor tissue and surrounding liver parenchyma were immersed for 2 to 3 hr in the same fixative at 4°. After fixation, the tissues were washed in cold phosphate buffer containing 0.25 M sucrose and postfixed for 1 hr at 4° in 1% osmium tetroxide in phosphate buffer, pH 7.4. The tissues were then dehydrated in graded concentrations of alcohols and embedded in Araldite.

For light microscopic study, sections 0.5 to 1 μm thick were cut with glass knives on a Porter-Blum MT1 ultramicrotome and stained with toluidine blue. Areas of tumor invasion were selected for electron microscopic studies. Ultrathin sections were cut on the same ultramicrotome, picked up on Formvar-coated grids, stained with uranyl acetate and lead citrate, and examined with a Philips 200 electron microscope.

RESULTS

Gross and Light Microscopic Observations. Seven to 10 days after implantation of the fragment of Novikoff hepatoma, a tumor nodule measuring from 5 to 15 mm in diameter was observed in the liver (Fig. 1). On section, the tumor tissue was well demarcated, soft, and grayish-white; occasional foci of necrosis were present.

Light microscopic examination of liver-tumor interzone showed some areas of liver compression and many areas of active invasion in which the tumor cells were in direct contact with hepatic cells without interposition of fibrous tissue (Fig. 2). The liver plates were disrupted and dissociated by the invading tumor cells (Fig. 2). Some hepatic cells surrounded by the tumor cells were degenerated or necrotic. In addition, there was a slight infiltration by lymphocytes, granulocytes, and monocytes in some areas of invasion.

Electron Microscopic Study. The tumor cells in the zone of invasion were isolated or in clusters. Their surface membrane frequently exhibited numerous microvilli and cytoplasmic processes (Figs. 3 to 5). The cytoplasm usually showed one or several Golgi complexes, numerous free ribosomes, and a small amount of poorly developed rough endoplasmic reticulum. The mitochondria were small, lacking in cristae, and often

1 This work was supported by a grant from the National Cancer Institute of Canada.
2 Present address: Department of Pathology, Faculty of Medicine, McGill University, Montreal 110, Canada.

Received July 21, 1972; accepted September 6, 1972.
Feridoun Babai and Gilles Tremblay

exhibited a matrix of low density. Lysosomes were rare and microbodies were not observed. Many of the tumor cells also contained lipid droplets and glycogen aggregates (Fig. 3), the latter being frequently concentrated in some areas of the cytoplasm.

A striking feature was the presence in many nuclei of tubular structures (Figs. 3 and 8) arising from deep invaginations of the inner nuclear membrane, as previously reported (3, 12).

**Hepatic Invasion by Tumor Cells.** The tumor cells were observed to invade the sinusoids, the intercellular spaces, and the hepatic cells themselves. Penetration through the hepatic cells was the pathway most frequently observed. The tumor cells extended microvilli and slender cytoplasmic processes that jutted out into the adjacent liver cells (Figs. 3 and 4). Some of these cytoplasmic processes were larger and extended more deeply into neighboring hepatic cells (Fig. 5). Finally, large portions of tumor cells could be seen protruding into the parenchymal cells (Fig. 3). During this process, the plasma membrane of the invaded hepatic cells was distorted and invaginated by the invading cells. In some areas, the surface of the tumor cells and that of the invaded cell appeared in close approximation; in other areas, the 2 surfaces remained separated by an intracellular space of varying width (Figs. 3 and 5). Furthermore, as reported elsewhere (21), tumor cells and adjacent hepatic cells were found in several instances to be attached by intercellular junctions of the macula adherens type.

Extension of tumor cell processes into the intercellular spaces between adjacent hepatic cells also occurred. However, junctional complexes often appeared to resist this infiltration (Fig. 5).

**Destruction of Liver Cells by Tumor Cells.** Even when distorted by the tumor-cell processes, some of the hepatic cells adjacent to the tumor cells appeared otherwise intact. Conversely, portions of other hepatic cells infiltrated by the tumor-cell processes were observed to undergo fragmentation with dissolution of the plasma membrane and spilling of cytoplasmic material into the intercellular spaces (Figs. 6 and 7). While this focal alteration took place in one invaded segment, the remaining larger part of the cells often appeared relatively uninjured (Fig. 6). Portions of liver cells were sometimes distorted by tumor cells into club-shaped protuberances still connected to the main mass of the cells by a narrow segment (Fig. 8).

