tional studies on rat liver fat-storing cell line and freshly isolated hepatocyte coculture system. Am J Pathol 1995;146:1508–1520.
[19] Loreal O, Levavasseur F, Fromaget C, Gros D, Guillouzo A, Clement B. Cooperation of Ito cells and hepatocytes in the deposition of an extracellular matrix in vitro. Am J Pathol 1993;143:538–544.
[20] Kristensen DB, Kawada N, Imamura K. Proteome analysis of rat hepatic stellate cells. Hepatology 2000;32:268–277.
[21] Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976;13:29–83.
[22] Gressner AM. Proliferation and transformation of cultured liver fat storing cells (perisinusoidal lipocytes) under conditions of beta-D-xyloside induced abrogation of proteoglycan synthesis. Exp Mol Pathol 1991;55:143–169.
[23] Yamada A, Matsumoto K, Iwanari H, Sekiguchi K, Kawata S, Matsuzawa Y. Rapid and sensitive enzyme-linked immunosorbent assay for measurement of HGF in rat and human tissues. Biomed Res 1995;16:105–114.
[24] Harlow E, Lane D. Immunoassays. Harlow E, Lane D, editors. Antibodies: a laboratory manual, New York, NY: Cold Spring Harbor Laboratory Press, 1983. pp. 555–599.
[25] Davidoson JS, Baumgarten IM. Glycyrrhetinic acid derivatives: a novel class of inhibitors of gap junctional intercellular communication. Structure–activity relationships. J Pharmacol Exp Ther 1988; 245:1104–1107.
[26] Nakamura T, Teramoto H, Ichihara A. Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. Proc Natl Acad Sci USA 1986;83: 6489–6493.
[27] Cioce V, Csaky KG, Chan AM, Bottro DP, Taylor WG, Jensen R, Aaronson SA, Rubin JS. Hepatocyte growth factor (HGF)/NK1 is a naturally occurring HGF/scatter factor variant with partial agonist/antagonist. J Biol Chem 1996;271:13110–13115.
[28] Bos M, Mendelsohn J, Kim YM, Albanell J, Fry DW, Baselga J. PD153035, a tyrosine kinase inhibitor, prevents epidermal growth factor receptor activation and inhibits growth of cancer cells in a receptor number-dependent manner. Clin Cancer Res 1997;3:2099–2106.
[29] Levitzki A, Gazit A. Tyrosine kinase inhibition: an approach to drug development. Science 1995;267:1782–1788.
[30] Sakaguchi K, Yanagishita M, Takeuchi Y, Aurbach GD. Identification of heparan sulfate proteoglycan as a high affinity receptor for acidic fibroblast growth factor in a parathyroid cell line. J Biol Chem 1991;266:7270–7278.
[31] Humphries DE, Silbert JE. Chlorate: a reversible inhibitor of proteoglycan sulfation. Biochem Biophys Res Commun 1988;154:365–371.
[32] Naka D, Ishii T, Shimomura T, Hishida T, Hara H. Heparin modulates the receptor-binding and mitogenic activity of hepatocyte growth factor on hepatocytes. Exp Cell Res 1993;209:317–324.
[33] Gallai M, Kovalszky I, Knittel T, Neubauer K, Armbrust T, Ramadori G. Expression of extracellular matrix proteoglycans perlecan and decorin in carbon-tetrachloride-injured rat liver and in isolated liver cells. Am J Pathol 1996;148:1463–1471.
[34] Sporn MB, Roberts AB. Role of retinoids in differentiation and carcinogenesis. Cancer Res 1983;43:3034–3040.

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Case report

Tumor thrombi in the portal vein system originating from gastrointestinal tract cancer

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Intraluminal tumor thrombus in the portal vein (PV) system originating from gastrointestinal (GI) tract cancer is a rare condition. There are two types of such thrombi, one arising indirectly from metastatic liver cancer and the other directly from the primary lesion. We report here three patients with the direct type and two with the indirect type; i.e., a total of five patients with gastric or large intestinal cancer with PV tumor thrombus. In all patients, the primary lesion was surgically resected; in two patients, the tumor thrombus was easily extirpated by direct opening of the PV. It is noteworthy that a patient whose tumor thrombus could not be treated died of cancer with liver failure, caused by expansive growth of the PV tumor thrombus, 4 months after the finding of the PV thrombus. Because PV tumor thrombus may, possibly, determine the patient's length of survival, in addition to causing cancer progression, surgical thrombectomy, combined with resection of the primary cancer and metastatic liver cancer, should be considered for prolongation of survival, if all macroscopic lesions can be controlled and if the tumor thrombus is a synchronous and recent one.

Key words: tumor thrombus, portal vein, colon cancer, gastric cancer, liver metastasis

Introduction

Portal thrombosis, produced by coagulation and portal vein involvement by neoplasms, has been reported as a cause of portal obstruction. Portal vein (PV) thrombosis has recently been detected by dynamic computed tomography (CT) in patients with liver cirrhosis; pancreatitis; appendicitis; gastrointestinal inflammatory diseases, such as ulcerative colitis; septicemia; thrombocytemia; and dehydration; and in patients after liver transplantation, splenectomy, estrogen therapy, and intra-arterial chemo-infusion therapy.[1–6] Portal vein involvement by neoplasms has been classified as either extrinsic portal vein occlusion or intraluminal tumor thrombus. The former is usually caused by pancreatic cancer, gallbladder cancer, and enlarged metastatic lymph nodes, while the latter is usually caused by hepatocellular carcinoma (HCC). Apart from being associated with HCC, intraluminal PV tumor thrombus has also been reported to be associated with renal cell carcinoma, cloacogenic carcinoma, and alpha-feto protein (AFP)-producing gastric cancer; namely, hepatoid adenocarcinoma with hepatic differentiation.[7–10]

Treatment of advanced HCC with tumor thrombus in the PV by hepatic resection with surgical thrombectomy or transcatheter arterial embolization (TAE) have been reported,[11,12] because PV tumor thrombus is fatal and the incidence is relatively high. By contrast, there are few reports as to treatment and clinical out come of GI tract cancer with tumor thrombus in the PV system. Furthermore, the few reports were not systemic ones but case reports. We report here five patients with gastric or colon cancer with tumor thrombus in the PV system, in all of whom the primary lesions were surgically resected and in two of whom tumor thrombi were surgically extirpated.

Case reports

Case 1

An 83-year-old woman with asymptomatic anemia was admitted for surgical treatment of Borrmann type 2

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gastric cancer at the lesser curvature of the cardia. Because the preoperative CT scan suggested a PV shunt located at the border of subsegments 4 and 5, angiography was performed. However, no abnormality was found in the PV system. Total gastrectomy was performed, with cholecystectomy, and dissection of group 1 and 2 regional lymph nodes. Pathological examination showed that the gastric cancer was moderately differentiated tubular adenocarcinoma (tub2), reaching the subserosal layer (ss), with no venous involvement (v0) or lymph node metastasis (n0), but with lymphatic duct involvement (ly1). The comprehensive stage was classified as stage Ib, and the surgical treatment was classified as a curative resection according to the general guidelines for gastric cancer proposed by the Gastric Cancer Study Group of Japan.[13]

Four months after the operation, she was admitted again because of anorexia and general malaise. CT scan showed obstruction of the PV, as shown in Fig. 1a. No metastatic liver cancer was found. Coagulopathy was ruled out by the findings for platelet count, prothrombin time, antithrombin III, and fibrinogen. Anti-cardiolipin antibody was negative. Estrogen therapy had not been employed. Emergent angiography revealed complete obstruction of the PV at the confluence of the superior mesenteric vein (SMV) and the splenic vein (SPV), caused by thrombi, as shown in Fig. 1b. Splenic venous blood did not flow into the PV, but drained through the inferior mesenteric vein. Although we tried to dissolve the thrombi by the infusion of urokinase through a catheter placed in the superior mesenteric artery (SMA), the thrombolysis failed. AFP was negative.

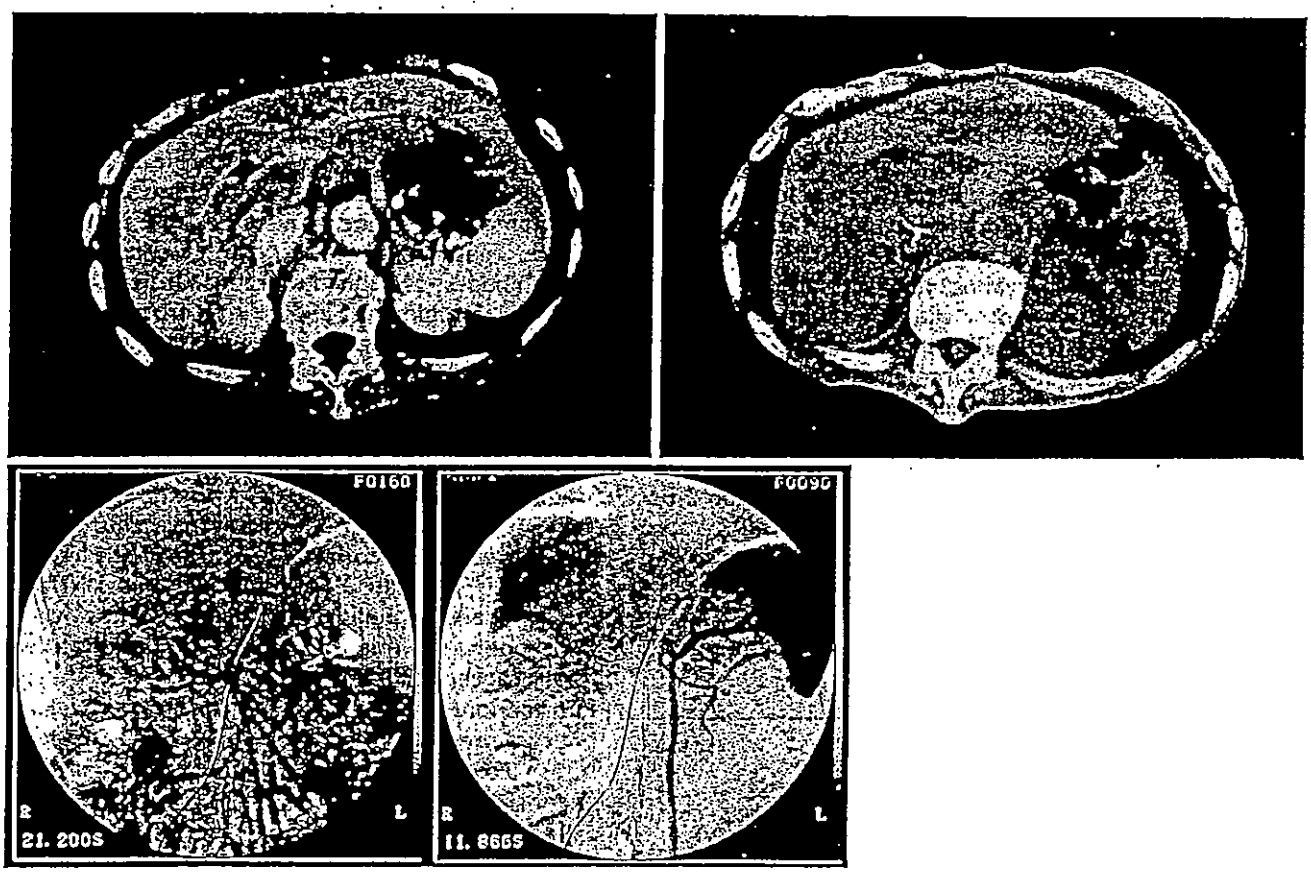

Fig. 1a–c. Case 1. a Computed tomography (CT) scan 4 months after total gastrectomy. The portal vein was completely obstructed by tumor thrombi, while the hepatic artery was stained well, indicating arterialized liver. Liver perfusion was inhomogeneous. b Abdominal angiography 4 months after total gastrectomy. *Left*; venous phase of superior mesenteric angiography showed that portal venous flow to the liver was disturbed at the confluence of the superior mesenteric vein and splenic vein. The liver portal vein tree was not visualized. *Right*; venous phase of celiac angiography showed that splenic venous blood drained through the inferior mesenteric vein because of the obstruction at the confluence. c Plain CT scan 21 days before death. Tumor thrombi occupied the portal vein system in the entire liver, which had a coral-like appearance. Massive ascites was found. Contrast media could not be used because of the patient's poor general condition and renal dysfunction

