1 Georgiou P, Tan E, Gouvas N, et al. Extended lymphadenectomy versus conventional surgery for rectal cancer: a meta-analysis. *Lancet Oncol* 2009; 10: 1053–62.

2 Ueno M, Oya M, Azekura K, et al. Incidence and prognostic significance of lateral node metastasis in patients with advanced low rectal cancer. *Br J Surg* 2005; 92: 756–63.

3 Kusters M, Beets GL, van de Velde CJH, et al. A comparison between the treatment of low rectal cancer in Japan and the Netherlands, with focus on the patterns of local recurrence. *Ann Surg* 2009; 249: 229–35.

4 Yano H and Moran BJ. The incidence of lateral pelvic side-wall nodal involvement in low rectal cancer may be similar in Japan and the West. *Brit J Surg* 2008; 95: 33–49.

5 Moriya Y, Sugihara K, Akasu T, et al. Nerve-sparing surgery with lateral node dissection for advanced lower rectal cancer. *Eur J Cancer* 1995; 31A: 1229–32.

6 Birgisson H, Pahlman L, Gunnarsson U, et al. Occurrence of second cancers in patients treated with radiotherapy for rectal cancer. *J Clin Oncol* 2005; 23: 6126–31.

Treatment of gastric cancer in Asia: the missing link

Conventional chemotherapy for gastric cancer is known to improve overall survival, quality of life (QOL), and the length of time a patient is free of symptoms compared with best supportive care,[1] but outcomes for advanced gastric cancer are still extremely poor. Although various combinations of platinum compounds and fluoropyrimidine derivatives improve patient outcomes, no accepted global standard exists for the treatment of gastric cancer. Additionally, there are marked geographical differences in the prevalence of types of gastric cancer, with intestinal-type distal gastric cancer related to *Helicobacter pylori* predominant in Asia, compared with the predominance of proximal and diffuse types of gastric cancer in Europe and North America. There are also marked regional differences in how gastric cancer is treated.

One common trend in chemotherapy is the replacement of intravenous infusion with oral administration, thus improving patient QOL and decreasing the length of time spent in hospital. In this issue of *The Lancet Oncology*, Boku and colleagues[2] show that S-1, an oral fluoropyrimidine derivative, is as effective as continuous infusion of fluorouracil for the treatment of advanced gastric cancer. S-1 contains tegafur (a prodrug of fluorouracil), 5-chloro-2,4-dihydropyrimidine (a reversible inhibitor of dihydropyrimidine dehydrogenase), and potassium oxonate. In phase 2 trials, S-1 showed good results in Japanese patients. It has recently been suggested that S-1 should be given in adjuvant settings to Asian patients with locally advanced gastric cancer after D2 dissection.[3] The SPIRITS trial[4] comparing S-1 plus cisplatin with S-1 alone, which started 2 years after the study by Boku and colleagues, showed that the combination of S-1 plus cisplatin seems to be more effective than S-1 monotherapy (p=0·04 for overall survival). Thus the study by Boku and colleagues is a missing link: together, the study by Boku and colleagues and the SPIRITS trial indicate that the combination of cisplatin plus S-1 should replace cisplatin plus fluorouracil as the first-line treatment of choice for Japanese patients with advanced gastric cancer. However, S-1 shows a different toxicity profile in patients in Europe and the USA, including severe diarrhoea and frequent neutropenia, and is therefore not always as effective as has been seen in Japan because of low dose intensity. Although the efficacy of S-1 plus cisplatin was similar to fluorouracil plus cisplatin in the FLAGS study,[5] oral administration of capecitabine, another fluoropyrimidine derivative, is recommended for western patients because of its efficacy and lower toxicity. Cisplatin plus capecitabine is non-inferior to fluorouracil plus cisplatin in advanced gastric cancer,[6] and capecitabine and oxaliplatin are as effective as fluorouracil and cisplatin in first-line triplet therapy with epirubicin for oesophagogastric cancer,[7] suggesting cisplatin plus capecitabine or capecitabine plus oxaliplatin plus epirubicin as a standard therapy for advanced gastric cancer or oesophagogastric cancer. Therefore, there are several different standards for the treatment of advanced gastric cancer throughout the world.

A combination regimen with platinum compounds, fluorouracil derivatives, and/or taxanes is usually more effective than monotherapy.[1] Boku and colleagues also examined whether the doublet of irinotecan plus cisplatin was more effective than fluorouracil, but noted that it was not (p=0·055). This may be partly due to the design of the three-group comparison, and relatively low statistical power. Nevertheless, triple therapy is hopefully more effective than monotherapy or doublet therapy. Several phase 2 studies have indicated that docetaxel plus cisplatin and fluorouracil is promising, despite its high toxicity.[1] However, targeted agents with more favourable toxicity profiles, such as trastuzumab, combined with cytotoxic agents might substantially improve survival and reduce toxic side-effects, as was seen in the ToGA

