Patterns of Regional Lymph Node Involvement in Intrahepatic Cholangiocarcinoma of the Left Lobe

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Lymph node involvement is an important prognostic factor in intrahepatic cholangiocarcinoma. Besides the nodes in the hepatoduodenal ligament, recent studies have suggested that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature can be affected when the primary tumor is located in the left hepatic lobe. However, the distribution of metastatic nodes has not been well described in this disease. Thirteen patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study. Lymphatic mapping was performed by means of both histologic examination and reverse transcriptase–polymerase chain reaction assays. Nodal involvement around the cardiac portion of the stomach or along the lesser gastric curvature (left pathway) was found in 7 (54%) of 13 patients by histologic examination or reverse transcriptase–polymerase chain reaction, whereas positive nodes in the hepatoduodenal ligament (right pathway) were found in 6 (46%) of 13 patients. Two patients (15%) had positive nodes only in the left pathway. Therefore, for a more accurate clinical staging of intrahepatic cholangiocarcinoma in the hepatic left lobe, lymph nodes around the cardiac portion of the stomach and along the lesser gastric curvature should be examined in addition to nodes in the hepatoduodenal ligament.
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KEY WORDS: Intrahepatic cholangiocarcinoma, lymph node, metastasis

Intrahepatic cholangiocarcinoma, a primary adenocarcinoma of the liver originating from the intrahepatic biliary epithelium, is the second most common primary hepatic malignancy, next to hepatocellular carcinoma.[1] The incidence of this malignancy is increasing rapidly worldwide.[2] In the United States, the age-adjusted mortality rate per 100,000 persons has increased from 0.15 to 0.66 in the past two decades.[3] For patients with this malignancy, only complete surgical resection provides the opportunity for cure and longer survival. However, despite recent advances in hepatobiliary surgery including safe major hepatectomy and extended lymphadenectomy with low perioperative mortality, outcomes with intrahepatic cholangiocarcinoma have been poor with 3-year survival ranging from 16% to 61% even in the patients who underwent curative resection.[4–8] Because the outcomes of patients with cholangiocarcinoma may be attributed to not only the difficulty of early detection but also the failure to select patients appropriately, precise staging of the disease should be emphasized to allow a better prognostic stratification of patients, and thus a better therapeutic approach in planning optimal management for patients with cholangiocarcinoma.

Lymph node involvement, which differentiates intrahepatic cholangiocarcinoma from hepatocellular carcinoma, is associated with poor prognosis in cholangiocarcinoma.[5–8] Precise knowledge of the mode of lymphatic spread is imperative to determine the

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extent of lymphadenectomy and to allow a better prognostic evaluation. It is well known that lymphatic drainage of the liver flows along the hepatoduodenal ligament and that nodes in this area are defined as regional.[9] Recently another lymphatic pathway across the lesser omentum from the hepatic bed to the stomach has been recognized.[10] Two reports have described metastatic lymph nodes around the cardiac portion of the stomach or along the gastric lesser curvature in patients with intrahepatic cholangiocarcinoma in the hepatic left lobe.[5,10] In these reports, however, lymphadenectomy around the cardia and gastric lesser curvature was not systemically performed, and the frequency of positive nodes in these areas remains obscure.

The present study was conducted to assess the pattern of lymphogenous tumor cell spread in patients with intrahepatic cholangiocarcinoma in the left hepatic lobe. We particularly focused on the incidence of nodal metastasis around the cardiac portion of the stomach and along the lesser gastric curvature. Not to ignore any minimum metastatic foci, we used molecular-based analysis for diagnosis of lymph node status in addition to the histopathologic approach. This previously established genetic detection system,[11] which uses carcinoembryonic antigen (CEA) and mammaglobin B (MMGB) as genetic markers, is an assay with a high sensitivity and a lower false negative rate for the detection of lymph node micrometastasis in cancer of the biliary tract.

PATIENTS AND METHODS

Patients and Surgical Samples

Thirteen consecutive patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study; written informed consent was obtained from all of them. All patients were treated with curative intent between 1997 and 2001 at Osaka University Hospital (Osaka, Japan) and Moriguchi Keijinkai Hospital (Osaka, Japan) by left lobectomy or extended left lobectomy of the liver; additional caudate lobectomy, if necessary, and lymphadenectomy along the hepatoduodenal ligament, around the cardiac portion of the stomach, and along the gastric lesser curvature were also performed. We collected 13 primary tumor tissues and 275 lymph nodes, and documented the location of each lymph node. Each lymph node was cut into two pieces. One piece was fixed in formalin and embedded in paraffin for routine histologic examination using hematoxylin and eosin staining, and the other piece was stored for reverse transcriptase–polymerase chain reaction (RT-PCR) assay. Tissue samples for molecular analysis were immediately frozen in liquid nitrogen after surgical resection at −80° C until ribonucleic acid (RNA) extraction.

RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction

RNA extraction was carried out with the use of TRIZOL reagent (Life Technologies, Vienna, Austria) in a single-step method, and purified total cellular RNA was quantitated and assessed for purity by means of ultraviolet spectrophotometry. Complementary deoxyribonucleic acid (cDNA) was generated from 1 μg RNA with avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, WI). The amplification of each specific RNA was performed in a 25 μl reaction mixture containing 2 μl of cDNA template, 1 × Perkin-Elmer (Norwalk, CT) polymerase chain reaction buffer, 1.5 mmol/L of $MgCl_2$, 0.8 mmol/L of deoxynucleotide triphosphate, 5 pmol of each primer, and 1 unit of Taq DNA polymerase (AmpliTaq Gold; Roche Molecular Systems, Inc., NJ). The polymerase chain reaction primers used for detection of porphobilinogen deaminase (PBGD), MMGB, and CEA have been previously described.[11–13] These primers were designed to flank intronic sequences in order to avoid false positive results due to amplification of contaminated genomic DNA. The polymerase chain reaction cDNA products of PBGD, MMGB, and CEA were 127, 245, and 160 base-pairs, respectively. The annealing temperature and cycles for the polymerase chain reaction were set up as follows: one cycle of denaturing at 95° C for 12 minutes, followed by 40 cycles (95° C for 1 minute, 62° C for 1 minute, and 72° C for 1 minute for PBGD and 95° C for 1 minute, 58° C for 1 minute, and 72° C for 1 minute for mammaglobin B) or 35 cycles (95° C for 1 minute and 72° C for 1.5 minutes for CEA) before a final extension at 72° C for 10 minutes. These polymerase chain reaction conditions were set up in a GeneAmp PCR System 9600 (Perkin-Elmer). Aliquots (8 μl) from each reaction mixture were size fractionated on 2% agarose gel and visualized with ethidium bromide staining. To verify the integrity of each RNA sample, PBGD as the housekeeping gene was amplified. Specimens that failed to amplify PBGD were not considered.

Evaluation of the Mode of Lymphatic Tumor Cell Spread

Each lymph node was evaluated by histologic analysis and RT-PCR assay separately. The results were marked on an anatomic map of each patient. To assess lymphogenous tumor cell spread from the primary lesion, we categorized the site of lymph nodes into the following three groups: (1) lymph nodes along the

right pathway; (2) lymph nodes along the left pathway; and (3) lymph nodes in any distant areas. Lymph nodes located in the hepatoduodenal ligament were considered nodes along the right pathway. Lymph nodes around the cardiac portion of the stomach and along the gastric lesser curvature were considered nodes along the left pathway (Fig. 1). The group of distant areas includes all nodes collected from retroperitoneal tissue along the celiac artery, superior mesenteric artery, aorta, inferior vena cava, or common hepatic artery.

RESULTS

Patient Characteristics

Patient characteristics are presented in Table 1. The median age of the 13 patients accrued for this study was 61 years (range 34 to 77 years). There were five men and eight women. The median tumor size was 4.5 cm (range 1.5 to 9.0 cm). On the basis of histologic findings, 12 tumors were confirmed to be adenocarcinoma and one tumor (in patient 7; see Table 1) was a mixed type of intrahepatic adenocarcinoma with hepatocellular carcinoma. Left lobectomy was performed in seven patients, left and caudate lobectomy in four patients, and extended left hepatectomy in two patients. None of the patients had any major surgical complications.

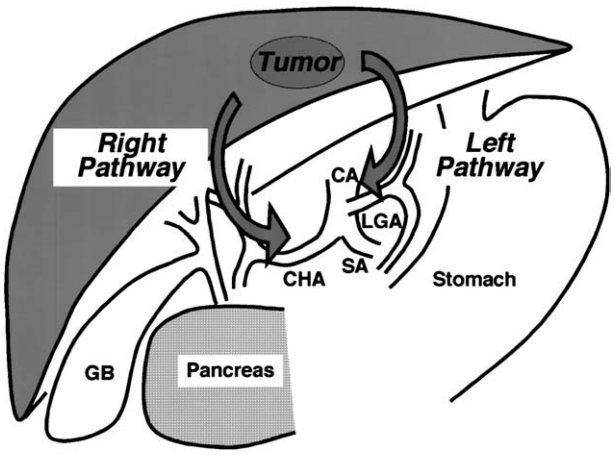

Fig. 1. Schematic of two possible drainage pathways. Two lymphatic pathways from the hepatic left lobe are shown: the right pathway through the hepatoduodenal ligament and the left pathway through the lesser omentum to the cardiac portion of the stomach and the gastric lesser curvature. CA = celiac artery; CHA = common hepatic artery; GB = gallbladder; LGA = left gastric artery; SA = splenic artery.