Ascites appeared thereafter. A subsequent CT scan showed that the PV thrombus had developed and was expansive in both diameter and length. The thrombus completely occupied the PV system in the entire liver, which had a coral-like appearance, as shown in Fig. 1c. Therefore, we clinically diagnosed the thrombus as tumor thrombus originating from gastric cancer. No adjuvant chemotherapy was employed, because of the patient's advanced age. She died of cancer and liver failure 4 months after the first finding of PV obstruction. Informed consent for pathological autopsy could not be obtained.

Case 2

A 69-year-old woman with heartburn, malaise, a palpable abdominal mass below the navel, and severe anemia was admitted for surgical treatment of transverse colon cancer with direct invasion to the stomach. Chest CT scan showed left lung metastasis. Abdominal CT scan showed a tumor with a lumen that was approximately 10 cm in size (which was compatible with transverse colon cancer with serosal invasion to the greater curvature of the antrum), and intraluminal tumor thrombi in the SMV, as shown in Fig. 2a. No definite liver metastasis was found. Angiography showed obstruction of the middle colic artery and SMV distal to the confluence of SMV and SPV by the tumor, as shown in Fig. 2b. However, PV flow to the liver was maintained through the SPV. En-bloc transverse colectomy and distal partial gastrectomy with gastroduodenostomy were performed. During ligation and dissection of the middle colic vein, tumor thrombi (confirmed by pathological examination) were shown at the cut ends of the small branches of this vessel. The root apparently linked to the trunk of the SMV. Pathological examination showed that a Borrmann type 3 colon cancer, 8 × 8 × 6.5 cm in size, was poorly differentiated adenocarci-

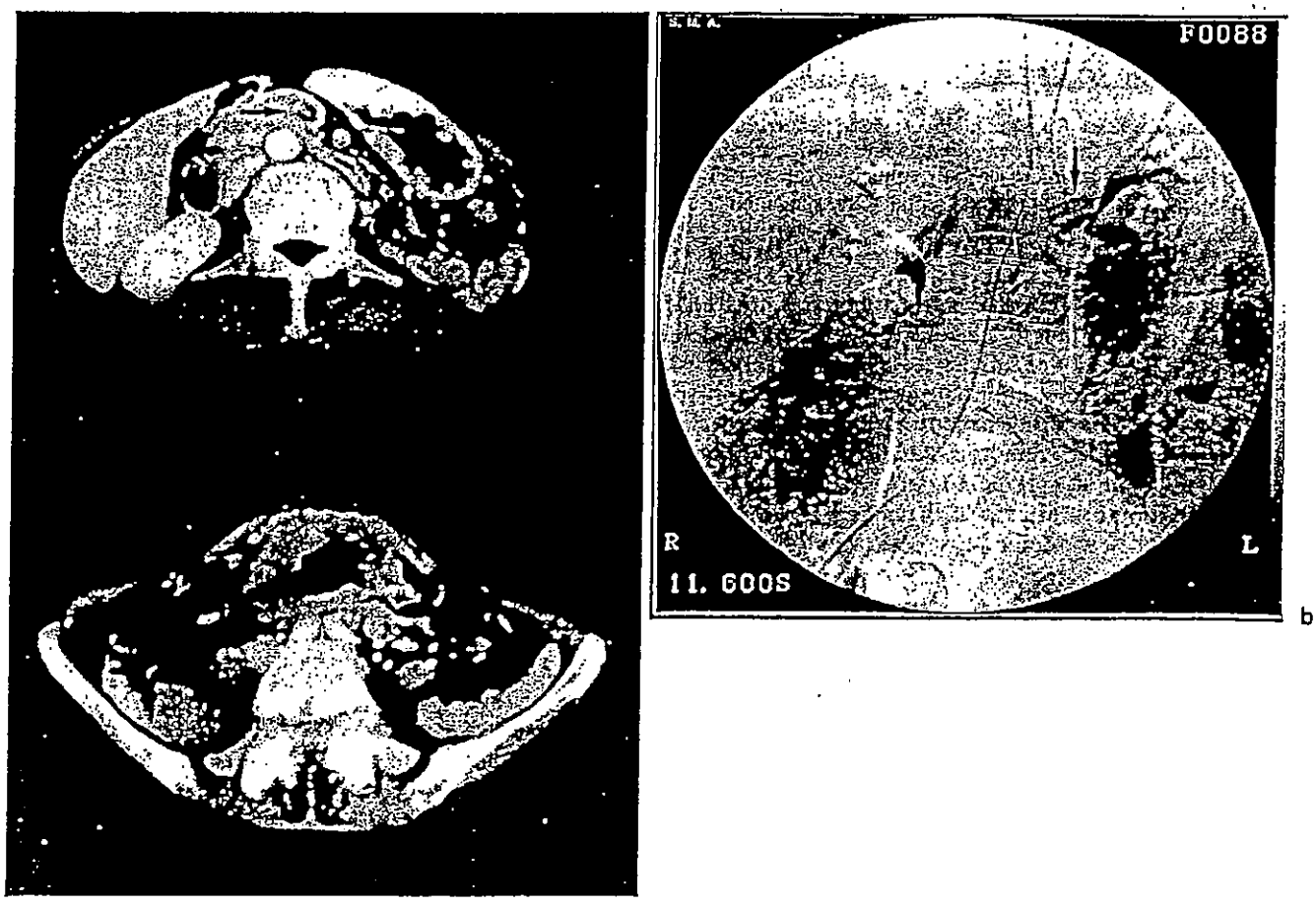

Fig. 2a,b. Case 2. a CT scans. *Upper*; the superior mesenteric vein running below the pancreas was occluded by tumor thrombi. *Lower*; large tumor with lumen indicates transverse colon cancer with direct invasion to the stomach. b Abdominal angiography. Late phase of celiac angiography showed that the portal venous flow to the liver was maintained by splenic venous flow. The portal vein tree in the liver was visualized well. *Arrow* indicates the splenic vein

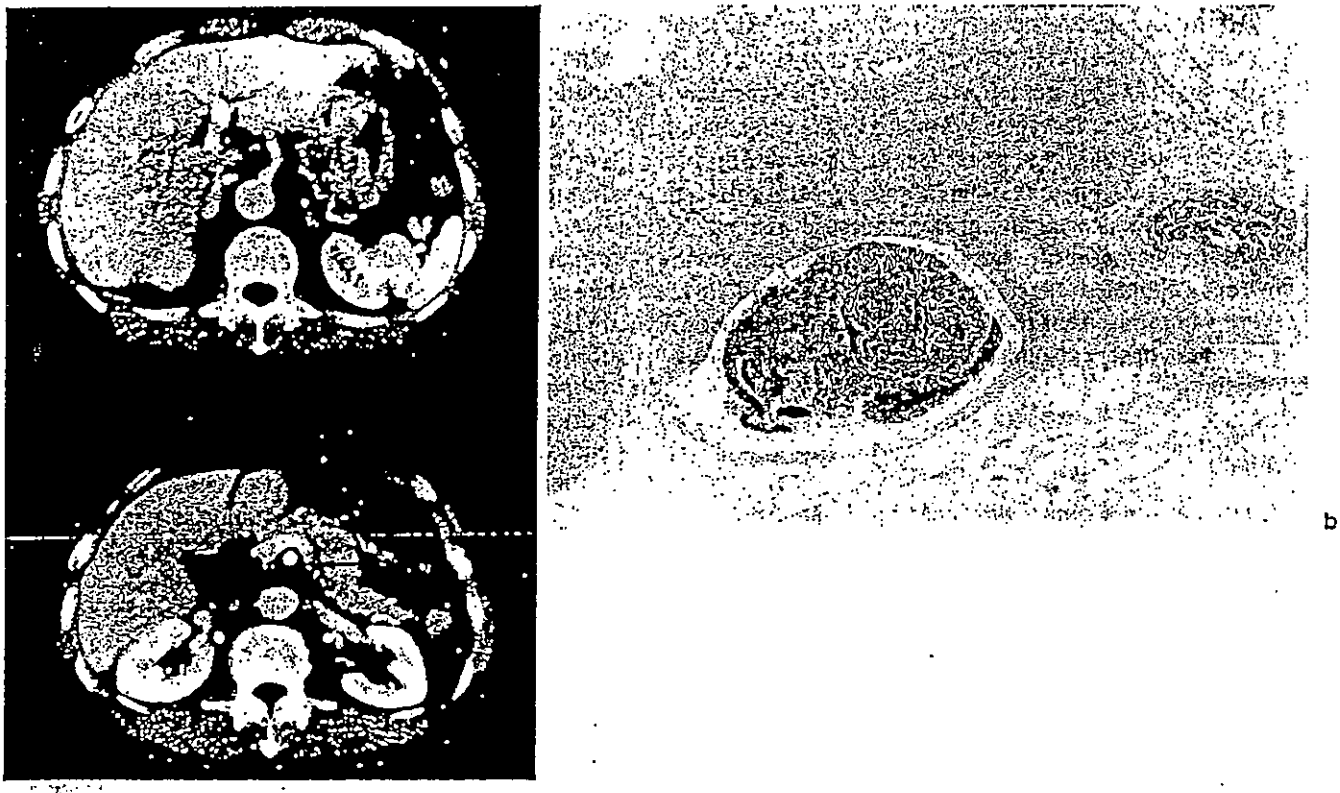

Fig. 3a,b. Case 3. **a** CT scans. *Upper*, gastric cancer, indicated by the thick gastric wall, invaded the pancreas body. *Lower*, the distal portion of the splenic vein was not stained, because of tumor thrombi. *Arrow* indicates the tumor thrombi in the splenic vein. **b** Pathological findings of the splenic vein tumor thrombi. The tumor thrombi were packed in the splenic vein. The section was obtained from the pancreatosplenectomy specimen

noma with direct invasion to the stomach (si), with ly2, v1, and n1, according to the general guidelines for colon cancer proposed by the Japanese Society for Cancer of the Colon and Rectum.[14] The patient's postoperative course was uneventful, except for obstruction of the duodenum, which required additional gastrojejunostomy. She did not agree to adjuvant chemotherapy. Thirteen months after the first operation, she died of peritonitis carcinomatosa with lung and liver metastases.