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ORIGINAL ARTICLE

Risk factors of lateral pelvic lymph node metastasis in advanced rectal cancer

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Abstract
Background To clarify the risk factors of lateral pelvic lymph node (LPLN) metastasis of rectal cancer, we examined associations between LPLN status and clinicopathological factors including LPLN status diagnosed by computed tomography (CT).
Methods We reviewed a total of 210 patients with advanced rectal cancer, of which the lower margin was located at or below the peritoneal reflection, who underwent preoperative CT with 5-mm-thick sections and lateral pelvic lymph node dissection at the National Cancer Center Hospital between February 1998 and March 2006.
Results Forty-seven patients (22.4%) had LPLN metastasis. Multivariate analysis showed that LPLN status diagnosed by CT, pathological regional lymph node status, tumor location, and tumor differentiation were significant risk factors for LPLN metastasis. Among 45 patients with well-differentiated adenocarcinoma who were LPLN-negative and in whom CT had found no regional lymph node metastasis, none had LPLN metastasis. On the other hand, among 13 patients with moderate or less differentiated lower rectal adenocarcinoma who were LPLN-positive and in whom CT had revealed regional lymph node metastasis, 12 (92.3%) had LPLN metastasis.
Conclusions LPLN status diagnosed by CT, pathological regional LN status, tumor location, and tumor differentiation are significant risk factors for LPLN metastasis. Using these factors, patients can be classified as having a low or high risk of LPLN metastasis.

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Keywords Rectal cancer · Lymph node dissection · Lateral pelvic lymph node · Risk factor

Introduction

Lateral pelvic lymph node dissection (LPLD) is widely performed for advanced lower rectal cancer in Japan, and the incidence of lateral pelvic lymph node (LPLN) metastasis has been demonstrated to be 15–30% [1–3]. In spite of the relatively high incidence of LPLN metastasis, most surgeons, except for those in Japan, do not perform LPLD, and instead adjuvant chemoradiotherapy and total mesorectal excision (TME) have become the standard therapy for rectal cancer. In order to clarify the indications for, and the possible benefits of, LPLD, a retrospective multicenter study was conducted in Japan, and this demonstrated that LPLD was effective for local control, and might be indicated for patients with T3–T4 lower rectal cancer [3]. The 5-year survival rate of patients with LPLN metastasis is about 40% [1–3], which is comparable with that of patients with resectable liver or lung metastasis. From this viewpoint, LPLN metastasis should be classified as distant metastasis, and resected if at all possible. Kim et al. demonstrated that LPLN metastasis is a major cause of local recurrence in patients who receive preoperative chemoradiotherapy without LPLD [4]. This indicates that LPLD should not be neglected even in the era of neo-adjuvant therapy for rectal cancer. Therefore, accurate preoperative diagnosis of pelvic lateral node metastasis is important. Although Yano et al. showed that conventional CT accurately predicted LPLN status [5], validation studies are necessary. In this study, therefore, we examined the association between clinicopathological factors, including CT diagnosis of lymph nodes and LPLN status, and

selected high-risk factors for LPLN metastasis, enabling classification of patients according to LPLN metastasis risk.

Patients and methods

Patients

We reviewed a total of 210 patients with advanced rectal cancer, of which the lower margin was located at or below the peritoneal reflection, who underwent preoperative computed tomography (CT) with 5-mm-thick sections and lateral pelvic lymph node dissection (LPLD) at the National Cancer Center Hospital between February 1998 and March 2006. All the patients underwent TME or tumor-specific mesorectal excision. Pelvic autonomic nerves were preserved completely or partially in 187 patients (89%). The patients were followed up at 3-monthly intervals for 2 years, and at 6-monthly intervals thereafter. Tumor markers were examined at every patient visit. CT of the liver and lung or abdominal ultrasonography with chest X-ray was performed at least every 6 months. Colonoscopy was performed twice within 5 years after surgery. Median follow-up time was 3.8 years. Six patients received preoperative or postoperative radiotherapy. Pathological stage III patients were given adjuvant chemotherapy.

Diagnosis

All the patients underwent preoperative CT with 5-mm-thick sections using intravenous contrast media, and lymph nodes more than 5 mm in diameter were considered positive (Fig. 1). A radiologist interpreted the CT images preoperatively, and one author (SF) interpreted the images postoperatively. The author finally determined the lymph node status. Lymph nodes were classified according to their location. Lymph nodes in the lateral pelvic area outside the pelvic plexus and hypogastric nerves along the internal ileac, external ileac, common ileac vessels, and in the obturator space were considered LPLN. Patients with LPLN metastasis were classified as stage III in this study. Lymph nodes in the area lying along the inferior mesenteric vessels were considered regional lymph nodes. Tumor size and annularity were determined preoperatively by colonoscopy, barium enema, or virtual colonoscopy. Depth of invasion (T) and tumor location were determined preoperatively by CT or magnetic resonance imaging (MRI), and tumor location was finally confirmed during surgery. All the cancers were biopsied and a pathological diagnosis obtained before surgery.

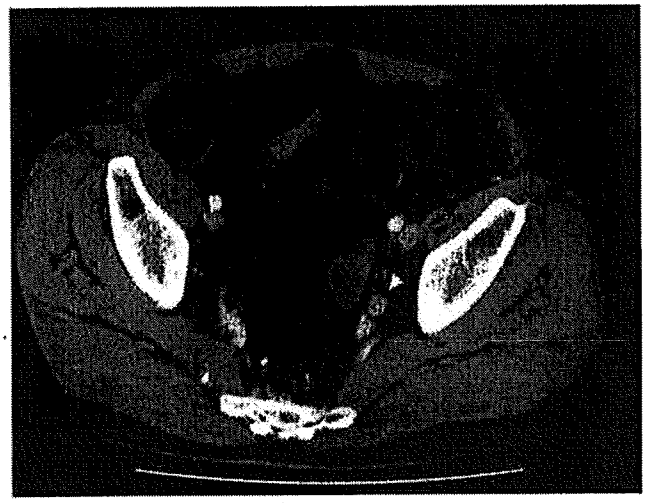

Fig. 1 Representative lateral pelvic lymph node swelling detected by CT. Left lateral pelvic lymph node swelling is seen (*arrowhead*). The lymph node diameter is 10 mm. This patient underwent lateral pelvic lymph node dissection and metastasis was found by pathological examination 201 × 285 mm