Lymph Node Metastasis

A total of 275 lymph nodes were harvested from 13 patients, ranging from 5 to 57 nodes per patient with a median value of 20 lymph nodes. Metastases were found in 27 nodes by means of histologic examination and in 51 nodes by RT-PCR assay (see Table 1). All 27 histologically positive nodes were also positive by RT-PCR assay. In addition to these 27 histologically metastasis-positive nodes, another 24 lymph nodes were positive by RT-PCR assay in lymph nodes that were negative according to histologic examination (Fig. 2). The genetic analysis, however, was not applicable for one patient (No. 10; see Table 1) because the primary tumor did not express any of two genetic markers required for RT-PCR. Two of the seven patients with node-negative disease by histologic examination were positive by RT-PCR assay (Nos. 1 and 6; see Table 1). In a patient-based analysis, 6 of 13 patients were node positive by histologic examination and 8 of 12 patients whose primary tumors were positive for either of two genetic markers were node positive by RT-PCR assay.

Anatomic Distribution of Lymph Node Metastases

As described in Patients and Methods, we drew the map of positive and negative nodes in each patient and then analyzed anatomic distribution of lymph node metastases. The summarized anatomic distribution of lymph node metastasis in each patient is shown in Table 1. Positive nodes in the right pathway were found in 5 (38%) of 13 patients by histologic examination and 6 (50%) of 12 patients by RT-PCR assay. In the left pathway, histologic nodal involvement was found in 4 (31%) of 13 patients and in 7 (58%) of 12 patients by RT-PCR assay. The number of patients with lymph nodes containing metastases in both pathways included three (23%) shown by histologic examination and five (38%) by molecular assay (Table 2). Metastasis-positive lymph nodes of distant areas were found in 3 (23%) of 13 patients by histologic examination and in 5 (42%) of 12 patients by RT-PCR assay (see Table 2). In the patients with positive lymph nodes in distant areas, positive nodes were also found in either the right or left pathway. Of all 13 patients, two had positive nodes only within the area along the left pathway by histologic or RT-PCR examination. Detailed anatomic mapping of lymph node metastasis in three patients whose positive nodes were limited to the area along the right and/or left pathway(s) are shown in Fig. 3.

Table 1. Summary of clinical features and nodal status in each patient

Patient	Age (yrs)	Sex	Tumor size (cm)	Operation	No. of lymph nodes sampled	No. of histologically positive nodes	No. of RT-PCR–positive nodes	Right pathway		Left pathway		Distant area	
								Histology	RT-PCR	Histology	RT-PCR	Histology	RT-PCR
1	58	F	5.0	Left and caudate lobectomy	57	0	13	(−)	(+)	(−)	(+)	(−)	(+)
2	77	F	2.5	Left and caudate lobectomy	10	0	0	(−)	(−)	(−)	(−)	(−)	(−)
3	70	M	1.5	Left lobectomy	5	0	0	(−)	(−)	(−)	(−)	NS	NS
4	60	F	7.0	Left lobectomy	10	7	9	(+)	(+)	(+)	(+)	(+)	(+)
5	67	M	3.0	Left lobectomy	20	1	1	(−)	(−)	(+)	(+)	(−)	(−)
6	65	F	6.2	Left lobectomy	31	0	1	(−)	(−)	(−)	(+)	(−)	(−)
7	61	M	9.0	Left lobectomy	14	7	10	(+)	(+)	(+)	(+)	(+)	(+)
8	72	F	3.5	Left and caudate lobectomy	20	0	0	(−)	(−)	(−)	(−)	(−)	(−)
9	67	M	4.5	Left lobectomy	20	0	0	(−)	(−)	(−)	(−)	(−)	(−)
10	71	M	3.5	Extended left lobectomy	34	0	NA	(−)	NA	(−)	NA	(−)	NA
11	34	F	8.0	Left and caudate lobectomy	13	8	10	(+)	(+)	(+)	(+)	(+)	(+)
12	60	F	6.5	Extended left lobectomy	25	3	4	(+)	(+)	(−)	(−)	(−)	(+)
13	63	F	4.2	Left lobectomy	16	1	3	(+)	(+)	(−)	(+)	(−)	(−)

NA = not applicable; NS = not sampled; RT-PCR = reverse transcriptase–polymerase chain reaction.