Case 3

A 73-year-old man with anemia was admitted for surgical treatment of residual gastric cancer with liver metastasis. He had received partial gastrectomy and retrocolic gastrojejunostomy for gastric polyps. Abdominal CT scan showed a thickened gastric wall that directly invaded the pancreas body, and tumor thrombi in the SPV, as shown in Fig. 3a. Total resection of the residual stomach, and combined resection of the pancreas body and tail, and the spleen, were performed. When the pancreas body was dissected in front of the SMA, tumor thrombi (confirmed by pathological examination) were extirpated from the SPV. Liver metastasis in subsegment 6 was treated by microwave coagulation therapy. Pathological examination showed that Borrmann type 3 gastric cancer at the lesser curvature of the cardia was well differentiated tubular adenocarcinoma (tub1) with serosal invasion (si), with lymph node metastasis along the splenic artery (n2), ly2, and v2. Figure 3b shows the tumor thrombi in the splenic vein. Repeated TAE with epirubicin was performed for the liver metastasis, and the patient continues to visit our outpatient clinic, 18 months after the operation.

Case 4

A 59-year-old man was admitted for surgical treatment of sigmoid colon cancer with liver metastasis. Abdominal CT scan showed two liver metastases, located in subsegment 8 and in the left lobe, with the tumor in the left lobe associated with left PV tumor thrombi, as shown in Fig. 4a. Abdominal angiography showed that the tip of the tumor thrombus reached the bifurcation through the left PV, as shown in Fig. 4b. Sigmoidectomy, with dissection of regional lymph nodes, left lobectomy, partial resection of subsegment 8, and extir-

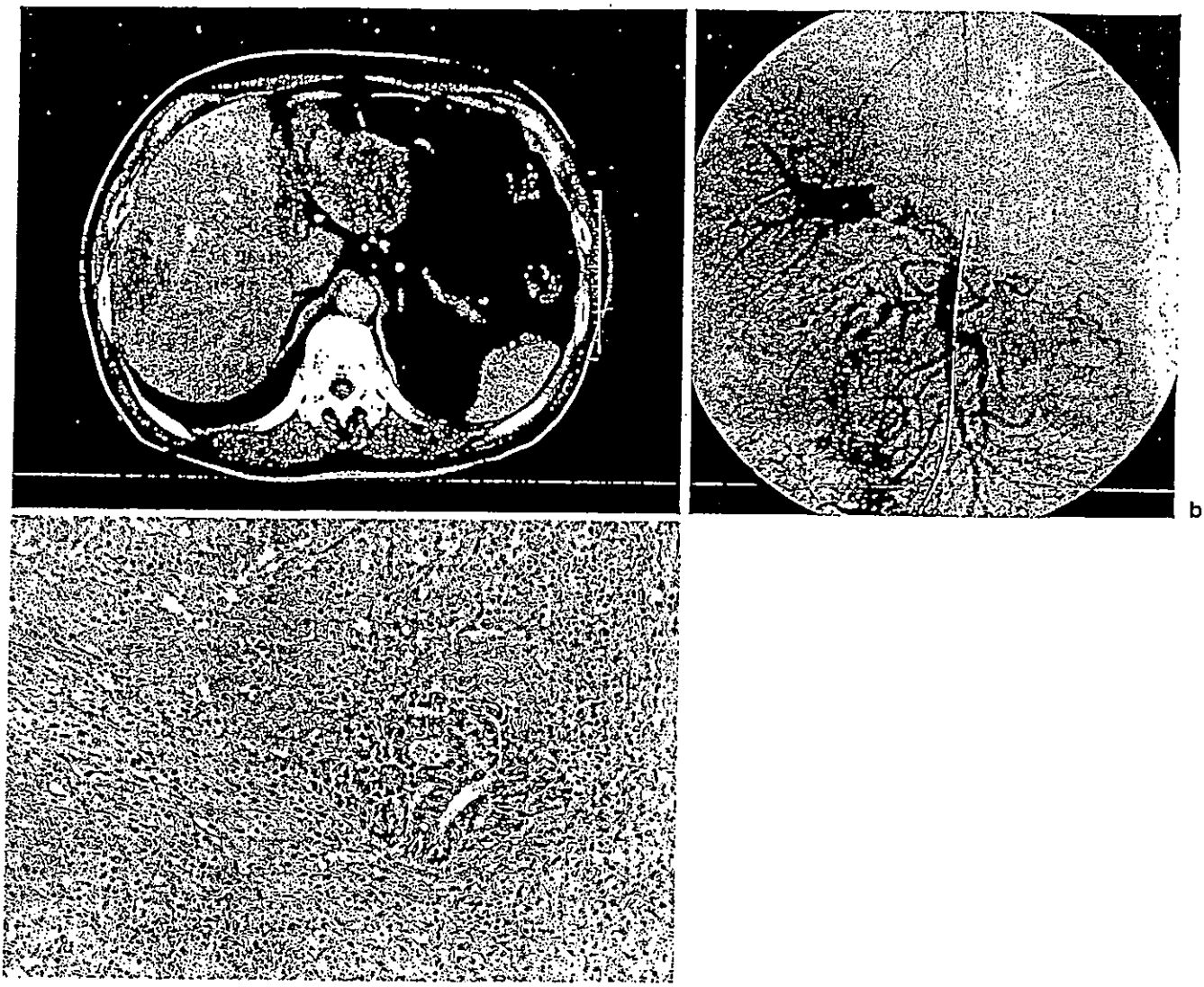

Fig. 4a–c. Case 4. a CT scan. Two metastatic liver tumors were located in subsegment 8 and in the left lobe. The tumor in the left lobe was associated with left portal vein tumor thrombi. The left lobe was atrophic because of left portal vein obstruction. *Arrow* indicates that the tip of the tumor thrombus reached the portal vein trunk. b Abdominal angiography. Late phase of superior mesenteric angiography indicates that the tip of the left portal vein tumor thrombus reached the bifurcation. c Pathological findings of the extirpated portal vein tumor thrombi. Adenocarcinoma proliferated in the fibrous tissue

pation of the tumor thrombus by direct opening of the PV under vascular control, were performed. However, small peritoneal disseminated nodules were encountered in the rectovesicular pouch. Pathological examination showed that Borrmann type 2 sigmoid colon cancer was moderately differentiated adenocarcinoma exposed to the serosa (se) with lymph node metastasis (n1). Figure 4c shows that the extirpated portal vein thrombi consisted mainly of fibrous tissue, in which adenocarcinoma proliferated. Repeated intraarterial chemoinfusion therapy was performed, and no liver metastasis was found. Bone metastases were treated by radiation therapy. Eleven months after the operation, we plan to treat peritoneal dissemination, with abdominal distension, by employing systemic chemotherapy.

Case 5

A 68-year-old man was admitted for surgical treatment of combined sigmoid colon and gastric cancer with bilateral multiple liver metastases. Sigmoidectomy and partial gastrectomy, with regional lymph node clearing, were performed. Surgical resection was not performed for the liver metastases. Borrmann type 2 sigmoid colon

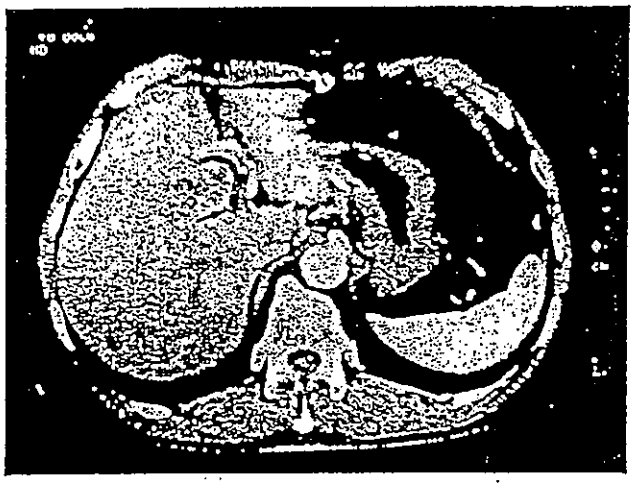

Fig. 5. Case 5. CT scan. Multiple liver metastases were detected, and the left portal vein was not stained because of tumor thrombi. *Arrow* indicates the tumor thrombi

cancer was well differentiated adenocarcinoma reaching the subserosal layer (ss) with ly2, v0, and n0; while Borrmann type 2 gastric cancer was well differentiated tubular adenocarcinoma reaching the proper muscle layer (pm), with ly1, v0, and n3. Subsequently, TAE with epirubicin was administered twice for multiple liver metastases, but a tumor thrombus in the left PV, linked to the metastatic liver cancer, grew, as shown in Fig. 5. Chemolipiodolization with epirubicin and lipiodol for the liver metastasis with PV tumor thrombus was performed only once, because the patient did not agree to further chemotherapy. He died of cancer 7 months after the first finding of the left PV tumor thrombus, i.e., 15 months after the operation.

The details of the five patients are summarized in Table 1.

Discussion

Intraluminal tumor thrombus in the PV system originating from gastrointestinal (GI) tract cancer is a rare condition. According to the 1997 *Annual of the pathological autopsy cases in Japan*,[15] the incidence of PV metastasis in gastric cancer, and in colon and rectal cancer, was reported to be 1.2% (29/2330) and 0.6% (9/1604), respectively. Two metastatic routes from GI tract cancer to the PV are possible: (1) intraluminal tumor thrombus originating directly from the primary lesion or originating indirectly from the liver metastatic lesion, and (2) extrinsic PV occlusion produced by enlarged metastatic lymph nodes. Because the incidence of liver metastasis in gastric cancer and colon and rectal cancer reported in the *Annual of the pathological autopsy cases in Japan*[15] was high, at 35.2% (821/2330) and 38.1% (611/1604), respectively, as compared with the incidence of PV metastasis, it is likely that the incidence of direct and indirect PV tumor thrombus (included in PV metastasis) is low. By contrast, intrahepatic metastasis and tumor thrombosis in the PV are common characteristics of HCC, because HCC is a hypervascular tumor with shunt formation from the hepatic artery to the PV. The Liver Cancer Study Group of Japan[16] has reported that the incidence of tumor thrombus formation in the first branch and trunk of the PV in surgically treated patients and in autopsy cases from 1994 to 1995 was 3.3% (131/3926) and 38.9% (222/570), respectively. The 1997 *Annual of the pathological autopsy cases in Japan*[15] reported that the incidences of PV involvement and liver metastasis in primary liver cancer, including intrahepatic bile duct cancer, were almost at the same level, at 11.9% (319/2671) and 14.3% (382/2671), respectively. These studies clearly indicated that both the direct formation of a tumor thrombus from GI tract cancer, and the indirect formation of a tumor thrombus via liver metastasis are rare, as compared with the incidence of tumor thrombus in primary liver cancer.