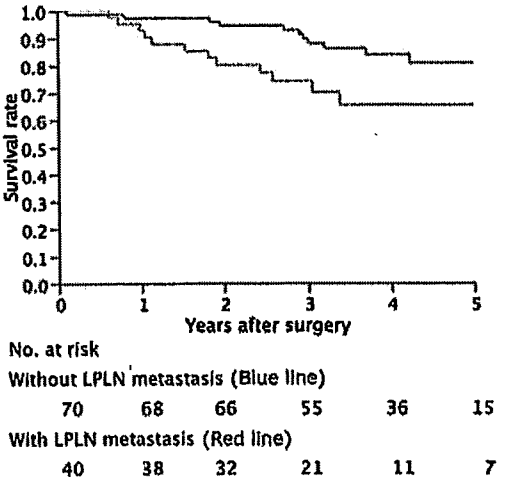

Fig. 2 Survival curves for patients with stage III rectal cancer with and without LPLN metastasis. 201×285 mm

Statistical analysis

Statistical analysis was carried out by the chi-squared test. Survival rates were calculated by the Kaplan–Meier method, and survival curves were compared by the log-rank test. A logistic regression model was used for multivariate analysis. Data differences between groups were considered statistically significant at $P<0.05$.

Results

Incidence of LPLN metastasis and prognosis

Among the 210 patients, 47 (22.4%) had LPLN metastasis. The survival curves for stage III patients are shown in

Fig. 2. The survival rate of stage III patients with LPLN metastasis was significantly poorer than that of stage III patients without LPLN metastasis (P=0.014). Although the follow-up period was insufficient, the estimated 5-year survival rate for the patients with LPLN metastasis was 54%. The incidence of local recurrence in stage III patients with LPLN metastasis was 22.5% (9/40) and that in stage III patients without LPLN metastasis was 10.0% (7/70). Although the incidence of local recurrence in stage III patients with LPLN metastasis was higher than that in stage III patients without LPLN metastasis, the difference was not statistically significant (P=0.074).

Table 1 Incidence of LPLN metastasis and preoperative clinicopathological factors

	LPLN metastasis positive (n=47)	LPLN metastasis negative (n=163)	P
Age (years)			0.749
<60	25	91	
≥60	22	72	
Sex			0.336
Male	30	116	
Female	17	47	
CEA (ng/ml)			0.072
≤5	25	110	
>5	22	53	
Tumor location			0.018
Ra	3	35	
Rb	44	128	
Clinical T			0.616
T1, 2	4	14	
T3	31	118	
T4	12	31	
Regional LN status			0.014
Negative	13	78	
Positive	34	85	
LPLN status			<0.001
Negative	18	147	
Positive	29	16	
Tumor size (cm)			0.673
≤5	22	82	
>5	25	81	
Annularity			0.197
≤2/3	23	97	
>2/3	24	66	
Tumor differentiation			<0.001
Well	14	92	
Moderate	26	66	
Poor, mucinous	7	5	

Ra tumor center located above the peritoneal reflection; *Rb* tumor center located below the peritoneal reflection

Table 2 Incidence of LPLN metastasis and postoperative clinicopathological factors

	LPLN metastasis positive (n=47)	LPLN metastasis negative (n=163)	P
Pathological T			0.058
T1, 2	4	38	
T3	40	111	
T4	3	14	
Pathological regional LN status			<0.001
Negative	7	84	
Positive	40	79	
Lymphatic invasion			<0.001
Negative	17	116	
Positive	30	47	
Venous invasion			0.002
Negative	11	80	
Positive	36	83	
Perineural invasion			0.001
Negative	27	131	
Positive	20	31	
Tumor budding			0.073
Negative	15	76	
Positive	32	87	

Associations of LPLN metastasis with clinicopathological factors

Associations of LPLN metastasis with preoperative clinicopathological factors are shown in Table 1. LPLN status and regional lymph node status diagnosed by CT, tumor location, and tumor differentiation were significantly associated with LPLN metastasis. Associations of LPLN metastasis with postoperative clinicopathological factors are shown in Table 2. Pathological regional lymph node status, lymphatic invasion, venous invasion, and perineural invasion were significantly associated with LPLN metastasis. Multivariate analysis showed that LPLN status diagnosed by CT, pathological regional lymph node status, tumor location, and tumor differentiation were significant risk factors for LPLN metastasis (Table 3).