**Phagocytic Activity of Tumor Cells.** The cytoplasm of tumor cells often contained cellular debris enclosed in vacuoles. In some vacuoles, the debris consisted of well-recognizable hepatic cell fragments including mitochondria and organized arrays of rough endoplasmic reticulum (Fig. 9). In other instances, the debris presented variable degrees of disintegration to the point of becoming unidentifiable. These vacuoles were frequently situated near aggregates of glycogen particles and lipid droplets (Figs. 3 and 8).

Erythrocytes enclosed in vacuoles (phagosomes) were also seen in the tumor cells (Fig. 3); such erythrocytes were intact or altered. Tumor cells appear to achieve capture of erythrocytes by extending cytoplasmic processes around them (Fig. 3). Unlike in the hepatic cells, intracellular penetration and fragmentation of erythrocytes by tumor cells were not seen. In addition, there was no indication of any close association of lysosomes with the phagocytic vacuoles.

**DISCUSSION**

The present study provides evidence that, when Novikoff tumor is transplanted in rat liver, the initial stage in the process of invasion is the projection of cytoplasmic processes from the tumor cells into the hepatic cells. This active extension of pseudopodal processes, followed by protrusion of larger cytoplasmic portions, is at times associated with fragmentation of hepatic cells. Moreover, the tumor cells are able to phagocytize hepatic cell debris and red blood cells.

The present findings on the early stage of invasion are consistent with previous observations. For instance, in their ultrastructural study of experimentally induced hepatic metastases of Walker carcinoma, Fisher and Fisher (7) observed the presence of pseudopodal cytoplasmic extensions of the tumor cells into adjacent hepatic cells. More recently, Locker et al. (17), studying the metastatic growth by Yoshida hepatoma in chick embryo liver, noted that tumor cells extended pseudopodia invaginating within the liver parenchyma. The occurrence of tumor cell pseudopodia indenting the plasma membrane of host cells has also been reported by Butterworth (5) in intrahepatic tumors produced by implanting Landschutz sarcoma in mouse liver. Similarly, electron microscopic investigations on experimentally induced and on human squamous cell carcinomas have revealed cytoplasmic protrusions from the tumor cells penetrating into the underlying connective tissue through gaps in the basement membrane (2, 9, 11, 19). These cytoplasmic protrusions were even found in papillomas and in precancerous squamous cell lesions (9, 19). Ozzello and Sanpittak (18) reported that the earliest detectable stage of invasion in intraductal carcinoma of the breast was characterized by neoplastic cytoplasmic protrusions into the stroma through gaps in the basement membrane. These observations and the present findings suggest that infiltration of neighboring structures by cytoplasmic processes from the tumor cells is a common and possibly a general mode of early invasion.

The mechanism by which tissues are destroyed by invading tumor cells is still unclear. One hypothesis is that tumor cells release toxic and lytic substances capable of damaging and destroying normal cells (20). The present morphological study does not provide evidence to invalidate or substantiate this hypothesis. Leighton (16) has proposed the concept of neoplastic blockade to explain the destruction of invaded tissue. According to this hypothesis, the tumor cells, by surrounding and covering the normal cells, produce a nutritional deprivation in the latter leading to their degeneration. In the present study, hepatic cells were often found to be extensively surrounded by tumor cells and it would seem reasonable to assume that such a process could eventually result in nutritional blockade and ultimate degeneration of the liver cells. However, a more active mode of
Liver Invasion by Novikoff Hepatoma

destruction was also observed. While some hepatic cells, although deformed and indented by the protruding pseudopods were otherwise intact, others appeared to undergo fragmentation and piecemeal necrosis. Whether the infiltration by tumor pseudopods is sufficient in itself to produce the piecemeal necrosis of hepatic cells or whether other deleterious factors such as nutritional deprivation are also involved cannot be determined at this time. However, the observation that the remaining part of the hepatic cells often appears uninjured would tend to favor the 1st possibility. Active destruction of hepatic cells has not been observed in the chick embryo liver invaded by Yoshida ascites hepatoma (17). However, Katsuta et al. (14), using cinemicrographic observation of mixed cultures of normal liver cells and hepatoma AH-130 cells, found that the tumor cells actively assaulted the normal cells by direct contact. Whether this aggressive behavior of Novikoff hepatoma and AH-130 hepatoma reflects a higher degree of malignancy remains to be determined.

