

Observation of microvascular casts of human hepatocellular carcinoma by scanning electron microscopy

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Summary: The microcirculation of hepatocellular carcinomas (HCCs) and surrounding tissue was observed three-dimensionally by scanning electron microscopy of vascular casts made from 10 livers at autopsy. The livers were perfusion-washed and cast with resin through both the hepatic artery and portal vein branches. The HCCs observed ranged from several millimeters to 3 cm in size. A vascular plexus proliferated around the HCC nodules in all cases. Both portal vein and hepatic artery branches proliferated markedly to form the plexus in 5 patients. These vessels communicated directly with the blood sinuses of the HCCs as feeder vessels. HCC cells replaced normal cells while maintaining the liver's trabecular structure in 2 cases. At the borders of these HCCs, there was direct communication between the hepatic sinusoids and the tumor blood sinuses. Efferent vessels of the tumors were generally difficult to identify but vessels resembling hepatic vein branches were detected in one 4-mm HCC nodule after microdissection. Thus, HCC was demonstrated to be supplied not only by the hepatic artery but also by the portal vein and hepatic sinusoids. This may be one of the reasons why cancer cells survive in the tumor margins and daughter nodules after trans-catheter arterial embolization of HCC. *Gastroenterol Jpn* 1991;26:319–328

Key words: blood supply; hepatocellular carcinoma; microcirculation; scanning electron microscopy; vascular cast

Introduction

Normal liver tissue is supplied by the hepatic artery and the portal vein, whereas malignant tumors of the liver are thought to receive their blood supply principally from the hepatic artery^{1,2}. Based on this premise, transcatheter arterial embolization (TAE) has been performed to treat hepatocellular carcinoma (HCC) and has been found to prolong survival^{3–5}. However, as our experience with TAE has grown, we have discovered that although the center of the tumor readily undergoes necrosis, cancer cells often survive at the tumor margins and in daughter nodules^{6,7}. It has therefore been suggested that hepatic tumors also receive blood via the peribiliary plexus or the portal vein in addition to the hepatic artery^{8,9}. However, the details of the microcirculation of

HCC remain unclear at present.

Accordingly, we made vascular casts of HCCs from autopsy specimens and studied their microvascular architecture three-dimensionally.

Subjects and Methods

The subjects of this study consisted of 10 patients with both HCC and liver cirrhosis, (9 males and 1 female with an average age of 55 ± 13 years, **Table 1**). Macroscopically, 2 tumors were of the nodular type, 4 were of the multinodular type, 3 were of the massive type, and 1 was of the diffuse type. Histologically, 8 of the 10 HCCs were of the trabecular type, 1 was of the pseudoglandular type, and 1 was a mixture of these 2 types (**Table 1**).

A tissue block including the tumor was excised from each liver at autopsy (**Fig. 1**). The block was

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Table 1 Vascular architecture of hepatocellular carcinoma

| Case | Age | Sex | Macroscopic classification | Histological classification | Tumor area | | Tumor surroundings | | Communication with blood sinuses | | Efferent vessels |
|------|-----|-----|----------------------------|-----------------------------|---------------|-------------------------------|----------------------------------|----------------------|----------------------------------|---|------------------|
| | | | | | Blood sinuses | Arterial branch proliferation | Portal vein branch proliferation | Portal vein branches | Sinusoids | | |
| 1 | 57 | M | Nodular type | Trabecular type | | ○ | | | | | |
| 2 | 55 | M | Nodular type | Trabecular type | | ○ | | | | | |
| 3 | 66 | F | Multinodular type | Trabecular type | | ○ | | | | | |
| 4 | 62 | M | Multinodular type | Trabecular type | ○ | ○ | | | | | |
| 5 | 61 | M | Multinodular type | Trabecular type | ○ | ○ | | | | | |
| 6 | 53 | M | Multinodular type | Trabecular type | ○ | ○ | ○ | | | ○ | |
| 7 | 62 | M | Massive type | Trabecular type | ○ | ○ | ○ | | | ○ | |
| 8 | 50 | M | Massive type | Trabecular-pseudoglandular | ○ | ○ | ○ | | | ○ | |
| 9 | 43 | M | Massive type | Pseudoglandular type | | ○ | ○ | | | | |
| 10 | 25 | M | Diffuse type | Trabecular type | ○ | ○ | ○ | ○ | ○ | ○ | ○ |

○; Detected in the vascular casts

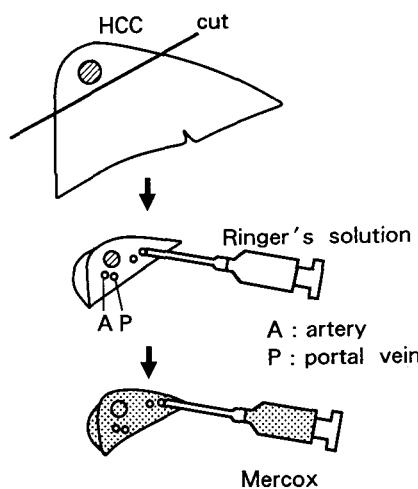


Fig. 1 The method of perfusion of tissue blocks including HCC tumors and injection of Mercox.

perfused with Ringer's solution through catheters inserted into the branches of the hepatic artery and portal vein connecting with the cut surfaces. Methacrylate resin (Mercox, Dainippon Ink & Chemicals Inc., Tokyo) was injected by hand, first into the hepatic artery branches and then the portal vein branches until the resin filled the vessels in the block. Each block was then immersed in water at 60°C for 2 hours to polymerize the resin^{10,11}. Tissue blocks were next immersed in 20% sodium hydroxide at 60°C for 24 hours, and

then washed in running water. The vascular cast thus obtained was placed in a synthetic detergent solution (Mama Lemon, Lion Corporation Tokyo, Japan) overnight at 60°C, then washed and dried at room temperature.