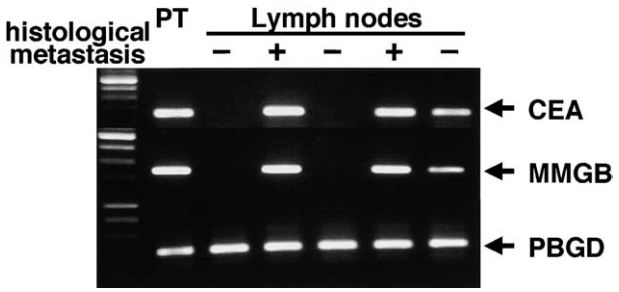

Fig. 2. Typical profile of detection of carcinoembryonic antigen (*CEA*) and mammaglobin B (*MMGB*) reverse transcriptase–polymerase chain reaction (RT-PCR) products in lymph nodes. *Upper*, CEA RT-PCR; *B*, MMGB RT-PCR; *lower*, Porphobilinogen deaminase (*PBGD*) RT-PCR. PT = primary tumor; − = histologically metastasis-negative node; + = histologically metastasis-positive node.

Table 2. Summary of patients with lymph node metastasis

	Histology (n = 13)	RT-PCR (n = 12)
Nodal metastasis (−)	7 (54%)	4 (33%)
Nodal metastasis (+)	6 (46%)	8 (67%)
Right pathway (+)	5 (38%)	6 (50%)
Left pathway (+)	4 (31%)	7 (58%)
Distant area (+)	3 (23%)	5 (42%)

RT-PCR = reverse transcriptase–polymerase chain reaction.

DISCUSSION

Negative nodal status for metastasis is one of the important favorable prognostic factors after hepatectomy for intrahepatic cholangiocarcinoma.[4–8] However, the distribution of metastatic nodes has not been well described in this disease. In the present study, we clearly demonstrated that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature were common sites of lymphatic metastases in patients with left intrahepatic cholangiocarcinoma. Lymph node metastasis in these regions were detected in 4 (31%) of 13 patients by histologic examination and in 7 (58%) of 12 patients by molecular examination, whereas metastases in the right pathway were detected in 5 (38%) of 13 patients by histologic examination and 6 (50%) of 12 patients by molecular examination. The frequency of the lymph node metastasis in the left pathway seems to