In our case 1, PV tumor thrombus was found after total gastrectomy for gastric cancer and because so massive as to occupy the entire liver, resulting in cancer death with liver failure. Such an extreme case, in terms of rapid and expansive progress, is rare. Case 1 died 4 months after the finding of the PV tumor thrombus, while case 2 survived for 13 months, in a tumor thrombus-bearing state after noncurative surgical treatment without thrombectomy. The difference in the survival times between the two patients could be explained in terms of the maintenance of portal flow, and liver function. Therefore, treatment for PV tumor thrombus should be encouraged if it appears that the thrombus would occlude the portal flow, and if collaterals via the pericholedochal plexus, or compensatory arterialization of the liver do not appear to be likely. Based on such a viewpoint, we performed extirpation of synchronous tumor thrombus, combined with surgical treatment of the primary lesion and metastatic liver tumor in cases 3 and 4, although the presence of tumor thrombus indicated tumor spread in the liver. Surgeons who are familiar with vascular surgery can open the PV system under vascular control and easily extirpate a tumor thrombus. Extirpation may be difficult, however, if the tumor thrombus has invaded the PV wall, or if the tumor thrombus is large and long, indicating that its presence is of long duration. However, in our two patients with recent tumor thrombi, the adhesion of the tumor thrombi to the PV wall was easily detached.

By contrast, the synchronous bilateral and multiple liver metastases, and the metachronous tumor thrombus, in case 5 appeared to contraindicate surgical treatment. In case 1, a second surgical thrombectomy was

Table 1. Summary of 5 cases of GI tract cancer with tumor thrombus in the PV system

Case no.	Age (years)/ sex	Primary lesion stage	Tumor thrombus in PV system	Liver metastasis	Surgical treatment	Life span (months)	
						After surgical treatment	After finding PV thrombus
1	86/F	Gastric cancer	Confluence—entire PV tree; direct; metachronous	None	Total gastrectomy	8	4
2	69/F	Transverse colon cancer; direct invasion to the stomach	SMV—confluence; direct; synchronous	Metachronous	Transverse colectomy; partial gastrectomy	13	—
3	73/M	Residual gastric cancer; direct invasion to the pancreas	SPV; direct; synchronous	Synchronous	Total gastrectomy; pancreatosplenectomy; SPV thrombectomy; MCT for liver metastasis	18 (alive)	—
4	59/M	Sigmoid colon cancer	Left PV—PV bifurcation; indirect; synchronous	Synchronous	Sigmoidectomy; left lobectomy; partial resection of S8; PV thrombectomy	11 (alive)	—
5	68/M	Double gastric and sigmoid colon cancer	Left PV; indirect; metachronous	Synchronous	Sigmoidectomy; gastrectomy	15	7

PV, portal vein; SMV, superior mesenteric vein; SPV, splenic vein; MCT, microwave coagulation therapy

not performed for the metachronous tumor thrombi, because they were already so expansive and, likely, of long duration. In case 2, extirpation of the synchronous tumor thrombi was not performed at the time of resection of the primary lesions, because the tumor thrombi had already invaded the small branches of the middle colic vein. In these two patients, chemotherapy for PV tumor thrombi was not given, because of the patient's advanced age (case 1) and because of lack of informed consent (case 2). Although there are a few case reports of successful chemotherapy for liver metastasis with tumor thrombus in the Japanese literature,[17] neither retrospective nor prospective studies in such advanced GI tract cancers have been reported, indicating that chemotherapy is basically not effective.

Tumor thrombus formation by HCC in the PV and hepatic vein is well recognized, because of its high incidence. The presence of PV thrombi indicates a poor prognosis, because of portal hypertension, rupture of esophageal varices, and liver failure. Depending on liver function and tumor size, number, and location, HCC has been treated by several modalities, e.g., surgical resection, microwave coagulation therapy, pure ethanol injection therapy, and TAE. However, the presence of tumor thrombi in the PV is a contraindication for TAE, because of the possibility of inducing liver necrosis by interrupting the dual blood supply through the PV and hepatic artery.[11] Other modalities cannot completely control tumor thrombi in the PV.[18] Therefore, hepatic resection, combined with extirpation of the tumor thrombus, has been performed to prolong survival as far as possible, even though curative resection cannot be achieved. Tanaka et al.[12] in their analysis, have reported that the median survival duration of patients with HCC with tumor thrombi in the first branch or portal trunk who were surgically treated was 10 months, while the survival duration in conservatively treated patients was 3 months.[12] The cumulative survival rate of patients with PV thrombus originating from GI tract cancer cannot be determined in our small patient group, whose backgrounds with respect to treatment, such as location of PV tumor thrombus and presence of collaterals, were not uniform. However, it is likely that patients with PV tumor thrombus originating from GI tract cancer survive longer than those with PV tumor thrombus associated with HCC. As a special case, it is noteworthy that our patient in whom PV tumor thrombus could not be treated died of cancer with liver failure caused by completely obstructed portal blood flow 4 months after the finding of the thrombus. In HCC, hepatic cause of death is classified according to the following three criteria:[19] (a) intrahepatic tumor occupies more than 50% of the entire liver; (b) tumor thrombus is present in the main portal trunk or inferior vena cava; and (c) there is rupture of intrahepatic nodules. Although liver failure in metastatic GI tract cancer is usually ascribed to a reduced liver mass, caused by large and numerous liver metastases, the presence of intraluminal PV tumor thrombi is likely to be fatal.

There is no absolute indication for surgical PV thrombectomy for GI tract cancer with PV tumor thrombus from the viewpoint of curative resection. Surgical thrombectomy can be a relative indication, as in our cases 3 and 4, when all lesions, including distant metastases, can be macroscopically resected or controlled, and when the portal vein tumor thrombus can be easily extirpated without the use of any temporary or permanent bypass.[20] Recurrence should be treated by a combination of local control (such as a second surgical resection or microwave coagulation therapy) and systemic control (such as chemotherapy). Thrombectomy is contraindicated (as in our cases 1, 2, and 5) when all macroscopic lesions cannot be controlled or when the thrombectomy would be associated with a high operative risk.

Conclusions

GI tract cancer has the potential to form a tumor thrombus in the PV system, either directly from the primary lesion, or indirectly from metastatic liver cancer at an advanced stage, as occurs in HCC. Because the presence of a PV tumor thrombus may, possibly, determine the patient's length of survival, in addition to causing cancer progression, surgical thrombectomy, combined with resection of the primary cancer and metastatic liver cancer, should be considered for to prolong survival, if all macroscopic lesions can be controlled and if the tumor thrombus is a synchronous and recent one.

References

1. Mathieu D, Vasile N, Grenier P. Portal thrombosis: dynamic CT features and course. Radiology 1985;154:737–41.
2. Zalcman M, Gansbeke DV, Matos C, Engelholm L, Struyven J. Sonographic demonstration of portal venous thromboses secondary to inflammatory diseases of the pancreas. Gastrointest Radiol 1987;12:114–6.
3. Chan S, Chan F, Chau E, Mok F. Portal thrombosis complicating appendicitis. J Comput Assist Tomogr 1988;12:208–10.
4. Reh TE, Srivisal S, Schmidt EH. Portal venous thrombosis in ulcerative colitis. J Comput Assist Tomogr 1980;4:545–7.
5. Wozney P, Zajko A, Bron KM, Point S, Starzl TE. Vascular complications after liver transplantation. AJR Am J Roentogenol 1986;147:657–63.
6. Takayasu K, Makuuchi M, Moriya Y. Portal vein obstruction complicating intra-arterial chemo-infusion for hepatic metastases. J Gastroenterol Hepatol 1990;5:708–13.
7. Adler J, Goodgold M, Mitty H, Gordon D, Kinkhabwala M. Arteriovenous shunts involving the liver. Radiology 1978;129: 315–22.

8. Heaston DK, Chuang VP, Wallace S, Santos L. Metastatic hepatic neoplasms: angiographic features of portal vein involvement. AJR Am J Roentgenol 1981;136:897–900.
9. Araki T, Suda K, Sekikawa T, Ishii Y, Hihara T, Kachi K. Portal venous thrombosis associated with gastric adenocarcinoma. Radiology 1990;174:811–4.
10. Ishikura H, Kirimoto K, Shamoto M, Miyamoto Y, Yamagiwa H, Itoh T, et al. Hepatoid adenocarcinoma of the stomach. Cancer 1986;58:119–26.
11. Yen FS, Wu JC, Kuo BI, Chen TZ, Lee SD. Transcatheter arterial embolization for hepatocellular carcinoma with portal vein thrombosis. J Gastroenterol Hepatol 1995;10:237–40.
12. Tanaka A, Morimoto T, Yamaoka Y. Implications of surgical treatment for advanced hepatocellular carcinoma with tumor thrombi in the portal vein. Hepatogastroenterology 1996;43:637–43.
13. Japanese Gastric Cancer Association, editors. Japanese classification of gastric carcinoma. 13th Ed. Tokyo: Kanehara; 1999.
14. Japanese Society for Cancer of the Colon and Rectum, editors. General rules for clinical and pathological studies on cancer of the colon, rectum and anus. Tokyo: Kanehara; 1998.
15. The Japanese Society of Pathology, editors. Annual of the pathological autopsy cases in Japan. Vol 40 (Jan–Dec 1997) (in Japanese). Aichi: Jinshikai; 1998.
16. Liver Cancer Study Group of Japan, editors. Thirteenth follow-up study of primary liver cancer in Japan (1994–1995) (in Japanese). Kyoto: Shinko; 1998.
17. Yoshida Y, Egami I, Onda M. A case of liver metastasis of gastric cancer with portal vein tumor thrombosis responding to chemotherapy with 5-FU and epirubicin. Jpn J Cancer Chemother 1995;22:1245–8.
18. Raoul JL, Guyader D, Bretagne JF, Duvanferrier R, Bourquet P, Bekhechi D, et al. Randomized controlled trial for hepatocellular carcinoma with portal vein thrombosis: intra-arterial iodine-131-iodized oil versus medical support. J Nucl Med 1994;35:1782–7.
19. Okusaka T, Okada S, Ishii H, Nose H, Nagahama H, Nakasuka H, et al. Prognosis of hepatocellular carcinoma patients with extrahepatic metastases. Hepatogastroenterology 1997;44:251–7.
20. Kumada K, Ozawa K, Okamoto R, Takayasu T, Yamaguchi T, Yamamoto Y, et al. Hepatic resection for advanced hepatocellular carcinoma with removal of portal vein tumor thrombi. Surgery 1990;108:821–7.