The vascular casts were examined and microdissected under a stereo-microscope. Casts were cut into sections of less than 10 mm to conform to the size of the specimen holder. After microdissection, sections were stained with 2% osmium tetroxide, mounted on a specimen holder, coated with gold or gold-palladium and examined under a scanning electron microscope (JSM U-3, JEOL) at an accelerating voltage of 5 kV. From 1 to 20 cancer nodules were examined per patient.

For histological examination, a block of the autopsy specimen was fixed in 10% phosphate-buffered formalin. Moreover, the tumor tissue injected with Mercox was sectioned before corrosion of the tissue in order to examine one side histologically and the other for microvascular study by SEM.

Results

SEM demonstrated connections between HCC blood sinuses and vessels surrounding the tumor in vascular casts from 6 patients, while in the remaining 4 it was difficult to trace the connections

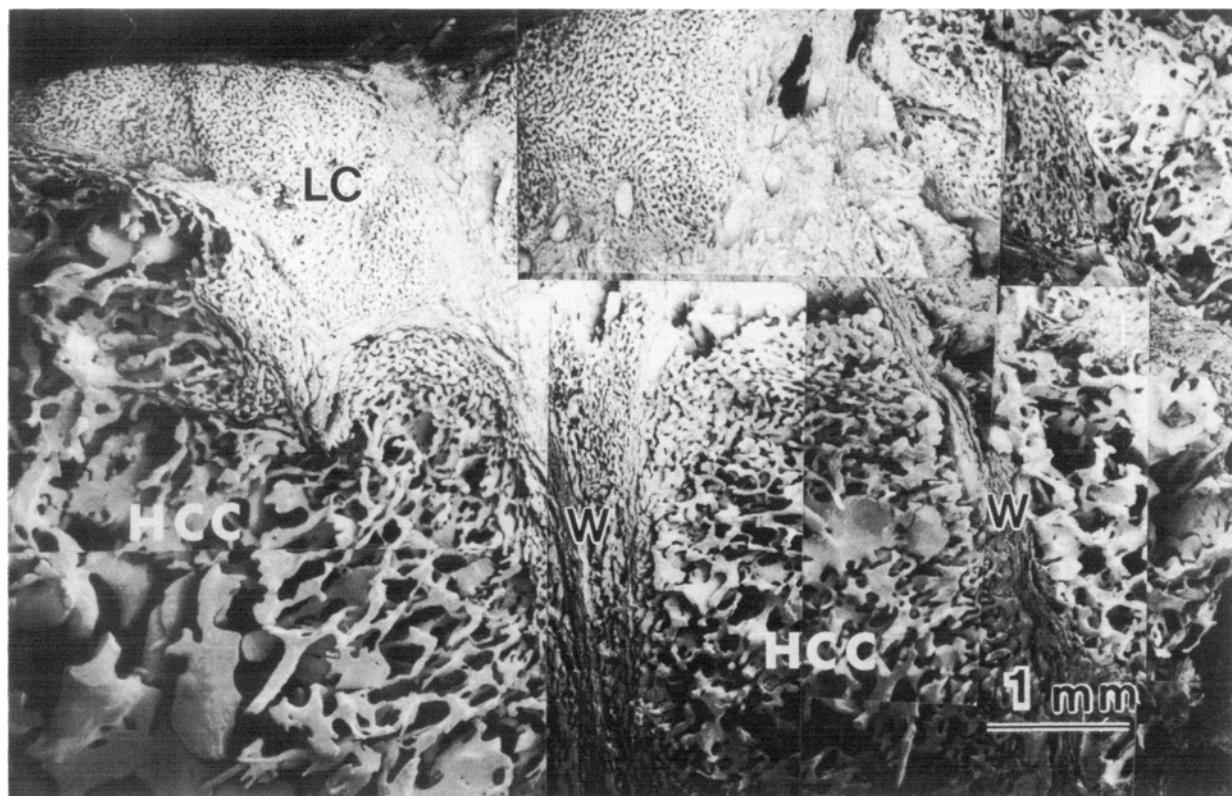


Fig. 2 Scanning electron micrograph of the cut surface of a vascular cast from a hepatocellular carcinoma (Case 5). In the cirrhotic area (LC), liver parenchyma exhibits evenly distributed sinusoids approximately 20 μm in diameter, whereas in the carcinomatous area (HCC, trabecular type) the blood sinuses are flatter, larger, and more sparsely and irregularly distributed. The blood sinuses become progressively larger as they get closer to the center of the tumor. There are no arteries penetrating directly into the tumor. The tumor nest is bordered by septa (W).