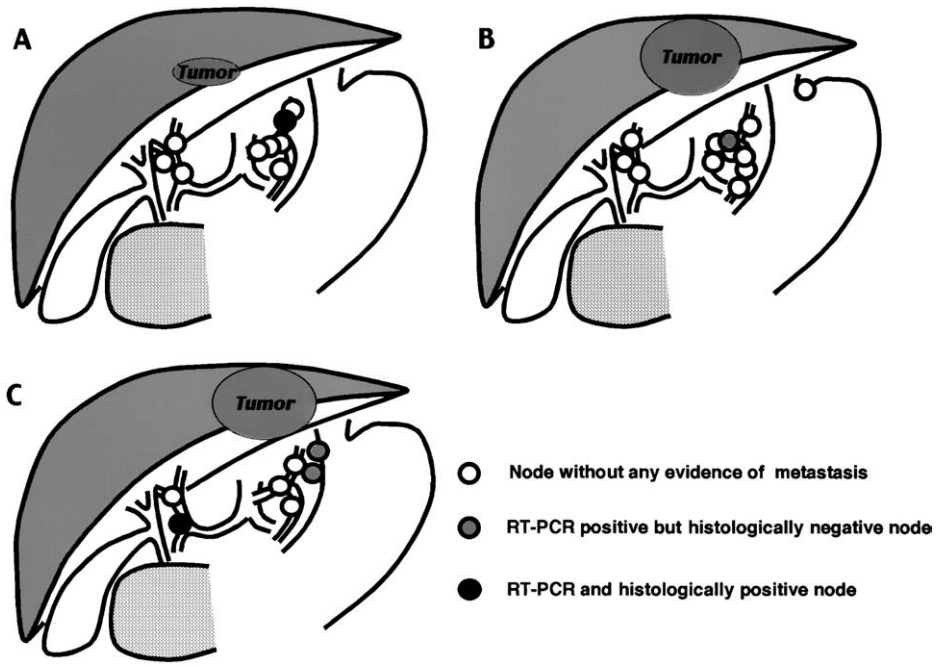

Fig. 3. Lymphatic maps of positive and negative nodes in three representative cases. **A**, Patient 5 in Table 1 had one positive node detected by both histopathologic examination and reverse transcriptase–polymerase chain reaction (*RT-PCR*) assay along the lesser gastric curvature. **B**, Patient 6 in the Table 1 had one positive node that was not detected by histologic examination but could be detected by RT-PCR. This node was also located in the left pathway. **C**, Patient 13 had three positive nodes. One was detected in the hepatoduodenal ligament by histologic examination and the others were detected in the connective tissue around the gastric cardia by RT-PCR.

be nearly equal to that in the right pathway, a pathway considered to be the primary regional nodal pathway in left intrahepatic cholangiocarcinoma.[14] Of note, two (29%) of seven patients with metastatic nodes had a positive node only along the left pathway. If no attention had been paid to the nodal status in that region, the stage of these patients would have been underestimated as metastasis-free disease. Furthermore, lymph nodes in distant areas, such as nodes in retroperitoneal tissue along the celiac artery, aorta, inferior vena cava, or common hepatic artery, were also affected in 3 (25%) of 13 patients by histologic examination and in 5 (42%) of 12 patients by molecular examination. All patients with positive nodes in any of these distant areas also had metastatic nodes in the right and/or left pathway, suggesting that tumor cells passed through either of these two pathways to spread to the distant area. On the basis of our findings, we propose that both the nodes along the left pathway and those along the right pathway should be classified as regional lymph nodes of intrahepatic cholangiocarcinoma arising in the left hepatic lobe.

According to the TNM staging system, which is applied to intrahepatic cholangiocarcinoma, the regional site of this disease is limited to the hepatoduodenal ligament regardless of where in the liver the primary tumor is located.[14] Nozaki et al.[10] had previously reported that intrahepatic cholangiocarcinoma in the left hepatic lobe displayed a different distribution of metastatic nodes from intrahepatic cholangiocarcinoma in the right lobe. The most important point in their article was that the lymph nodes around the cardiac portion and along the lesser curvature of the stomach were affected, as well as nodes in the hepatoduodenal ligament, if the primary tumors were located in the left lobe, as shown in the present study. However, as mentioned in their article, it was possible that they missed small metastatic foci within the clinically unremarkable lymph nodes because only enlarged lymph nodes were sampled and examined histopathologically. In the present study we evaluated patients who had intrahepatic cholangiocarcinoma that was limited to the left hepatic lobe and offered the same manner of lymphadenectomy to all patients. To avoid missing any metastatic lymph nodes, we removed the entire connective tissue in the hepatododenal ligament area and the lesser omentum, along the lesser gastric curvature, and around the cardiac portion of the stomach and then sampled all lymph nodes in the resected specimen. Furthermore, to detect metastasis more accurately, we used not only a histologic examination but also a molecular-based analysis.[11] Thus the present findings may be more reliable than those in some previous studies.[5,10]

In recent years many analyses based on molecular techniques have been developed to evaluate minimal residual cancer.[15] We applied a RT-PCR assay with two molecular markers[11] to assess the presence of small metastatic foci (micrometastasis) in lymph nodes that were not detected by histologic examination. Indeed, of the 214 histologically negative nodes in six patients, we detected lymph node "micrometastatses" in 24 nodes from two patients by RT-PCR assay. Thus we believe that our highly sensitive RT-PCR assay with two molecular markers enabled us to more accurately analyze the lymphatic spread of cancer cells from the left hepatic lobe.

From a clinical point of view, whether the presence of positive nodes along the left pathway correlates with postoperative prognosis remains unknown. Despite the short follow-up period, five of the seven patients with lymph node metastasis either in the right or left pathway had a recurrence within 1 year after surgery, whereas only one of five patients without any evidence of lymph node metastasis had a recurrence. This observation suggests the importance of lymph node staging for optimal adjuvant treatment after surgery. Further study with more patients and long-term analyses are needed to reveal the clinical implications of nodal status in patients with left intrahepatic cholangiocarcinomas.

CONCLUSION

For more accurate clinical staging of the patients with intrahepatic cholangiocarcinoma in the left hepatic lobe, lymph nodes around the cardia and along the gastric lesser curvature should be considered regional lymph nodes in addition to those along the hepatoduodenal ligament.

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