Laboratory/Clinical Translational Research

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Prognostic Impact of Multiple Allelic Losses on Metastatic Recurrence in Hepatocellular Carcinoma after Curative Resection

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Key Words
Chromosomal change · Chromosome 13q · Chromosome 16q · Chromosome 17p · Loss of heterozygosity · Hepatocellular carcinoma · Recurrence · Metastasis · Hepatectomy

Abstract
Loss of heterozygosity (LOH) on chromosomes 13q, 16q and 17p has been associated with the progression of hepatocellular carcinoma (HCC). To investigate the prognostic impact of such LOH, we examined the metastasis-free survival of curatively resected HCC cases, in whom these LOHs were analyzed. Among the 49 HCC patients examined, the frequency of LOHs was 28% on 13q, 33% on 16q and 40% on 17p. The patients were followed up for metastatic recurrence after surgery and for analysis of the relationship between chromosomal changes and patients' metastasis-free survival. Univariate survival analysis showed the presence of LOH on 16q, 17p and the number of chromosomes with LOH were significantly and negatively associated with metastasis-free survival, indicating that patients with LOH on multiple chromosomes had a poorer prognosis after surgery than those with LOH on a single chromosome or no LOH. Multivariate Cox survival analysis identified the presence of LOH on 16q and the number of chromosomes with LOH as the most significant independent negatively predictive factors for metastasis-free survival. These findings indicate that accumulation of chromosomal changes is associated with metastatic behavior, and that LOH on 16q was the most useful prognostic indicator for metastasis after curative resection of HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the major causes of cancer-related deaths, and hepatectomy is a useful therapy for HCC. However, there are some patients with small tumors who develop early metastasis, while others with advanced tumors have good prognoses [1]. Recent advances in molecular genetics have shown that many oncogenes and tumor suppressor genes were involved in hepatocarcinogenesis [2–4]. Among them, mu-

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Table 1. Initial clinicopathological profiles and chromosomal changes of the cases with metastatic recurrence

Age/ sex	Liver		HCC					Chromosomes			Observation	
	virus	fibrosis	size cm	vascular invasion	number of tumors	stage	differentiation	13q	16q	17p	period days	outcome
62/M	C	4	3.5	–	single	II	poorly	LOH	LOH	LOH	1,001	MI
62/M	C	4	1.4	–	single	I	moderately	HET	HOM	LOH	91	MI
59/M	C	1	2.3	–	multiple	IV	moderately	HET	HET	HET	444	MI
66/F	B	4	2.0	–	single	I	moderately	HOM	LOH	LOH	545	MI
65/M	C	4	6.0	–	multiple	III	moderately	HET	HET	LOH	81	MI
59/M	C	2	3.0	–	single	II	well	LOH	HET	LOH	420	MI
62/F	C	3	1.6	+	single	I	moderately	LOH	HET	LOH	508	MI
50/M	B	4	7.5	+	single	III	poorly	HET	LOH	HET	63	MI
65/M	NBNC	3	3.0	+	multiple	III	poorly	HET	HET	HET	1,037	MI
56/M	C	4	1,6	–	multiple	II	moderately	HET	HOM	LOH	210	MI
74/M	NBNC	1	12.0	+	multiple	II	well	HET	HET	LOH	98	MI
56/M	C	1	5.0	–	single	II	moderately	LOH	LOH	HOM	1,430	MI
50/M	C	1	6.0	+	single	III	poorly	LOH	LOH	HOM	120	PV, MI
64/F	C	1	6.9	+	single	IV	poorly	LOH	LOH	HET	90	PV, MI
65/M	C	3	7.0	+	multiple	III	moderately	HET	HET	HET	527	PV, lung, MI
41/M	NBNC	3	6.0	+	single	III	poorly	HOM	LOH	LOH	173	IVC, MI
52/M	B	3	4.0	–	multiple	III	moderately	LOH	HET	HOM	2,035	IVC, MI
76/F	C	2	8.0	–	single	II	moderately	LOH	LOH	LOH	247	bone, MI
47/F	B	3	5.0	–	single	II	moderately	HET	LOH	LOH	152	bone, MI
75/M	C	4	6.5	+	multiple	III	moderately	HET	HOM	HET	365	LN, MI
61/M	C	4	5.0	–	multiple	II	well	HET	HET	LOH	527	PD, MI

Infection of hepatitis virus was examined with serum HBsAg and serum HCVAb. Fibrosis grading of the background liver was classified according to the criteria of Desmet et al. [11], and the stage of HCC was determined according to the classification given in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [9]. B = Positive for HBsAg; C = positive for HCVAb; NBNC = negative for both HBsAg and HCVAb; LOH = heterozygous alleles, heterozygous deletion (bold type); HET = heterozygous allele, both alleles preserved; HOM = homozygous alleles, LOH cannot be assessed; MI = multiple intrahepatic recurrence; PV = portal vein thrombosis; IVC = thrombosis in the inferior vena cava; lung = lung metastasis; bone = bone metastasis; LN = lymph node metastasis; PD = peritoneal dissemination.

tation of the tumor suppressor *p53* gene, and expression of the p73 (homologue of p53) and $p27^{Kip1}$ proteins have been reported as poorly prognostic markers for surgically treated HCC patients [5–7].

We reported that loss of heterozygosity (LOH) on chromosomes 13q, 16q and 17p was frequently observed in advanced HCC [8], suggesting that these genetic changes play an important role in the acquisition of aggressive tumor phenotypes. However, no relationships between these LOHs and prognosis of HCC have yet been established. Therefore, we followed up curatively operated HCC patients with or without LOH on 13q, 16q and 17p at the time of surgery, examined the relationship between metastasis-free survival and the presence of these LOHs in the HCC cases. We report here on the prognostic significance of LOH on 13q, 16q and 17p after surgery for HCC.

Materials and Methods

Patients

Forty-nine patients with first emergence of HCC who underwent curative resection of HCC at the Kyoto University Hospital between October, 1990, and September, 1998 were included in this study. All samples were obtained by surgical resection, and the LOHs were analyzed with informed consent. Macroscopic and ultrasonographic examination during surgery showed that no tumor remained in any of the remnant livers. Histological studies were performed at the Clinical Pathology Department of the Hospital, and the stage of HCC was determined according to the classification given in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [9]. The stage of non-cancerous chronic liver disease was identified according to the Child-Pugh classification [10], and the fibrosis grading of the background liver was also evaluated according to the criteria of Desmet et al. [11] (F0, no fibrosis; F1, mild fibrosis; F2, moderate fibrosis; F3, severe fibrosis, and F4, cirrhosis). Hepatitis B surface antigen (HBsAg) was measured by the HB Reversecell test (Yamanouchi, Tokyo, Japan) and hepatitis C virus antibody (HCVAb) by the HCVAb PHA test (Dinabott, Tokyo, Japan).

Follow-Up

After surgery, the recurrence of HCC was monitored by means of abdominal ultrasonography, computed tomography or magnetic res-

Table 2. Initial clinicopathological profiles and chromosomal changes of the cases without metastatic recurrence

Age/ sex	Liver		HCC					Chromosomes			Observation	
	virus	fibrosis	size cm	vascular invasion	number of tumors	stage	differentiation	13q	16q	17p	period days	outcome
64/M	C	2	1.0	–	single	I	well	HET	HET	HET	2,022	MC
57/F	C	3	3.0	–	single	II	poorly	HOM	HET	HET	1,331	MC
68/F	C	1	2.1	+	single	II	moderately	HET	HET	HET	1,747	MC
67/M	C	3	3.0	–	multiple	III	moderately	HET	HET	HET	485	MC
73/M	C	4	5.0	–	single	II	moderately	HET	HET	HET	426	MC
61/M	C	3	1.7	–	single	I	moderately	HET	HET	HET	610	MC
58/M	C	4	5.5	+	multiple	III	moderately	LOH	HET	HET	485	MC
57/M	C	3	5.0	+	single	III	modertely	HET	HET	HET	850	MC
67/M	C	4	4.0	–	single	II	well	HET	HET	HET	877	MC
71/M	C	3	1.0	–	single	I	well	HET	HET	HET	573	MC
71/M	C	1	7.0	–	multiple	III	moderately	LOH	LOH	HOM	60	liver failure
48/M	B	4	5.0	+	multiple	IV	well	HET	LOH	LOH	46	MOF
67/M	B	2	2.0	–	single	I	poorly	HET	LOH	LOH	180	sepsis
65/M	C	3	7.0	+	multiple	III	poorly	LOH	HET	HET	25	peritonitis
65/M	C	3	5.5	–	multiple	III	well	HET	HET	HET	154	colon
72/M	C	2	1.8	+	single	I	moderately	LOH	HET	HET	3,389	prostate
63/M	C	4	4.0	–	single	II	moderately	HET	LOH	LOH	255	disease free
63/F	C	4	3.0	–	single	II	well	HET	LOH	HET	280	disease free
56/M	C	4	5.0	–	single	II	moderately	HET	HET	HET	83	disease free
62/M	B	3	6.5	+	multiple	III	moderately	LOH	LOH	HET	63	disease free
57/M	B	3	5.0	–	multiple	II	well	HET	HET	HET	270	disease free
57/M	C	4	3.0	+	multiple	III	moderately	HET	HET	HET	485	disease free
74/M	C	4	11.0	–	multiple	III	moderately	HET	HET	HET	293	disease free
67/M	NBNC	1	12.0	+	single	III	moderately	HET	HET	LOH	1,379	disease free
76/M	C	3	2.0	–	single	I	well	HET	HET	HET	1,516	disease free
63/M	NBNC	3	2.0	–	multiple	I	well	HET	HET	HET	1,163	disease free
77/F	NBNC	ND	7.5	+	single	II	well	HET	HET	LOH	90	disease free
65/M	C	ND	5.0	–	single	II	well	HET	HET	LOH	150	disease free

Infection of hepatitis virus was examined with serum HBsAg and serum HCVAb. Fibrosis grading of the background liver was classified according to the criteria of Desmet et al. [11], and the stage of HCC was determined according to the classification given in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [9]. ND = Not determined; B = positive for HBsAg; C = positive for HCVAb; NBNC = negative for both HBsAg and HCVAb; LOH = heterozygous alleles, heterozygous deletion (bold type); HET = heterozygous allele, both alleles preserved; HOM = homozygous alleles, LOH cannot be assessed; MC = multicentric HCC; MOF = multiorgan failure; colon = emergence of colon cancer; prostate = emergence of prostate cancer.

onance imaging every 3 months or when clinically indicated. Serum α-fetoprotein (AFP) levels were also examined every month. Chest radiograms, abdominal angiography and bone scintigrams were performed when necessary. We defined recurrence as the emergence of new lesions with radiological features typical for HCC, and the type of recurrence was defined by at least two imaging methods. The vascularities of the initial and recurring HCCs were also assessed with angiography. If the diagnosis of the secondary tumors could not be confirmed to be HCCs, we re-evaluated the size of the tumors 3 months after the emergence of the lesions, because it is often difficult to distinguish well-differentiated HCCs from dysplastic nodules or large regenerative nodules with imaging alone. The end-points included: (1) first recurrence (31 patients); (2) death (4 patients), and (3) emergence of other cancers (2 patients). The other 12 patients did not show any recurrence during the observation periods. In this study, extrahepatic recurrences, recurrences with vascular invasion and multiple hypervascular intrahepatic recurrences were defined as metastatic recurrences. Twenty-one patients were considered to have metastatic recurrence (table 1), all of whom showed multiple intrahepatic metastasis, 3 portal vein thrombosis and 2 thrombosis in the inferior vena cava. Other types of recurrence were bone metastasis (2 patients), lymph node metastasis, lung metastasis and intraperitoneal dissemination (1 patient each; table 1). On the other hand, 10 patients had solitary intrahepatic recurrences. All of them were less than 2.0 cm in diameter, developed in different hepatic segments from initial tumors and were considered as multicentric HCCs for the following reasons. Two HCCs were resected again at the time of recurrence and histologies were confirmed to be well-differentiated HCCs (a 64-year-old male and 57-year-old female; table 2). These two HCCs were classified as multicentric according to the criteria described in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [9]. Seven patients, 1 female (68 years old) and 6 males (57, 58, 61, 67, 67 and 73 years old; table 2) were confirmed to have hypovascular recurring HCCs with angiography, although their primary tumors were hypervascular. Since it is reported that arterial blood supply of HCCs increases as the tumors develop and arterialization in HCCs is generally accompanied by hypervascularity [12], the hypovascular tumors of these 7 patients

were classified as multicentric HCCs. One recurring HCC (a 71-year-old male; table 2) was detected in the caudate lobe as a hypovascular tumor using angiography, depicted as a hyperechoic nodule with abdominal US and had high signal intensity on T_1-weighted MRI. These imaging characteristics were consistent with the finding that recurring HCCs were well differentiated and considered as a multicentric HCC [13]. As the primary cancer of this case was well-differentiated HCC and 1.0 cm in diameter, the classification of this case was also supported by the evidence that no metastasis from the early HCC defined as a small tumor composed solely of a well-differentiated lesion has been detected [14]. All eight nodules, which were considered as multicentric HCCs following imaging, had grown in size 3 months after the emergence of the lesions and were diagnosed as HCCs rather than dysplastic nodules or large regenerative nodules.