Incidence of LPLN metastasis according to risk factors

In order to identify patients at low risk and high risk for LPLN metastasis preoperatively, patients were classified into four groups according to the significant risk factors of LPLN metastasis. Although pathological regional lymph node status was a significant risk factor for LPLN metastasis, regional lymph node status diagnosed by CT

Table 3 Multivariate analysis of clinicopathological factors associated with LPLN metastasis

	Odds ratio (95% C.I.)	P
LPLN status (positive/negative)	28.00 (9.19–102.46)	<0.001
Pathological regional lymph node status (positive/negative)	7.21 (2.19–28.08)	0.002
Tumor location (Rb/Ra)	12.56 (2.35–107.87)	0.009
Tumor differentiation (moderate, others/well)	4.05 (1.47–12.23)	0.009

C.I. confidence interval

was used for the classification, because pathological lymph node status was not clarified preoperatively. Tumors located at Ra (tumor center located above the peritoneal reflection) and tumors located at Rb (tumor center located below the peritoneal reflection) were analyzed separately, and other risk factors were used for the classification. Group I was the group with no risk factors. Group II was the group with negative LPLN status diagnosed by CT but with at least one of the other two risk factors. Group III was the group with positive LPLN status diagnosed by CT but without at least one of the other two risk factors. Group IV was the group with all of the risk factors. Incidences of LPLN metastasis according to this classification are shown in Table 4. Irrespective of tumor location, no patients (0/45) had LPLN metastasis in group I. On the other hand, in group IV, 50.0% (2/4) of the patients with Ra tumors and 92.3% (12/13) of the patients with Rb tumors had LPLN metastasis. When pathological regional lymph node status was used for this classification instead of regional lymph node status diagnosed by CT, 75 patients were classified into group I or group II without pathological lymph node metastasis, and these patients also had no LPLN metastasis.

Discussion

The incidence of LPLN metastasis in patients with advanced lower rectal cancer is 15–30% [1–3]. Although the prognosis of patients with LPLN metastasis is poor, the 5-year survival rate is 40%, being comparable to that of patients with resectable liver or lung metastasis. Sugihara et al. estimated that LPLD would improve the 5-year survival rate of patients with T3–T4 lower rectal cancer by 8% [3]. Therefore, LPLD for patients with LPLN metastasis should be considered. Because accurate diagnosis of LPLN metastasis is difficult, LPLD is routinely performed in Japan for stage II or III rectal cancer located at or below the peritoneal reflection. However, it is still unproved whether LPLD is necessary for patients without LPLN metastasis. In order to acquire level 1 evidence, we are currently performing a clinical trial to compare TME alone with TME plus LPLD for rectal cancer patients without LPLN metastasis (JCOG0212) (ClinicalTrials.gov Identifier NCT00190541). Because accurate preoperative diagnosis of LPLN metastasis is important for treatment of lower rectal cancer, we selected four high-risk factors for LPLN metastasis and were able to estimate the incidence of LPLN metastasis using a combination of these factors. Patients without LN metastasis diagnosed by CT and with well-differentiated adenocarcinoma have no LPLN metastasis, and would not require LPLD. On the other hand, more than 80% of patients with LPLN metastasis diagnosed by CT and with moderate or less differentiated adenocarcinoma have LPLN metastasis, and should undergo LPLD. Therefore, our classification is thought to be useful for determining the indications for LPLD.

Late adverse effects of LPLD are sexual and urinary dysfunction [6]. Recently, TME plus LPLD with autonomic nerve preservation has been performed in Japan, and the incidences of sexual and urinary dysfunction following this treatment have been comparable to those after TME [7–9]. Because the oncological outcome of TME plus LPLD with autonomic nerve preservation is also comparable to that without autonomic nerve preservation [10], the former has become the standard therapy for rectal cancer in Japan. However, when patients have LPLN metastasis or if the tumor has invaded the autonomic nerves, nerve preservation is not possible. Therefore, the autonomic nerves were not preserved in 11% of the patients in this series.

Sex, tumor location, depth of invasion, mesorectal LN status, tumor differentiation, and tumor size are reported to be factors associated with LPLN metastasis [3, 11]. Although our findings were comparable, these previous reports did not take into account LPLN status diagnosed by

Table 4 Incidence of LPLN metastasis according to risk factors

	Incidence of LPLN metastasis
Ra (n=38)	
Group I (n=7)	0.0% (0/7)
Group II (n=27)	3.7% (1/27)
Group III (n=0)	–
Group IV (n=4)	50.0% (2/4)
Rb (n=172)	
Group I (n=38)	0.0% (0/38)
Group II (n=93)	18.3% (17/93)
Group III (n=28)	53.6% (15/28)
Group IV (n=13)	92.3% (12/13)

CT. As demonstrated in the present study, LPLN status diagnosed by CT was the most important risk factor associated with LPLN status. Therefore, accurate diagnostic imaging is important. In this study, the sensitivity, specificity, and accuracy of LPLN status diagnosis using CT were 62%, 90%, and 84%, respectively. Arii et al. demonstrated that the accuracy of LPLN status diagnosis using MRI was 83%, whereas that using CT was 77% [12]. Matsuoka et al. reported that MRI diagnosis of LPLN status had 67% sensitivity, 83% specificity, and 78% accuracy [13]. These results were comparable to ours. On the other hand, Yano et al. showed that CT diagnosis of LPLN status had 95% sensitivity, 94% specificity, and 95% accuracy [5]. However, because the number of patients they examined was small (n=39) and patients who did not undergo LPLD were excluded, the results were not directly comparable with other studies. Quadros et al. reported the preliminary results of LPLN detection using lymphoscintigraphy and blue dye [14]. However, the sensitivity and specificity were 17% and 79%, respectively. Tada et al. demonstrated the effectiveness of ultrasonographic examination for determining LPLN status, the sensitivity, specificity, and accuracy being 75%, 94%, and 93%, respectively [15]. Although this result was excellent, there were some problems and limitations; for example, obturator space lymph nodes were sometimes overlooked, and the use of ultrasonography in obese patients was difficult.