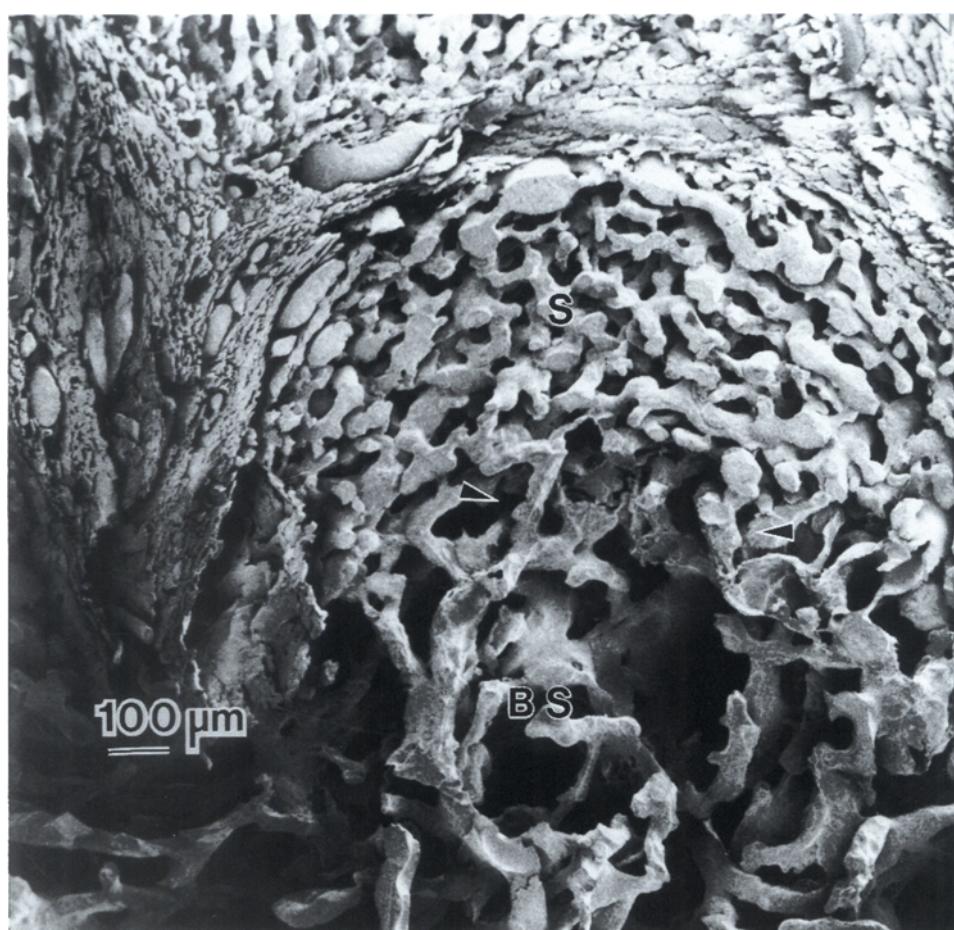
because resin had leaked around the tumor or because the tumor was necrotic (Table 1). In contrast, sinusoids in cirrhotic nodules were well demonstrated by the casting process.

The HCC blood sinuses were flat and sparsely distributed, while sinusoids in nodules of cirrhosis were approximately 20 μm in diameter and evenly distributed, which made it easy to separate them from the tumor regions (Fig. 2). Sinusoids of cirrhosis nodules communicated directly with HCC blood sinuses in 2 cases, in which the tumors were of the trabecular type and showed a replacing growth pattern^{12,13} (Figs. 3 and 4).

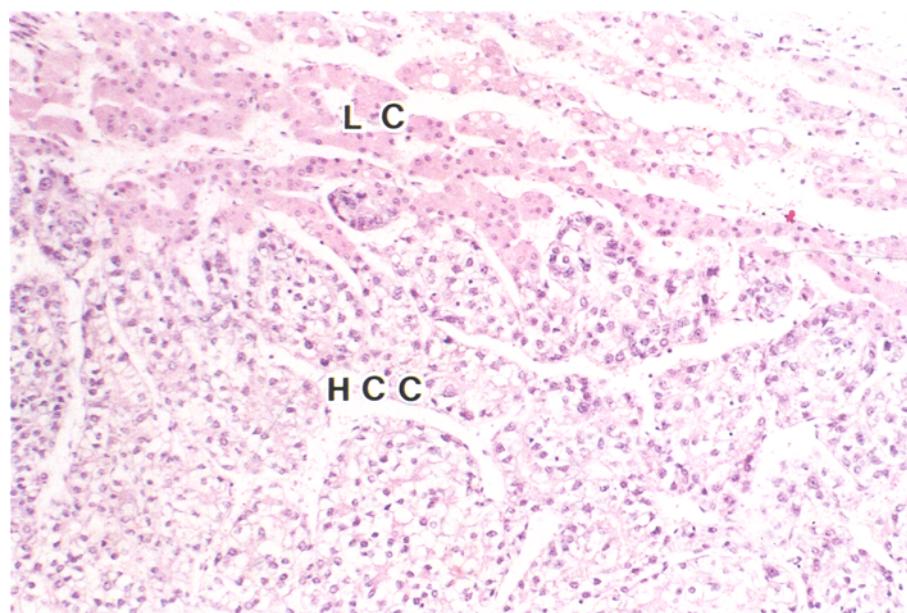
Vascular plexuses formed as the result of vessel proliferation, were observed surrounding the HCC nodules in all 10 patients (Table 1). The proliferating vessels varied in diameter and were

derived mostly from the hepatic artery and less often from the portan vein (Fig. 5). Sometimes it was difficult to determine the origin. The arterial branches communicated directly with the tumor nodules in all patients (Fig. 6). Portal vein branches also formed vascular plexuses at the tumor margins in 5 cases (Fig. 7). These proliferating portal vein branches were observed to connect directly with HCC blood sinuses in 3 cases (Fig. 7). Such portal vein branches varied in diameter and frequently anastomosed with each other (Fig. 8).