Four patients died of liver failure, multiorgan failure, sepsis or bacterial peritonitis, and 2 patients developed prostate or colon cancer. The remaining 12 patients were disease free until the end of the observation periods (table 2). These 28 cases were treated as censored at the date of diagnosis for univariate or multivariate analyses. Among the 49 patients that underwent curative resection, 22 were treated with transcatheter arterial embolization before surgery, and the others did not receive any preoperative treatment. Clinicopathological profiles of the patients are listed in tables 1 and 2.

LOH Analysis

We evaluated LOHs at six loci on 13q, five on 16q and six on 17p. LOHs at D13S1, RB1, D13S3, D16S7, TP53, D17S31 and D17S5 were analyzed with polymorphic probes pHU10 (D13S1), p7F-12 (D13S1), p123M1.8 (RB1), p88R2.5 (RB1), p68RS2.0 (RB1), p9A7 (D13S3), p79.2.23 (D16S7), pR4-2 (TP53), pMCT35.1 (D17S31) and pYNZ22 (D17S5). We also analyzed the *Bst*U1 polymorphism at codon 72 of the *p53* gene with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedure. Details of isolation of high-molecular-weight DNA from tumorous and non-tumorous tissues, Southern blot analysis and PCR-RFLP analysis have been described previously [8, 15]. LOHs at D13S171, D13S153, D13S159, D16S136, D16S503, D16S515, D16S3091, D17S849, D17S831 and D17S799 were analyzed with microsatellite markers which allow for chromosomal positions and sequences to be referred to the genome database [http://www.gdb.org] and cooperative linkage center [http://www.chlc.org]. The amplified fragments were separated by electrophoresis on 12% denaturating polyacrylamide gel and stained with ethidium bromide for UV detection. The intensity of the band was quantified with densitometry (Densitograph AE-6905C, ATTO, Tokyo, Japan). Only the cases with a density of the fainter band of <50% of that of normal tissues were determined as having LOH.

Statistics

Metastasis-free survival curves for the 49 curatively resected cases were estimated with the Kaplan-Meier method, and univariate findings of the parameters were also analyzed with the log-rank test. The joint effect of the covariables was examined with the stepwise Cox proportional-hazards regression model to select significant independent variables. For each analysis, $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed on a personal computer using StatView for Macintosh (version 5.0 StatView, Cary, N.C., USA).

Table 3. Univariate and multivariate analyses of LOHs and initial clinicopathological parameters for metastasis-free survival

Variables	Univariate	Multivariate	
	p value	p value	p value
LOH on 13q	0.343	NI	NI (0.081)
LOH on 16q	<0.001	0.005	NI (0.019)
LOH on 17p	<0.001	NI	NI (0.018)
Size	0.004	0.011	
Vascular invasion	0.320	NI	
Number of tumors	0.419	0.837	
Stage	0.045		NI (0.016)
Differentiation	0.153	0.666	NI (0.190)
Age	0.224	0.012	NI (0.323)
Sex	0.442		
Virus	0.693		
Fibrosis grading	0.904		
Child-Pugh classification	0.649		
Serum AFP value	0.348	0.021	NI (0.410)
Preoperative TAE	0.521		

Univariate (log-rank test) and multivariate analyses (stepwise Cox proportional-hazard regression model) of LOHs and initial clinicopathological parameters for metastasis-free survival. Standard prognostic factors, age and serum AFP value, all of which had p values <0.419 (the p value for the number of tumors), were included in Cox's model. In the multivariate analysis, only p values of independent predictive factors determined with Cox's model are shown in bold type. NI = No independent value. With the multivariate analysis, using LOHs, stage, differentiation grading, age and serum AFP value as covariables, none of the factors were established as independent, and p values are listed in parentheses.

For these analyses, age, tumor size, serum AFP value and degree of fibrosis in the background liver were divided into two or three groups; age: ≥60 and <60 years; tumor size: ≤2.0, 2.1–5.0 and >5.0 cm; serum AFP value: ≤20, 21–200 and >200 ng/ml; fibrosis grading: group with F1 or F2 and group with F3 or F4. TAE = Transcatheter arterial embolization.

Results

In this study, we found LOH in 28% (13/46) of the cases on 13q, in 33% (15/46) on 16q and in 40% (18/45) on 17p. Frequencies of LOH at each of the loci were as follows: 22% (4/18) at D13S1, 41% (12/29) at RB1, 39% (5/13) at D13S3, 25% (3/12) at D13S171, 27% (3/11) at D13S153, 25% (3/12) at D13S159, 30% (9/30) at D16S7, 27% (3/11) at D16S136, 17% (2/12) at D16S503, 20% (2/10) at D16S515, 18% (2/11) at D16S3091, 42% (10/24) at TP53, 53% (8/15) at D17S31, 35% (8/23) at D17S5, 33% (4/12) at D17S849, 39% (5/13) at D17S831 and 36% (4/11) at D17S799.

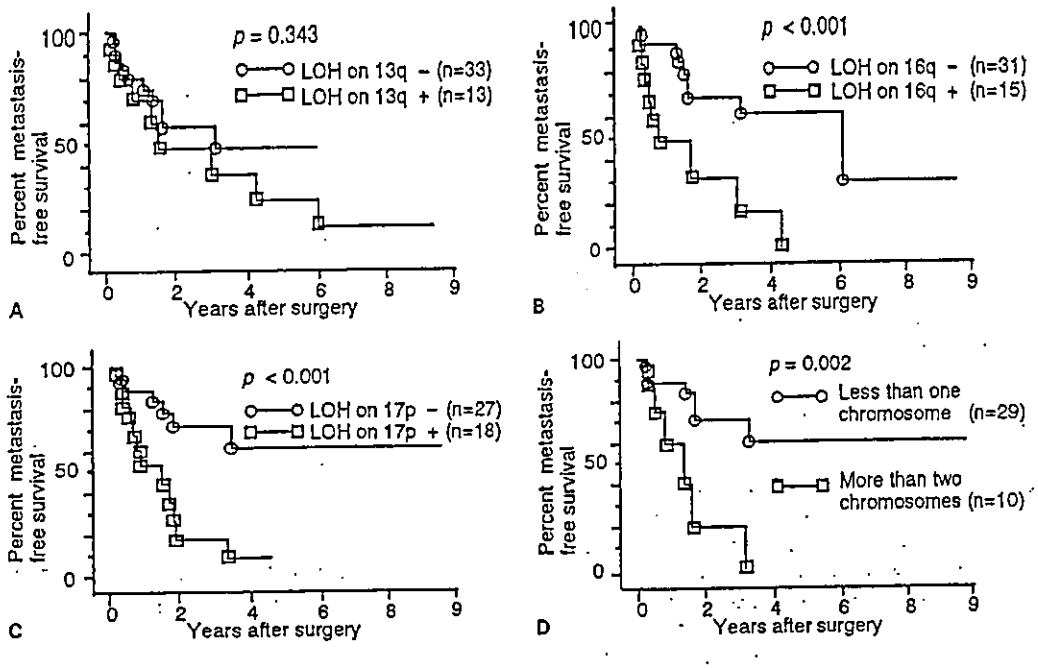

Fig. 1. Kaplan-Meier curves for metastasis-free survival of patients who underwent curative resection of HCC classified according to the presence or absence of LOH on chromosome 13q (**A**), 16q (**B**) and 17p (**C**) and the number of chromosomes with LOH (**D**).

Among the 49 patients examined, 21 had secondary metastatic HCCs and 10 were considered to have multicentric HCCs as secondary intrahepatic tumors. Among the 21 patients with metastatic recurrence, 14 were positive for HCVAb, 4 were positive for HBsAg and 3 were negative for both HCVAb and HBsAg (table 1). On the other hand, all 10 patients with multicentric HCCs were positive for HCVAb and negative for HBsAg (table 2). In the group with metastatic recurrence, the fibrosis grading of the background liver was not associated with the recurrence-free survival by the log-rank test (F1 or F2 vs. F3 or F4; $p = 0.592$). Whereas, among 10 patients with multicentric recurrences, 2 with mild or moderate fibrosis of the background liver (F1 or F2) had longer recurrence-free survival than those with severe fibrosis or cirrhosis (F1 or F2 vs. F3 or F4; $p = 0.025$ by the log-rank test).

Next, we treated 10 cases with multicentric HCCs, 4 deceased cases, 2 cases with other cancers and 12 disease-free cases as censored at the date of diagnosis, and analyzed the association between LOH and metastasis-free survival of 49 patients who underwent curative resection for HCC (fig. 1). Univariate survival analyses showed that LOH on 16q, and 17p, tumor size and stage were significantly associated with metastasis-free survival ($p < 0.001$, $p < 0.001$, $p = 0.004$ and $p = 0.045$, respectively; table 3, fig. 1). Although not statistically significant, vascular invasion, differentiation, age and serum AFP value also tended to be associated with metastasis-free survival ($p = 0.320$, $p = 0.153$, $p = 0.224$ and $p = 0.348$, respectively; table 3). Of the 49 cases, 39 were informative for all three chromosomes. We then examined the relationship between the number of chromosomes with LOH and metastasis-free survival of these 39 patients. Cases with LOH on multiple chromosomes showed significantly shorter metastasis-free survival than those with LOH on a single chromosome or no LOH ($p = 0.002$; table 4, fig. 1). Preoperative transcatheter arterial embolization did not improve metastasis-free survival after surgery ($p = 0.521$; table 3), which was consistent with the finding of a previous study [16].