A meta-analysis of mesenteric lymph node diagnosis has indicated that the sensitivity and specificity of CT, MRI, and endoscopic ultrasonography are compatible [16]. Matsuoka et al. also demonstrated that multidetector-row CT was as equally effective as MRI for local staging of rectal cancer [17]. We preliminarily examined the capacity of MRI for diagnosis of lymph node status, and found that its sensitivity was higher and its specificity lower than that of CT, with roughly comparable accuracy. The use of new criteria for lymph node status instead of size [18], or a new MRI contrast agent [19], has been reported to yield better sensitivity and specificity for MRI diagnosis of mesenteric lymph nodes. However, further examinations will be necessary to establish an optimal approach for diagnosis of lymph node status using imaging modalities.

If patients with LPLN metastasis do not undergo LPLD, they would suffer LPLN or local recurrence. Kim et al. showed that adjuvant preoperative radiotherapy without LPLD was unable to control LPLN metastasis and local recurrence [4]: lateral pelvic recurrence was observed in 2.3%, 12.5%, and 68.8% of patients with LPLN measuring <5, 5–10, and≥10 mm, respectively, determined by MRI. On the other hand, Quadros et al. showed that patients who received preoperative adjuvant chemoradiotherapy did not develop LPLN metastasis [14]. A small randomized study that compared adjuvant radiotherapy with LPLD also suggested that LPLD was unnecessary for patients who underwent preoperative radiotherapy [20]. Syk et al. demonstrated that LPLN metastasis was not a major cause of local recurrence of rectal cancer [21]. A comparative study demonstrated that the local recurrence rate in Korean patients who received adjuvant chemoradiotherapy without LPLD was lower than that in Japanese patients who underwent LPLD alone [22]. Moreover, the local recurrence rate in patients with LPLN metastasis has been reported to be 25.6% [3]. In our study, the local recurrence rate in patients with LPLN metastasis was 22.5%, which was significantly higher than that in patients without LPLN metastasis. These facts suggest that LPLD alone is not sufficient for local control in patients with LPLN metastasis. Therefore, a combination of adjuvant radiotherapy with LPLD is thought to be important for treatment of advanced rectal cancer, and a randomized study is required to determine whether LPLD is necessary for patients with LPLN metastasis receiving preoperative chemoradiotherapy.

In conclusion, LPLN status diagnosed by CT, pathological regional LN status, tumor location, and tumor differentiation are significant risk factors for LPLN metastasis. Using these factors, patients can be classified as having a low or a high risk of LPLN metastasis. This classification suggests that LPLD should be considered in patients with advanced lower rectal cancer.

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References

1. Fujita S, Yamamoto S, Akasu T, Moriya Y (2003) Lateral pelvic lymph node dissection for advanced lower rectal cancer. Br J Surg 90:1580–1585
2. Ueno M, Oya M, Azekura K, Yamaguchi T, Muto T (2005) Incidence and prognostic significance of lateral lymph node metastasis in patients with advanced low rectal cancer. Br J Surg 92:756–763
3. Sugihara K, Kobayashi H, Kato T, Mori T, Mochizuki H, Kameoka S, Shirouzu K, Muto T (2006) Indication and benefit of pelvic sidewall dissection for rectal cancer. Dis Colon Rectum 49:1663–1672
4. Kim TH, Jeong SY, Choi DH, Kim DY, Jung KH, Moon SH, Chang HJ, Lim SB, Choi HS, Park JG (2008) Lateral lymph node metastasis is a major cause of locoregional recurrence in rectal cancer treated with preoperative chemoradiotherapy and curative resection. Ann Surg Oncol 15:729–737
5. Yano H, Saito Y, Takeshita E, Miyake O, Ishizuka N (2007) Prediction of lateral pelvic node involvement in low rectal cancer by conventional computed tomography. Br J Surg 94:1014–1019
6. Hojo K, Sawada T, Moriya Y (1989) An analysis of survival and voiding, sexual function after wide iliopelvic lymphadenectomy in patients with carcinoma of the rectum, compared with conventional lymphadenectomy. Dis Colon Rectum 32:128–133