Efferent vessels of the HCCs were difficult to identify except in Case 9, where the tumor was of pseudoglandular type with encapsulated growth (Fig. 9). In this case, efferent vessels, not connected with hepatic arteries nor portal veins, were

**Fig. 3**

Higher magnification of the area left of center in Figure 2. There is direct continuity (arrowhead) between the liver cirrhosis sinusoids (S) in the upper half and the tumor blood sinuses (BS). (Cf. Fig. 4).

**Fig. 4**

Histological picture of the same case as in Figure 3 (HE stain, $\times 35$). The lower half shows thick trabecular type hepatocellular carcinoma (HCC), which grows compressing the cirrhotic liver cell cords (LC) in the upper half, and exhibits replacement-type proliferation at the frontier.

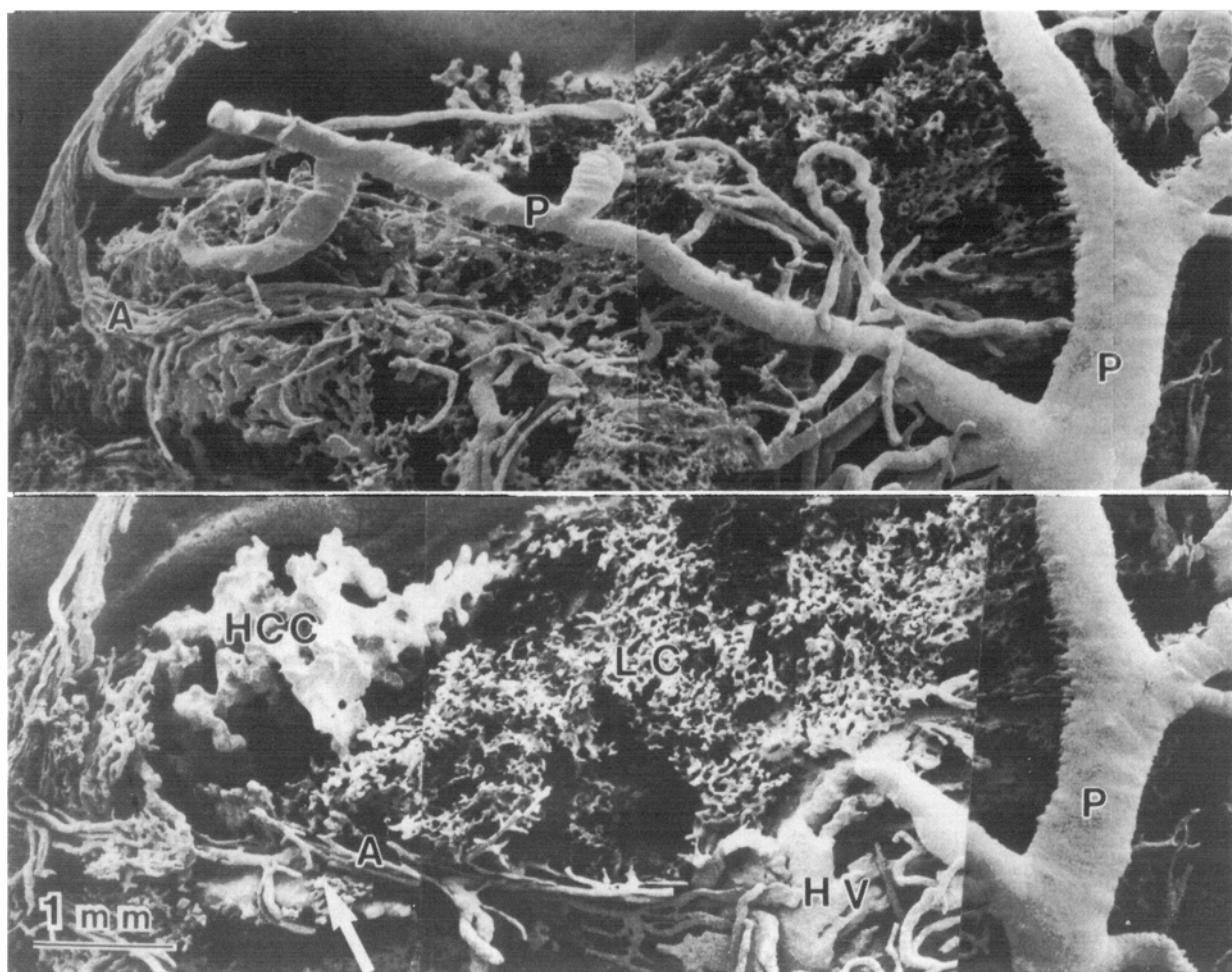


Fig. 5 Vascular cast of the liver (Case 6). The lower picture shows the interior of the upper specimen after microdissection. Portal vein (P) branches from 100 to 200 μm vessels around the cancer nodule (HCC) creating a vascular network. An arterial network (A) also surrounds the tumor nodule. LC: cirrhotic area; HV: hepatic vein.

detected after repeated microdissection of the blood sinuses (**Figs. 10 and 11**). These vessels were 100–200 μm in diameter, formed a network that was closer to the tumor nodule than the afferent vessels, and communicated with the tumor blood sinuses (**Fig. 11**).