Next, we performed multivariate analysis with the stepwise Cox proportional-hazards regression model to define independent risk factors, using standard prognostic factors (tumor size, vascular invasion, number of

Table 4. Univariate (log-rank test) and multivariate analyses (stepwise Cox proportional-hazard regression model) of the number of chromosomes with LOH and initial clinicopathological parameters for metastasis-free survival

Variables	Univariate	Multivariate	
	p value	p value	p value
Number of chromosomes with LOH	0.002	0.018	0.015
Tumor size	0.021	0.026	
Vascular invasion	0.481	NI	
Number of tumors	0.428	0.601	
Stage	0.010		NI
Differentiation	0.197	0.493	NI
Age	0.123	0.165	NI
Serum AFP	0.405	NI	NI

Of the 49 patients, 39 were informative for all three chromosomes and were included in these analyses. In the multivariate analysis, only p values of independent predictive factors determined with Cox's model are shown in bold type. NI = No independent values. The number of chromosomes with LOH was divided into two groups, less than one chromosome and more than two. Tumor size, age and serum AFP value were divided into two or three groups; tumor size: ≤2.0, 2.1–5.0 and >5.0 cm; age: ≥60 and <60 years; serum AFP: ≤20, 21–200 and >200 ng/ml.

tumors and differentiation), age, serum AFP as well as LOHs on 13q, 16q and 17p for covariables. Variables showing independent negatively predictive values for metastasis-free survival were the presence of LOH on 16q, tumor size, tumor number, differentiation, age and serum AFP value, and amongst all the presence of LOH on 16q was the most significant negatively predictive value for metastasis-free survival (p = 0.005, table 3). With the use of LOH, stage (depending on the tumor size, vascular invasion and number of tumors), differentiation, age and serum AFP value as covariables, LOH on 16q, 17p and stage seemed to be important factors for the metastatic recurrence, although they were not independent predictive values (table 3). Multivariate analysis of the cases informative for the three chromosomes, using size, vascular invasion, number of tumors, differentiation, age, serum AFP value and the number of chromosomes with LOH as covariables, showed that the number of chromosomes with LOH, tumor size, number of tumors, differentiation and age were independent predictive values. Among them, the number of chromosomes with LOH was the most significant negative predictor for metastasis-free survival (p = 0.018, table 4). Finally, with the use of the number of chromosomes with LOH, stage, differentiation, age and serum AFP value as covariables, the number of chromosomes with LOH was recognized as an independent predictive value (p = 0.015; table 4).

Discussion

Hepatectomy is an effective and curative therapy for HCC, although it is difficult to determine the prognosis of HCC after surgery. Recent advances in molecular biology have clarified a number of alterations in cancer-related genes in human HCC, although only few studies have evaluated the prognostic value of such alterations. In this study, we have demonstrated that the number of chromosomes with LOH and LOH on 16q can help predict metastasis after curative resection of HCC.

Recently, Chen et al. [17] used comparative genomic hybridization to analyze 31 pairs of initial and recurrent HCCs and reported that initial HCCs that subsequently relapsed accumulated more chromosomal aberrations than those that developed de novo HCCs. Our study, which demonstrated that the number of chromosomes with LOH was significantly and negatively associated with metastasis-free survival, also supports the idea that accumulation of chromosomal changes will provide useful information for evaluating the biological behavior of HCC.

Among the LOHs examined in this study, those on 16q and 17p were significantly associated with metastasis-free survival, as was expected from our previous studies that showed LOHs on these chromosomes were associated with progression of HCC [8], whereas LOH on 13q was not. The tumor suppressor *RB* and *p53* genes are located on 13q and 17p [18, 19], however, the relationship between p53 or RB abnormalities and patients' prognoses are still controversial. Studies have shown p53 alteration to be a poor prognostic indicator for survival in HCC cases after surgery [5, 20], but other studies failed to identify p53 abnormality as a valuable prognostic factor [6]. Concerning the relationship between RB aberration and survival, Naka et al. [20] reported that no significant survival differences was observed in resected HCC cases with or without RB expression. Our findings suggest that LOH on 17p, which was frequently accompanied by the *p53* mutation, was associated with metastatic recurrence in human HCC.

LOH on 16q has been reported for a variety of cancers, and many studies have reported the association of LOH

on 16q with metastasis of the tumor [21]. Furthermore, the introduction of human chromosome 16 suppressed metastasis of a highly metastatic prostate cancer cell line [22]. These findings indicate the possibility that LOH on 16q may enhance the metastatic potential in primary cancers. Recent analyses showed the presence of several tumor suppressor genes, including *cadherin* genes, on chromosome 16 [23, 24]. Since the disturbance of intracellular adhesion is important for the invasion and metastasis of tumor cells, cadherins may be considered prime candidates for inactivation in HCCs with LOH on 16q. The *E-cadherin* gene is reported to be suppressed by CpG methylation of the promoter [25]. However, Slagle et al. [26] reported an association between the loss of one copy of the *E-cadherin* gene and its reduced expression in HCC cases. Therefore, it is interesting to compare the expression of E-cadherin with LOH on 16q and CpG methylation of the promoter in HCC. In addition, since reduced E-cadherin expression reportedly correlates with recurrence and survival in breast and esophageal cancers, it may be useful to examine the relationship between E-cadherin expression and the prognosis of HCC [27, 28].

Recently, Koike et al. [29] reported that the fibrosis grading of the background liver was the risk factor contributing to HCC recurrence among HCV-related HCC, but not in HBV-related and non-B, non-C related HCC. In the present study, we also confirmed that all cases with multicentric HCCs were positive for HCVAb and that the recurrence-free interval was associated with the degree of fibrosis in the background liver in these cases.

Our study suggests that the accumulation of LOH on 13q, 16q and 17p is associated with the metastatic potential of HCC. Furthermore, LOH on 16q could be the most significant marker for metastatic recurrence after curative surgery. Liver transplantation is now regarded as a curative therapy for HCC, although indications for total liver resection combined with transplantation need to be determined more effectively [30]. For these reasons, the findings presented here may provide useful information for the assessment of prognosis and selection of surgical therapy for HCC.

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References

1 Ikeda K, Saitoh S, Tsubota A, Arase Y, Chayama K, Kumada H, Watanabe G, Tsurumaru M: Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. Cancer 1993;71:19–25.

2 Nishida N, Fukuda Y, Ishizaki K: Molecular aspects of hepatocarcinogenesis and their clinical implications. Int J Oncol 1994;4:615–622.

3 Nishida N, Fukuda Y, Komeda T, Kita R, Sando T, Furukawa M, Amenomori M, Shibagaki I, Nakao K, Ikenaga M, Ishizaki K: Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. Cancer Res 1994;54:3107–3110.

4 Nishida N, Fukuda Y, Ishizaki K, Nakao K: Alteration of cell cycle-related genes in hepatocarcinogenesis. Histol Histopathol 1997;12: 1019–1025.

5 Honda K, Sbisà E, Tullo A, Papeo PA, Saccone C, Poole S, Pignatelli M, Mitry RR, Ding S, Isla A, Davies A, Habib NA: p53 mutation is a poor prognostic indicator for survival in patients with hepatocellular carcinoma undergoing surgical tumour ablation. Br J Cancer 1998;77: 776–782.

6 Tannapfel A, Wasner M, Krause K, Geissler F, Katalinic A, Hauss J, Mössner J, Engeland K, Wittekind C: Expression of p73 and its relation to histopathology and prognosis in hepatocellular carcinoma. J Natl Cancer Inst 1999;91: 1154–1158.

7 Ito Y, Matsuura N, Sakon M, Miyoshi E, Noda K, Takeda T, Umeshita K, Nagano H, Nakamori S, Dono K, Tsujimoto M, Nakahara M, Nakao K, Taniguchi N, Monden M: Expression and prognostic roles of the G1-S modulators in hepatocellular carcinoma: p27 independently predicts the recurrence. Hepatology 1999;30:90–99.

8 Nishida N, Fukuda Y, Kokuryu H, Sadamoto T, Isowa G, Honda K, Yamaoka Y, Ikenaga M, Imura H, Ishizaki K: Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in the advanced stages of human hepatocellular carcinoma. Int J Cancer 1992;52:862–868.

9 Liver Cancer Study Group of Japan: The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, ed 4, revised. Tokyo, Kanehara Press, 2000.

10 Child CG: The liver and portal hypertension, Philadelphia, Saunders, 1964.

11 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ: Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 1994;19:1513–1520.

12 Bhattacharya S, Davidson B, Dhillon AP: Blood supply of early hepatocellular carcinoma. Semin Liver Dis 1995;15:390–401.

13 Monzawa S, Omata K, Shimazu N, Yagawa A, Hosoda K, Araki T: Well-differentiated hepatocellular carcinoma: Finding of US, CT and MR imaging. Abdom Imaging 1999;24:392–397.

14 Sakamoto M, Hirohashi S, Shimosato Y: Early stages of multistep hepatocarcinogenesis: Adenomatous hyperplasia and early hepatocellular carcinoma. Hum Pathol 1991;22:172–178.

15 Nishida N, Fukuda Y, Kokuryu H, Toguchida J, Yandell DY, Ikenaga M, Imura H, Ishizaki K: Role and mutational heterogeneity of the p53 gene in hepatocellular carcinoma. Cancer Res 1993;53:368–372.

16 Yamasaki S, Hasegawa H, Kinoshita H, Furukawa M, Imaoka S, Takasaki K, Kakumoto Y, Saitsu H, Yamada R, Oosaki Y, Arii S, Okamoto E, Monden M, Ryu M, Kusano S, Kanematsu T, Ikeda K, Yamamoto M, Saoshiro T, Tsuzuki T: A prospective randomized trial of the preventive effect of preoperative transcatheter arterial embolization against recurrence of hepatocellular carcinoma. Jpn J Cancer Res 1996;87:206–211.