The hypothesis that cancer cells possess significant phagocytic properties has been largely discarded (6, 23). In the present study, while some of the vacuoles seen in the cytoplasm of tumor cells could possibly have represented an autophagic process, others were undoubtedly of phagocytic origin as evidenced by their content of erythrocytes and of well-recognizable hepatic cell fragments. In their ultrastructural studies of lymphomas, Bernhard and Leplus (4) found that the cells of some reticulum cell sarcomas have phagocytic activity, but this could merely reflect the persistence in the tumor cells of a specific functional propensity. More directly related to the present findings is the work of Wood et al. (25) who, using a rabbit ear chamber and time-lapse cinemicrography to study the in vivo behavior of anaplastic V₂ carcinoma cells, observed the engulfment of an erythrocyte by a tumor cell. Moreover, in an ultrastructural study of breast carcinoma, Goldenberg et al. (10) noted that phagocytosed leukocytes were often found within carcinomatous breast cells. The leukocytes were contained in membrane-bound vacuoles and ranged from well preserved to barely recognizable cell debris. These and the present observations raise the possibility that phagocytic activity of malignant cells might be a more common phenomenon than was hitherto recognized.

The findings of the present study appear to indicate the active and destructive character of the process of liver invasion by Novikoff tumor. Clearly, further work with other tumors and other host tissues is required before their exact significance can be evaluated. Nevertheless, these findings are in accord with the accumulated evidence pointing to the role of the surface membrane of tumor cells in invasion (1).

REFERENCES

22. Weiss, L. The Cell Periphery, Metastasis and Other Contact
Fig. 1. Section of liver, showing a tumor nodule (T) on the 9th day of transplantation. × 1.5.

Fig. 2. Zone of invasion seen in light microscopy. Liver-cell plates (arrows) are disrupted by invading tumor cells. Toluidine blue, × 400.

Figs. 3 to 9. Electron micrographs of the zones of invasion.

Fig. 3. Low magnification of a zone of invasion. A tumor cell (TC1) infiltrates deeply into an adjacent hepatic cell (HC). This tumor cell shows intranuclear tubular structures (Ts) and areas of glycogen (G). A 2nd tumor cell (TC2) extends multiple cytoplasmic processes into an adjacent hepatic cell (long arrows). This tumor cell (TC2) contains an erythrocyte (E1) enclosed in a vacuole and disintegrated cell debris (Cd) in an area of glycogen. There is a Golgi apparatus (Go) and many lipid droplets (L) in the cytoplasm. A 2nd erythrocyte (E2) is being phagocytized by extension of thin cytoplasmic processes (short arrows). × 8,000.

Fig. 4. A tumor cell (TC) extends microvilli that protrude into the adjacent hepatic cell (HC). × 14,000.

Fig. 5. Large cytoplasmic processes from a tumor cell (TC) protrude deeply into the 2 neighboring hepatic cells (HC). The intercellular space showing a desmosome (arrow) is not infiltrated by the tumor processes. × 11,000.

Fig. 6. Portion of a hepatic cell (HC) infiltrated by cytoplasmic processes from a tumor cell (TC) is undergoing fragmentation. Some of the hepatic fragments (arrows) show necrotic changes and are being surrounded by cytoplasmic processes of the tumor cell. × 19,000.

Fig. 7. Portion of an invaded hepatic cell (HC) is undergoing fragmentation. Arrow, dissolution of plasma membrane and liberation of necrotic cytoplasmic material into the invagination of the tumor cell (TC). × 22,000.

Fig. 8. Portion of a hepatic cell (HC) is surrounded and in 1 area thinned out by 2 tumor cells. One of the tumor cells contains cell debris (Cd) partially surrounded by glycogen. The nucleus shows tubular structures (Ts). × 17,000.

Fig. 9. Electron micrograph showing a tumor cell (TC) and an invaded hepatic cell (HC). The tumor cell shows 2 vacuoles, 1 containing disintegrated cell debris (Cd) and the other enclosing a recognizable hepatic fragment (HCD). × 12,000.
Liver Invasion by Novikoff Hepatoma
Ultrastructural Study of Liver Invasion by Novikoff Hepatoma

Feridoun Babai and Gilles Tremblay


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/12/2765

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.