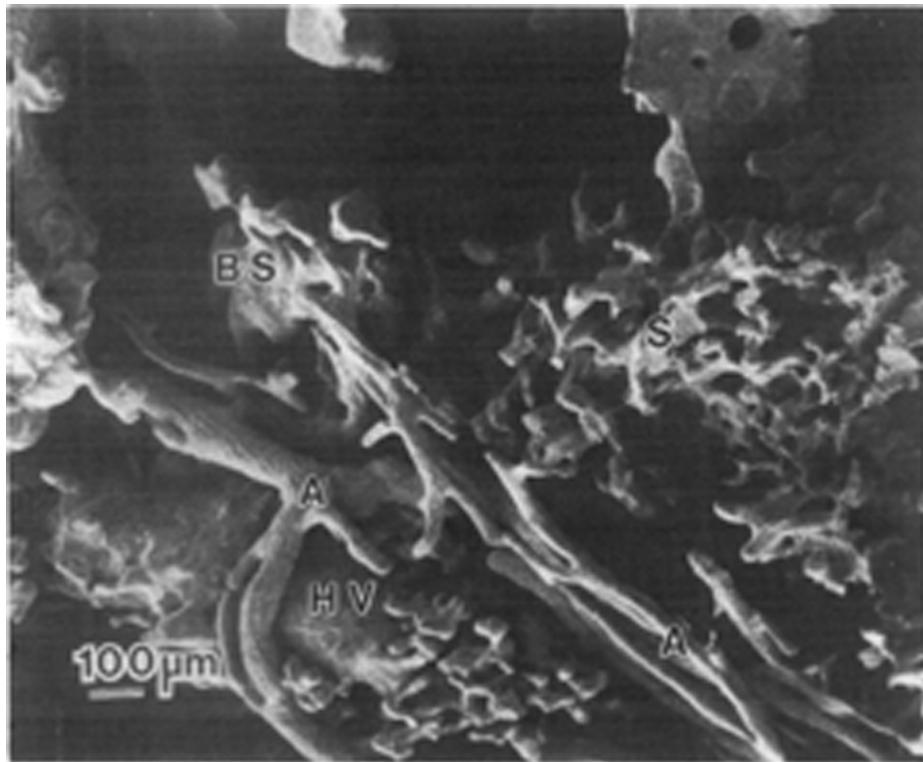
Discussion

The vascular architecture of liver cancer has been studied mainly by the use of various vascular injection methods. However, specimens obtained using India ink injection^{1,14}, colored gelatin injection^{1,2} or microangiography¹⁵ can only be ob-

served two-dimensionally. In contrast, SEM observation of vascular casts from HCCs is able to demonstrate the three-dimensional interrelationships of the microvascular plexus¹⁰.

It has been believed that liver tumors, whether primary or metastatic², chemically-induced¹⁶ or transplanted¹⁷ were principally supplied by arteries on the basis of animal experiments. However, it was demonstrated that portal vein branches as well as arterial branches supplied the HCCs in the patients we investigated.

Portal vein branches are known to be distorted and flattened in liver cirrhosis^{18,19}; however, they are not generally believed to proliferate to form a

**Fig. 6**

Enlargement of the area marked with a white arrow in the lower part of Figure 5. The arterial branch (A) communicates directly with the tumor blood sinus (BS) as well as with the cirrhotic sinusoid (S). HV: hepatic vein.

vascular plexus. In 5 out of 10 of our patients, we observed portal vein branch proliferation forming a pluxus around the tumors, and in 3 cases we found direct connections between these portal vein branches and the HCC blood sinuses. In these 3 cases, the portal vein branches anastomosed with each other around the tumors and formed a vascular plexus like that of the arterial branches. Sakamoto⁸ using the colored gelatin method has reported, that the portal vessels seen at the margins of small HCCs were remnant portal vein branches of non-cancerous tissue and acted as efferent tumor vessels. Wright¹ demonstrated connections between tumor blood sinuses and portal vein branches in metastatic liver cancer by the India ink injection method, and he also postulated that these portal vein branches were efferent vessels. Even today, there are authors with a similar interpretation of the role of the portal vein branches around HCCs^{8,15}. In contrast, Breedis and young² and Honjo and Suzuki²⁰ demonstrated that portal vein branches partly supplied

the tumor in their investigations of HCC, while Lin et al²¹ reached the same conclusion using the silicon injection method in metastatic cancer. We confirmed their findings three-dimensionally in this study of small HCC nodules.

Connections between tumor blood sinuses and hepatic sinusoids have been demonstrated by Sugihara et al²² in HCC with a replacing growth pattern^{12,13} by transmission electron microscopy. The authors used SEM to demonstrate similar connections three-dimensionally. At the borders of HCCs which show replacement growth pattern and continually change to hepatic parenchyma, when the direct arterial blood supply to the tumor is interrupted by TAE the borders may still be nourished by blood from the sinusoids.

The present study supports the concept that direct blood supply to HCCs from portal vessels or sinusoids is the mechanism which spares liver tumor margins from necrosis when TAE is performed.