7. Moriya Y, Sugihara K, Akasu T, Fujita S (1995) Nerve-sparing surgery with lateral node dissection for advanced lower rectal cancer. Eur J Cancer 31A:1229–1232
8. Sugihara K, Moriya Y, Akasu T, Fujita S (1996) Pelvic autonomic nerve preservation for patients with rectal carcinoma. Oncologic and functional outcome. Cancer 78:1871–1880
9. Havenga K, Enker WE, McDermott K, Cohen AM, Minsky BD, Guillem J (1996) Male and female sexual and urinary function after total mesorectal excision with autonomic nerve preservation for carcinoma of the rectum. J Am Coll Surg 182:495–502
10. Moriya Y, Sugihara K, Akasu T, Fujita S (1995) Patterns of recurrence after nerve-sparing surgery for rectal adenocarcinoma with special reference to loco-regional recurrence. Dis Colon Rectum 38:1162–1168
11. Ueno H, Mochizuki H, Hashiguchi Y, Ishiguro M, Miyoshi M, Kajiwara Y, Sato T, Shimazaki H, Hase K (2007) Potential prognostic benefit of lateral pelvic node dissection for rectal cancer located below the peritoneal reflection. Ann Surg 245:80–87
12. Arii K, Takifuji K, Yokoyama S, Matsuda K, Higashiguchi T, Tominaga T, Oku Y, Tani M, Yamaue H (2006) Preoperative evaluation of pelvic lateral lymph node of patients with lower rectal cancer: comparison study of MR imaging and CT in 53 patients. Langenbecks Arch Surg 391:449–454
13. Matsuoka H, Nakamura A, Masaki T, Sugiyama M, Nitatori T, Ohkura Y, Sakamoto A, Atomi Y (2007) Optimal diagnostic criteria for lateral pelvic lymph node metastasis in rectal carcinoma. Anticancer Res 27:3529–3533
14. Quadros CA, Lopes A, Araujo I, Fahel F, Bacellar MS, Dias CS (2006) Retroperitoneal and lateral pelvic lymphadenectomy mapped by lymphoscintigraphy and blue dye for rectal adenocarcinoma staging: preliminary results. Ann Surg Oncol 13:1617–1621
15. Tada M, Endo M (1995) Ultrasonographic examination for lateral lymphatic spread and local recurrence of rectal cancer. Preoperative detection and evaluation. Dis Colon Rectum 38:1047–1052
16. Bipat S, Glas AS, Slors FJ, Zwinderman AH, Bossuyt PM, Stoker J (2004) Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging—a meta-analysis. Radiology 232:773–783
17. Matsuoka H, Nakamura A, Masaki T, Sugiyama M, Takahara T, Hachiya J, Atomi Y (2003) A prospective comparison between multidetector-row computed tomography and magnetic resonance imaging in the preoperative evaluation of rectal carcinoma. Am J Surg 185:556–559
18. Brown G, Richards CJ, Bourne MW, Newcombe RG, Radcliffe AG, Dallimore NS, Williams GT (2003) Morphologic predictors of lymph node status in rectal cancer with use of high-spatial-resolution MR imaging with histopathologic comparison. Radiology 227:371–377
19. Koh DM, Brown G, Temple L, Raja A, Toomey P, Bett N, Norman AR, Husband JE (2004) Rectal cancer: mesorectal lymph nodes at MR imaging with USPIO versus histopathologic findings—initial observations. Radiology 231:91–99
20. Nagawa H, Muto T, Sunouchi K, Higuchi Y, Tsurita G, Watanabe T, Sawada T (2001) Randomized, controlled trial of lateral node dissection vs. nerve-preserving resection in patients with rectal cancer after preoperative radiotherapy. Dis Colon Rectum 44:1274–1280
21. Syk E, Torkzad MR, Blomqvist L, Ljungqvist O, Glimelius B (2006) Radiological findings do not support lateral residual tumour as a major cause of local recurrence of rectal cancer. Br J Surg 93:113–119
22. Kim JC, Takahashi K, Yu CS, Kim HC, Kim TW, Ryu MH, Kim JH, Mori T (2007) Comparative outcome between chemoradiotherapy and lateral pelvic lymph node dissection following total mesorectal excision in rectal cancer. Ann Surg 246:754–762

Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma

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The relationship between overexpression of glypican (GPC)-3 that is specific for hepatocellular carcinoma (HCC) and the prognosis has not yet been clarified. We attempted to determine the expression profile of GPC3 in association with the clinicopathological factors by immunohistochemical analysis in HCC patients and investigated the potential prognostic value of GPC3 by comparing the survival rate between the GPC3-positive and GPC3-negative HCC patients. Primary HCC tissue samples ($n = 107$) obtained from patients who had undergone hepatectomy between 2000 and 2001 were analyzed. GPC3 expression was less frequently observed in well-differentiated HCC than in moderately and poorly differentiated HCC, the difference in the frequency being statistically significant. GPC3-positive HCC patients had a significantly lower 5-year survival rate than the GPC3-negative HCC patients (54.5 vs 87.7%, $P = 0.031$). Among 80 of the 107 (74.6%) patients with initial treatment who underwent hepatectomy, none of GPC3-negative HCC patients ($n = 16$, 20.0%) died during the follow-up period. No deaths were noted in the GPC3-negative HCC patients among the 71 (88.7%) patients with moderately and poorly differentiated HCC. Multivariate analysis identified GPC3 expression ($P = 0.034$) as an independent prognostic factor for the overall survival. We showed that GPC3 expression is correlated with a poor prognosis in HCC patients. (*Cancer Sci* 2009; 100: 1403–1407)

Hepatocellular carcinoma (HCC) is one of the most common malignancies and is ranked as the third most common cause of cancer-related death worldwide. HCC is generally associated with a poor prognosis, the 5-year survival rate after surgery has been reported to be as low as 25–39%, and systemic therapy with cytotoxic agents provides only marginal benefit.[1] Even in those patients in whom the tumor has been successfully removed, the 2-year recurrence rate can be as high as 50%.[2,3] Several clinicopathological factors including poor levels of differentiation of the cancer cells, large size of the tumor, portal venous invasion, and intrahepatic metastasis have been shown to contribute to the poor prognosis in patients of HCC. Despite the critical need for better methods for the diagnosis and treatment of HCC, the mechanisms underlying the development of HCC remain unclear.