17 Chen Y-J, Yeh S-H, Chen J-T, Wu C-C, Hsu M-T, Tsai S-F, Chen P-J, Lin C-H: Chromosomal change and clonality relationship between primary and recurrent hepatocellular carcinoma. Gastroenterology 2000;119:431–440.
18 Godbout R, Dryja TP, Squire J, Gallie BL, Phillips RA: Somatic inactivation of genes on chromosome 13 is a common event in retinoblastoma. Nature 1983;304:451–453.
19 Isobe M, Emanuel BS, Givol D, Oren M, Croce CM: Localization of gene for human p53 tumor antigen to band 17p13. Nature 1986;320:84–85.
20 Naka T, Toyota N, Kaneko T, Kaibara N: Protein expression of p53, $p21^{WAF1}$, and Rb as prognostic indicators in patients with surgically treated hepatocellular carcinoma. Anticancer Res 1998;18:555–564.
21 Driouch K, Dorion-Bonnet F, Briffod M, Champeme MH, Longy M, Lidereau R: Loss of heterozygosity on chromosome arm 16q in breast cancer metastases. Genes Chromosom Cancer 1997;19:185–191.
22 Mashimo T, Watabe M, Cuthbert AP, Newbold RF, Rinker-Schaeffer CW, Helfer E, Watabe K: Human chromosome 16 suppresses metastasis but not tumorigenesis in rat prostatic tumor cells. Cancer Res 1998;58:4572–4576.
23 Kremmidiotis G, Baker E, Crawford J, Eyre HJ, Nahmias J, Callen DF: Localization of human cadherin genes to chromosome regions exhibiting cancer-related loss of heterozygosity. Genomics 1998;49:467–471.
24 Huntsman DG, Caldas C: Assignment of the E-cadherin gene (CDH1) to chromosome 16q22.1 by radiation hybrid mapping. Cytogenet Cell Genet 1998;83:82–83.
25 Kanai Y, Ushijima S, Hui AM, Ochiai A, Tsuda H, Sakamoto M, Hirohashi S: The E-cadherin gene is silenced by CpG methylation in human hepatocellular carcinomas. Int J Cancer 1997;71:355–359.
26 Slagle BL, Zhou YZ, Birchmeier W, Scorsone KA: Deletion of the E-cadherin gene in hepatitis B virus-positive Chinese hepatocellular carcinomas. Hepatology 1993;18:757–762.
27 Charpin C, Garcia S, Bonnier P, Martini F, Andrac L, Choux R, Lavaut M-N, Allasia C: Reduced E-cadherin immunohistochemical expression in node-negative breast carcinomas correlates with 10-year survival. Am J Clin Pathol1998;109:431–438.
28 Tamura S, Shiozaki H, Miyata M, Kadowaki T, Inoue M, Matsui S, Iwazawa T, Takayama T, Takeichi M, Monden M: Decreased E-cadherin expression is associated with haematogeneous recurrence and poor prognosis in patients with squamous cell carcinoma of the oesophagus. Br J Surg 1996;83:1608–1614.
29 Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, Hamamura K, Imai Y, Yoshida H, Shiina S, Omata M: Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus. An analysis of 236 consecutive patients with a single lesion. Hepatology 2000;32:1216–1223.
30 Yamamoto J, Iwatsuki S, Kosuge T, Dvorchik I, Shimada K, Marsh JW, Yamasaki S, Starzl TE: Should hepatomas be treated with hepatic resection or transplantation? Cancer 1999;86:1151–1158.

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Effectiveness of Endoscopic Nasobiliary Drainage for Postoperative Bile Leakage after Hepatic Resection

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Abstract. The effectiveness of endoscopic nasobiliary drainage (ENBD) for postoperative bile leakage after hepatic resection was investigated retrospectively. Between 1997 and 2002 a series of 486 hepatectomies without biliary reconstruction were performed. Bile leakage was divided into two categories. Type A was defined as bile leakage communicating with the main bile tree fistulographically or endoscopic cholangiographically, and type B was bile leakage without such a patency of bile flow. Bile leakage developed in 31 patients (6.4%) (types A/B = 16/15). Type A frequently occurred at the major Glisson's sheath. In contrast, most type B cases occurred at the peripheral bile duct at the cut surface of the liver. Among the type A patients, 10 of 11 were effectively treated with ENBD. For the type B patients, 12 of 15 patients were successfully treated with intraabdominal drainage via surgical drains inserted during the operation or percutaneous tubes newly inserted for biliary fluid collection. ENBD was effective in two of three type B patients. The duration of bile leakage significantly shortened after initiation of ENBD in type A patients (15.3 ± 6.9 vs. 25.8 ± 13.2 days, $p < 0.05$). The classification based on communication with the main bile tree is useful for determining therapeutic strategy. Type A leakage has a good indication for ENBD, whereas type B can be treated with intraabdominal drainage in most cases, although ENBD may be effective in some intractable type B cases. It is preferable to initiate ENBD as early as possible to shorten the duration of bile leakage and the subsequent hospital stay.

Despite recent advances in liver surgery, bile leakage still is a common cause of major morbidity after hepatic resection, with an incidence of 4% to 17% [1–5]. Postoperative bile leakage results in prolongation of the hospital stay. Furthermore, bile retention in the dead space after hepatic resection sometimes leads to the development of the intraperitoneal septic complications that are known to be a cause of postoperative liver failure and death. Therefore it is necessary to provide an effective therapeutic modality for bile leakage that can be instituted early after its onset.

Endoscopic treatments have been reported to be effective for intractable bile leakage after cholecystectomy [6, 7], liver transplantation [8, 9], liver injury [10], and hepatectomy [11–14]. Endoscopic treatments consist of endoscopic sphincterotomy, endoscopic biliary stenting, and endoscopic nasobiliary drainage (ENBD). In this study, we clarify the therapeutic effect of ENBD on persistent bile leakage after hepatic resection.

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Patients and Methods

A total of 544 patients underwent hepatic resection in the Department of Gastroenterological Surgery at Kyoto University Hospital from April 1997 to December 2002. Those with hepatectomies plus biliary reconstruction were excluded from the study. The remaining 486 patients were retrospectively reviewed. Altogether, 44 of them underwent repeated hepatectomy. The indications for hepatic resection and the incidence of postoperative bile leakage are shown in Table 1.

Surgical Techniques

Tumors were resected anatomically in principle, and the type of hepatectomy was determined by the assessment of preoperative liver function, functional residual liver volume, location of the tumor, number of tumors, and adjacent major hepatic vessels. Prior to hepatic parenchymal transection, hepatic blood inflow into the resected segments was occluded by the standard controlled method or Glisson's pedicle transection method. The hepatic parenchyma was transected using the Cavitron Ultrasonic Surgical Aspirator (CUSA) System 200 Console (Valley Lab, Boulder, CO, USA) and bipolar cautery equipped with a water dripping system as described previously [15]. Bile exsorption at the cut surface or at the stump of the Glisson's sheath was repaired with Prolene 6-0 sutures. Thereafter, diluted indigo carmine was injected through the cystic duct as a bile leak test to exclude occult bile leakage. Intraoperative cholangiography was usually performed to confirm the integrity of the bile duct. A transcystic duct tube (C-tube) or a retrograde transhepatic biliary drainage (RTBD) tube was placed prophylactically in selected patients with major bile exsorption repaired during the operation. Fibrin glue or sheet was applied to the raw surface of the liver to prevent bile leakage when deemed necessary by the opera-

Table 1. Indication for hepatic resection and incidence of postoperative bile leakage.

Indication for resection	No. of patients	No. with bile leakage
Malignant neoplasia	463	28 (6.0%)
Hepatocellular carcinoma	351	21 (6.0%)
Cholangiocellular carcinoma	18	0
Metastatic liver cancer	88	7 (8.0%)
Others[a]	6	0
Benign lesions	23	3 (13.0%)
Liver cyst	7	2 (28.6%)
Hemangioma	4	1 (25.0%)
Others[b]	12	0
Total	486	31 (6.4%)

[a]Other malignant neoplasia consisted of biliary cystadenocarcinoma ($n = 3$), hepatic hilar cholangiocarcinoma ($n = 2$), and gallbladder cancer.
[b]Other benign lesions consisted of a dysplastic nodule ($n = 5$), focal nodular hyperplasia ($n = 2$), intrahepatic cholelithiasis ($n = 2$), MALToma, angiomyolipoma, and adrenal adenoma.

tor. Surgical drainage tubes were removed when the discharge was serous and not biliary, usually between the fifth and seventh postoperative days.

Definitions

Bile leakage was diagnosed based on the following postoperative findings: (1) continuous biliary discharge from drainage tubes irrespective of its bilirubin concentration; or (2) delayed biliary fluid collection (biloma) at the cut surface of the liver, with the patient having clinical symptoms such as fever, pleural effusion, or an elevated serum C-reactive protein (CRP) level after removing the drainage tubes. This fluid collection was usually detected by ultrasonography or computed tomography (CT) and confirmed by percutaneous puncture, drainage, or both. Bile leakage that ceased spontaneously within 1 week from the onset was excluded from this study. The onset of leakage was defined as the date on which biliary discharge from drainage tubes or any clinical symptom associated with bile leakage was detected.

Bile leakage was divided into two categories. Type A was bile leakage recognized to have communication with the main bile tree (seen by fistulography) or to have extravasation of contrast medium, as seen by endoscopic retrograde cholangiopancreatography (ERCP). Type B was bile leakage without such communication with the main bile tree.

ENBD

A XEMEX ENBD tube (Zeon Medical, Tokyo, Japan) was inserted. The ENBD tube was placed peripheral to the leakage site if possible, principally to avoid displacement. Sphincterotomy was not performed in our ENBD cases.

Statistical Analysis

All data were expressed as the mean ± standard deviation. The incidence of bile leakage was statistically analyzed by the χ^2 test or Fisher's exact test. The durations of bile leakage before and after initiation of ENBD in the type A patients were analyzed with the paired t-test. A value of $p < 0.05$ was considered significant.

Table 2. Incidence of postoperative bile leakage for each type of hepatectomy.

Operation	No. of patients	No. with bile leakage	Type A/B
Left trisectionectomy	8	3 (37.5%)*	1/2
Central bisectionectomy	13	4 (30.8%)*	2/2
Right anterior sectionectomy	20	4 (20.0%)*	4/0
Left hepatectomy	74	5 (6.8%)	2/3
Right trisectionectomy	15	1 (6.7%)	1/0
Right hepatectomy	123	8 (6.5%)	3/5
Right posterior sectionectomy	41	2 (4.9%)	1/1
Segmentectomy	27	1 (3.7%)	0/1
Partial hepatectomy	133	3 (2.3%)	2/1
Left lateral sectionectomy	24	0	
Left medial sectionectomy	8	0	
Total	486	31 (6.4%)	16/15

*$p < 0.05$, compared with partial hepatectomy.

Results

Incidence

Postoperative bile leakage occurred in 31 of 486 patients (6.4%), 16 of whom were allocated to type A and 15 to type B. The incidence of bile leakage was not significantly different between malignant and benign diseases [28/463 (6.0%) vs. 3/23 (13.0%), respectively] (Table 1) and between primary hepatectomy and repeat hepatectomy [28/442 (6.3%) vs. 3/44 (8.6%), respectively]. The incidences of bile leakage for the various types of hepatectomy are shown in Table 2. The terminology of hepatic anatomy and resections is based on the liver terminology guidelines of the International Hepato-Pancreato-Biliary Association [16]. Bile leakage after left trisectionectomy, central bisectionectomy, or right anterior sectionectomy was observed in 11 patients. Their incidences were significantly higher than that for nonanatomic partial hepatectomy ($p < 0.05$). Type A bile leakage occurred more frequently than type B bile leakage in these 11 patients (7/11 vs. 4/11 for types A and B, respectively).

Leakage Site

The types of bile leakage and the sites of leakage are shown in Table 3. More than half of type A bile leakage occurred at the major Glisson's sheath (a first- or second-order biliary branch). In contrast, no type B leakage occurred at the major Glisson's sheath.

Treatments for Bile Leakage

Two type A patients were successfully treated with interventional therapy alone using a surgical drainage tube (Table 4). In two patients, biliary decompression tubes placed prophylactically during the operation were effective; one was a T-tube for a bile duct injury after hepatic hilar lymph node dissection and the other was a C-tube placed after repair of major bile exsorption at the stump of the left hepatic duct. In the other 12 patients, bile leakage was persistent; 11 patients were treated with ENBD. Subsequently, bile leakage healed without any additional therapeutic modalities. The other patient was treated with percutaneous transhepatic biliary drainage (PTBD) because ENBD failed. Twelve patients with type B leakage were treated successfully with interventional therapy us-