As efferent vascular network system belong-

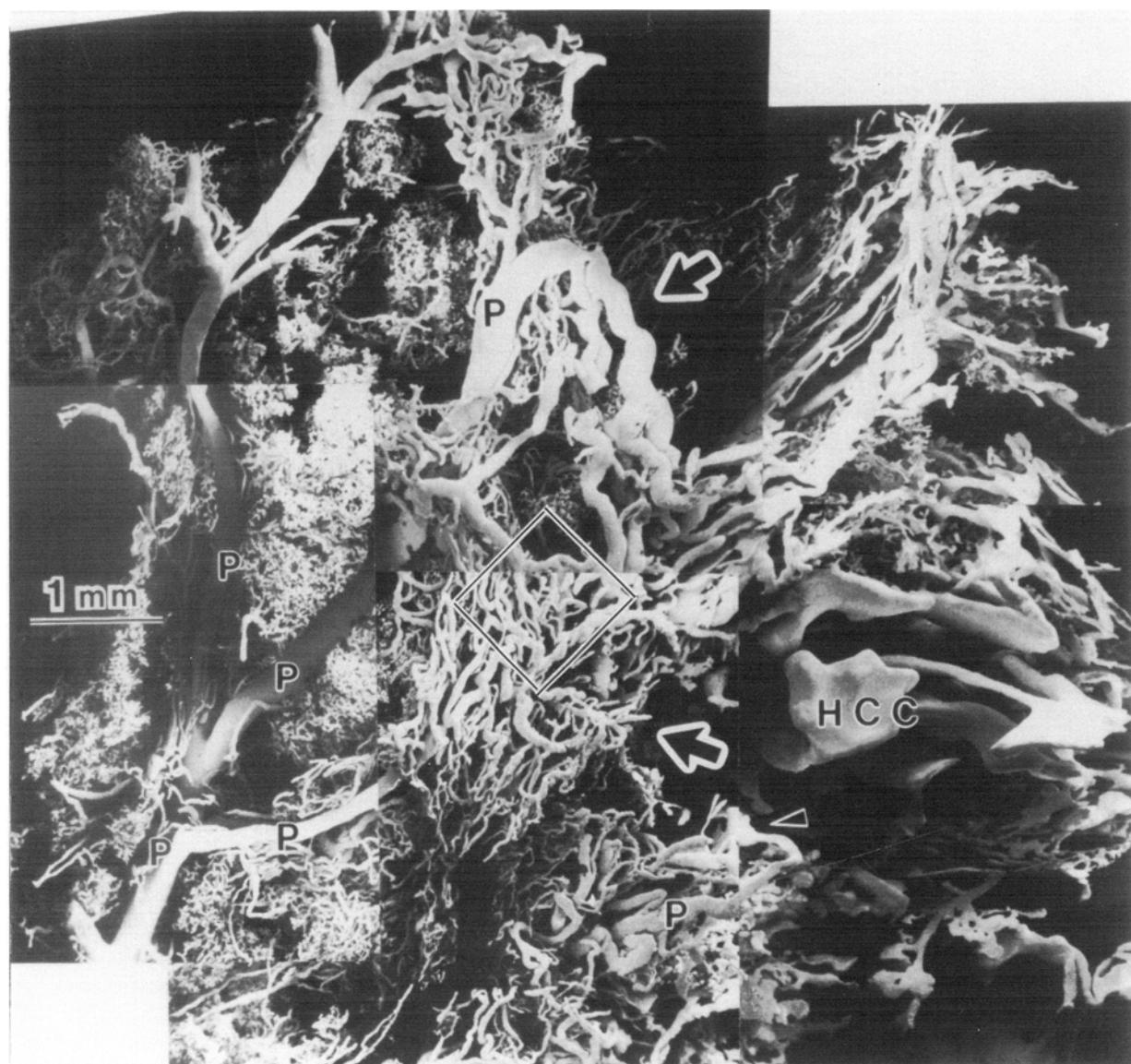
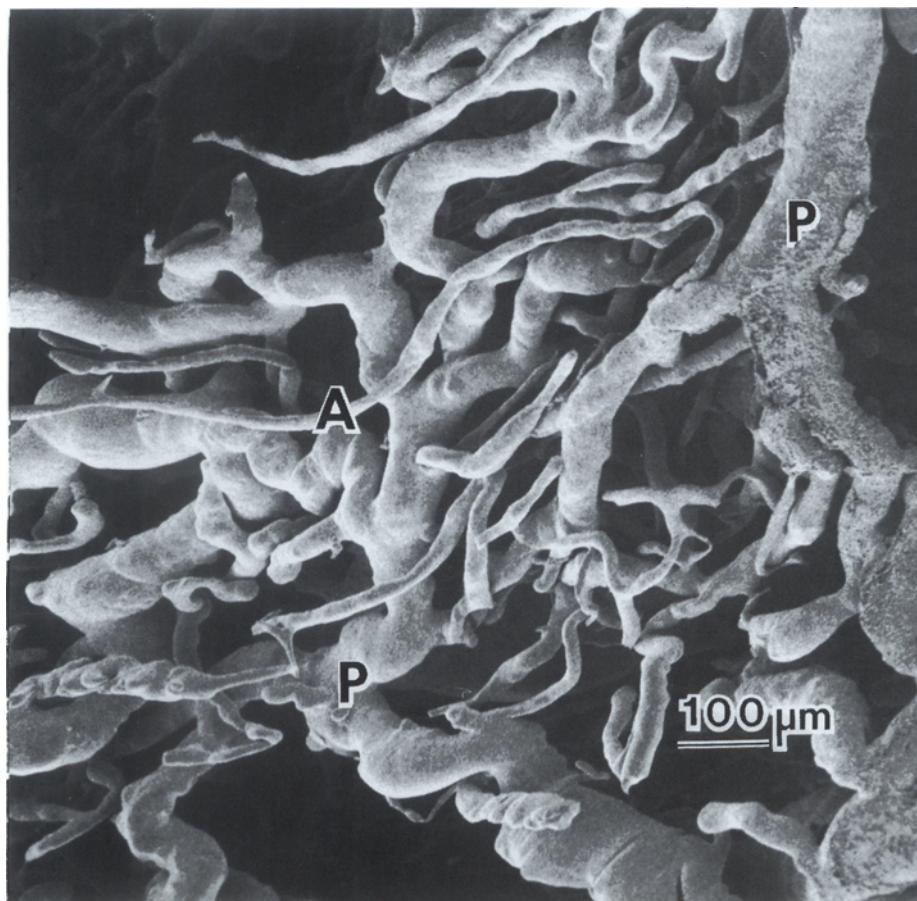