Glypican (GPC)-3 was discovered as a potential serological and histochemical marker that is specific for HCC. GPC3 is a member of the glypican family and belongs to a group of heparan sulfate proteoglycans bound to the outer surface of the cell membrane through a glycosylphosphatidylinositol anchor.[4] In mammals, this family comprises six members, GPC1 to GPC6. GPC are released from the cell surface by a lipase called Notum to regulate the signaling of Wnts, Hedgehogs, fibroblast growth factors, and bone morphogenetic proteins.[5–9] Depending on the context, their functions exerted may either be stimulatory or inhibitory through these pathways. GPC3 has been detected in the placenta and fetal liver, but not in other adult organs. During hepatic carcinogenesis, GPC3 appears in the HCC tissue and is released into the serum.[10–12] In addition, its expression has also been reported in melanoma.[13–15]

A dramatic elevation of GPC3 expression has been reported in a large proportion of HCC, as determined by cDNA microarray analysis, whereas its expression has been shown to be less frequent in preneoplastic or entirely absent in non-neoplastic liver tissue.[16–18] This has led to the notion that GPC3 may have diagnostic usefulness as a marker of differentiation or a specific tumor marker in the case of HCC. However, until now, the relationship between GPC3 overexpression and the prognosis of HCC has not been clarified.

In the present study, we attempted to determine the tumor expression profile of GPC3 in association with clinicopathological factors in HCC patients by immunohistochemical analysis. We also investigated the potential prognostic value of GPC3 by analyzing the survival rate of GPC3-positive and GPC3-negative HCC patients. By elucidating the association between the GPC3 expression level in HCC tumors and the survival rate of the patients, we concluded that the GPC3 expression level is correlated with a poor prognosis in HCC patients.

Materials and Methods

Patients and tumor tissue samples. Primary HCC tissue samples ($n = 107$) were obtained from patients who underwent hepatectomy at the National Cancer Center Hospital East between 2000 and 2001. The histological types were assigned according to the criteria of the World Health Organization classification. Liver tissue sections prepared from the surgically resected tumors and adjacent parenchyma fixed in 10% formalin and embedded in paraffin were retrieved from the files of the Department of Pathology at our institution.

Immunohistochemical staining. Sections 6 μm thick were prepared from the paraffin-embedded blocks. The sections were deparaffinized in xylene and rehydrated through ethanol to water. Endogenous peroxidase activity was blocked using 3% H_2O_2 in methanol for 20 min. For antigen retrieval, sections were heated in 10 mM citrate buffer (pH 6.0) with microwave at 95°C for 15 min. The slides were then allowed to cool down, and the prediluted primary monoclonal anti-GPC3 antibody (dilution 1 : 300; Biomosaics, Burlington, VT, USA) was added to cover each slide, and the slides were incubated for 2 h at room temperature. Thereafter, the slides were washed three times in TBS–Tween 20 for 5 min each. Mouse Envision Polymer-horseradish

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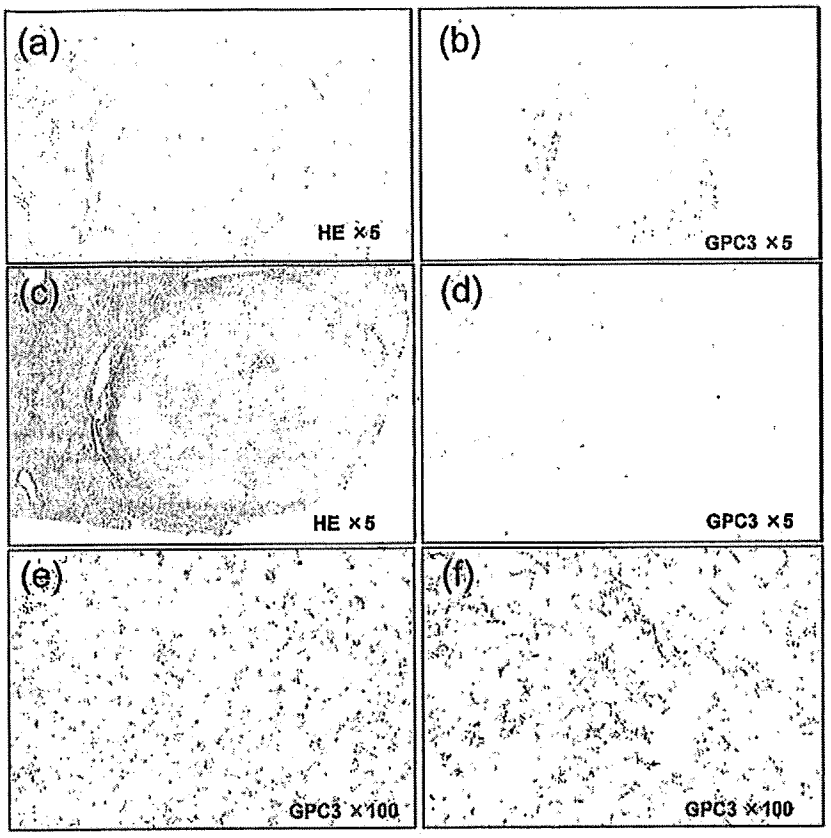

Fig. 1. Glypican (GPC)-3 expression and localization is hepatocellular carcinoma (HCC)-specific. (a,c) Microscopic view of a HE-stained sections of resected HCC. (b,d) HCC sections were stained for GPC3 expression with anti-GPC3 monoclonal antibody. (e) HCC displays prominent bile-canalicular immunostaining. (f) Membranous and cytoplasmic staining of liver tumor cells are shown.

peroxidase (DakoCytomation, Carpenteria, CA, USA), was used as the secondary antibody for 30 min at room temperature followed by three washes in TBS–Tween 20 for 5 min each. Finally, the visualization signal was developed by the addition of 3,3-diaminobenzidine tetrahydrochloride (DakoCytomation) to each slide, followed by incubation for 2 min. Slides were then washed in distilled water, counterstained with hematoxylin, and dehydrated.