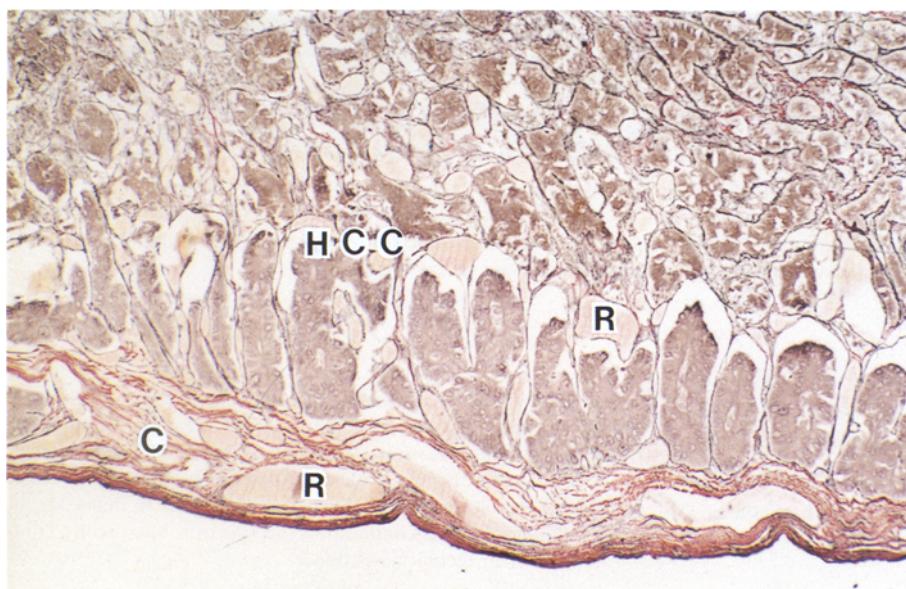
Fig. 7 Portal vein network surrounding a nodule of hepatocellular carcinoma (Case 7). The tumor (HCC) is on the lower right, and the cirrhotic region occupies the left half. Three branches of the portal vein (P) are seen in the lower left-hand region, which communicate with each other intimately at the tumor margins forming a vascular network (arrows). A $100 \mu\text{m}$ the portal vein branch enters the cancer nodule and communicates directly with a tumor blood sinus (arrowhead).

ing to the tumor was detected in addition to the afferent vessels in case 9. These efferent vessels formed a vascular network inside the network of afferent vessels and drained blood from the HCC sinuses. They resembled the efferent vessels of regenerating nodules of liver cirrhosis¹⁹.

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**Fig. 8**

Enlargement of the area within the frame in Figure 7. The proliferating portal vein branches (P) are irregular in diameter, and form a network of anastomoses with each other. A: arterial branch.

**Fig. 9**

Histological appearance of the complementary specimen to that shown in Figures 10 and 11 (Case 9, silver stain, $\times 100$). A thin fibrous capsule (C) is formed around a pseudo-glandular type hepatocellular carcinoma (HCC). The capsule contains many vessels showing the injected resin (R).

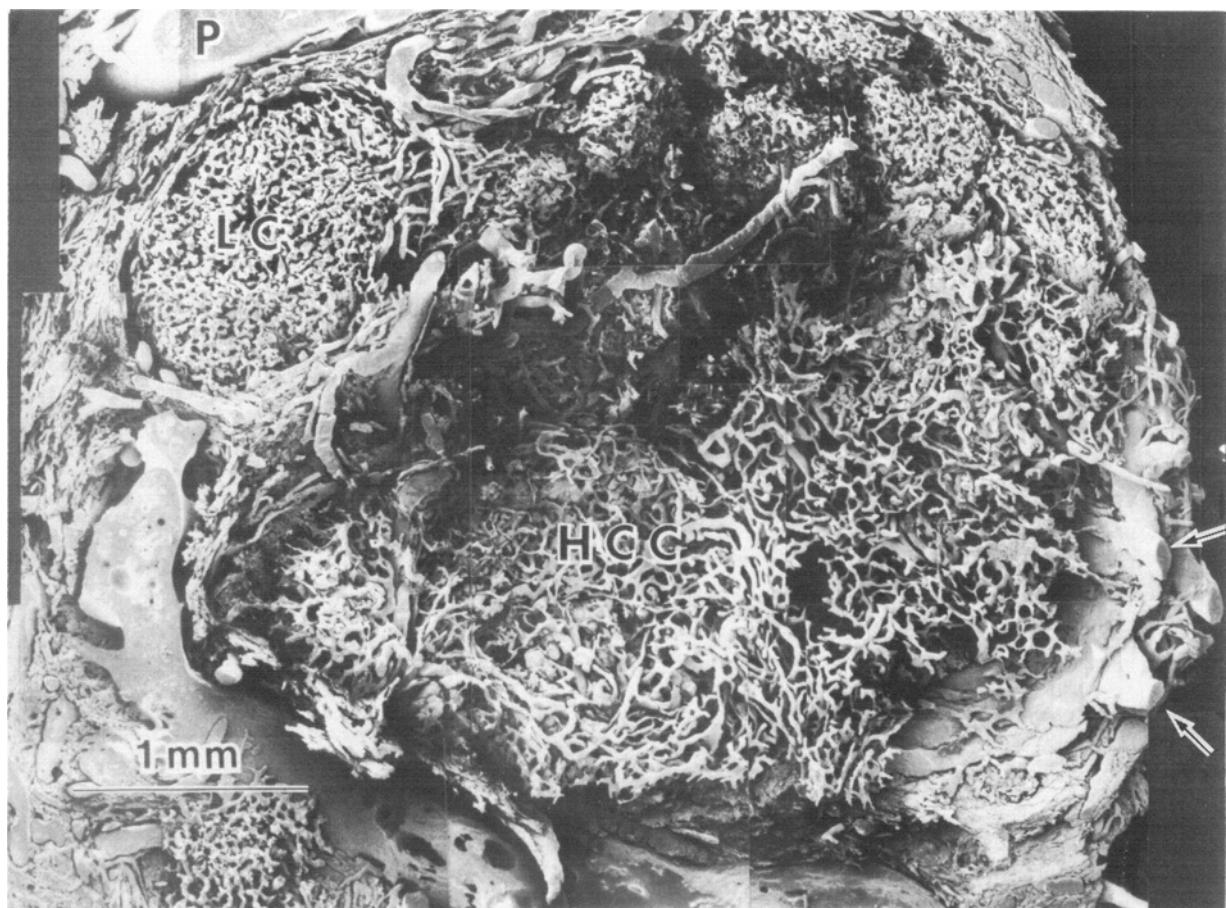


Fig. 10

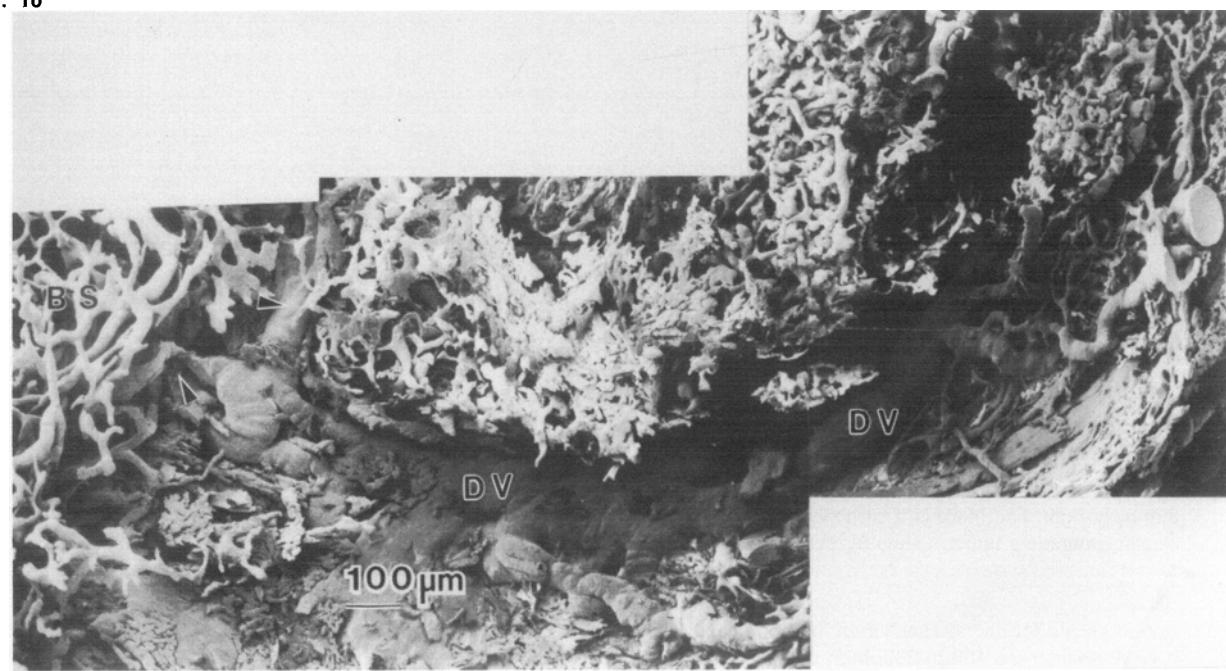


Fig. 11

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Fig. 10 Vascular cast of the hepatocellular carcinoma specimen corresponding to Figure 9. A 4-mm tumor nodule (HCC) is seen on the lower right, and there is a cirrhotic area (LC) and portal vein branch (P) in the upper left corner. Several flattened vessels cluster around the tumor nodule (arrows, lower right).

Fig. 11 Appearance after microdissection of the border region of the tumor shown in Figure 10. The blood vessels (DV) exposed after microdissection are 100 to 200 μ m in diameter and are in a flaccid state. They anastomose with each other to form a network which communicates with a tumor blood sinus (BS) (arrowheads), and are believed to be efferent vessels.