For the immunohistochemical analysis of GPC3, we evaluated only the area of GPC3-positive staining in one slide in each patient, including the HCC lesion and adjacent non-cancerous lesion. At first, to analyze GPC3 expression, the results of immunohistochemical staining were classified according to the area of GPC3-positive staining cells as follows: −, negative (<10%); +/−, weakly positive (10–30%); and +, positive (>30%). Finally, in this study, we classified two groups between GPC3-negative (<10%) and GPC3-positive (>10%). The expression of GPC3 was judged to be positive when the percentage of immunoreactive cells was semiquantitatively assessed as being ≥10% in focal lesions. The slides were examined independently by two observers (H. Shirakawa and T. Nakatsura) and then collectively by a pathologist (M. Kojima).

Analysis of the correlation of GPC3 expression with various clinicopathological factors. The correlation of GPC3 expression with various clinicopathological factors was analyzed. Overall survival was calculated from the date of surgery to the date of death.

Statistical analysis. The differences in the level of GPC3 expression were tested by the χ^2-test and the means of each subgroup were compared using Student's *t*-test. Survival analyses were carried out according to the Kaplan–Meier method and the differences were assessed using the log-rank test. Follow-up time was censored if the patient was lost to follow up. Cox proportional-hazards analysis was used for univariate and multivariate analyses to explore the effects of the variables on survival. *P*-values of less than 0.05 were considered to be significant.

Results

Glypican-3 expression in HCC. In order to characterize the expression of GPC3 in HCC, 107 surgical specimens were analyzed immunohistochemically. The mean and median follow-up period were 3.4 ± 2.0 years and 3.5 years respectively. GPC3 expression was detected in 87 of the surgically resected tumor specimens (81.3%) (Fig. 1a,b), but not in the remaining 20 specimens (18.7%) (Fig. 1c,d). In most of the GPC3-positive cases, the protein expression was localized mainly in the cellular cytoplasm (Fig. 1e) with some amount detected on the cell membrane (Fig. 1f). The results of the immunohistochemical analysis were evaluated in relation to the pathological findings and follow-up data. There was no correlation between GPC3 expression and any of the clinicopathological features, except that the GPC3 expression increased with increasing degree of dedifferentiation of the cancer cells (Table 1). GPC3 expression was less frequently observed in well-differentiated HCC than in moderately or poorly differentiated HCC; the difference in frequency was statistically significant. Thus, an increase in GPC3 expression was correlated with increasing aggressiveness of the cancer cells, which was accompanied by dedifferentiation of the cells.

Correlation between GPC3 expression and patient survival. In order to determine the prognostic value of GPC3, the overall survival was compared between GPC3-positive and GPC3-negative HCC patients. The GPC3-positive HCC patients had a significantly lower 5-year survival rate than the GPC3-negative HCC patients (54.5 vs 87.7%, $P = 0.031$; Fig. 2a). After surgery,

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Table 1. Correlation between glypican (GPC)-3 expression and clinicopathological features of patients with hepatocellular carcinoma

Variable	GPC3 expression		*P*-value
	Positive ($n = 87$)	Negative ($n = 20$)	
Age (years) (mean ± SD)	63.6 ± 9.7	60.2 ± 11.8	0.169
Sex (male/female)	67/20	18/2	0.321
HBsAg status (positive/negative)	26/61	3/17	0.283
HCV status (positive/negative)	50/37	12/8	0.999
ICG R15 (%) (mean ± SD)	15.9 ± 8.1	15.5 ± 7.6	0.823
AFP (ng/mL) (mean)	6710	463	0.198
PIVKA-II (mAU/mL) (mean)	7370	5900	0.823
Tumor occurring (primary/recurrence)	64/23	16/4	0.753
Number of tumor (solitary/multiple)	64/23	11/9	0.172
Resection procedure (trisegmentectomy, lobectomy, or segmentectomy/subsegmentectomy or partial resection)	22/65	7/13	0.378
Operation time (min.) (mean ± SD)	310 ± 165	263 ± 119	0.248
Intraperative blood loss (mL) (mean)	2910	1500	0.356
Perioperative transfusion (present/absent)	45/42	9/11	0.767
Tumor size (mm) (mean ± SD)	54.7 ± 41.9	53.0 ± 31.2	0.861
Histological tumor differentiation (well/moderately and poorly)	6/81	6/14	0.032
pStage (UICC) (I/II/III)	35/41/11	6/10/4	0.577
Portal vein involvement (present/absent)	39/48	8/12	0.885
Hepatic vein involvement (present/absent)	9/78	1/19	0.750
Bile duct involvement (present/absent)	11/76	1/19	0.557
Intrahepatic metastasis (present/absent)	18/69	6/14	0.545
Non cancerous tissue (cirrhosis/non-cirrhosis)	36/51	4/16	0.075
Postoperative recurrence (present/absent)	70/17	16/4	0.963

AFP, alpha-fetoprotein; HBsAg, hepatitis B s antigen; HCV, hepatitis C virus; ICG-R15, indocyanine green-retention at 15 min; PIVKA-II, protein induced by vitamin K absence II; UICC, International Union against Cancer.

HCC recurrence was observed in 86 (80.4%) of the 107 patients. In the majority (97.7%) of patients with recurrence, the recurrence was observed in the residual liver. Among these 86 patients, 43 (50%) and seven (8.1%) developed multinodular and extrahepatic recurrence respectively. Although no correlations were observed between these recurrence patterns and GPC3 expression, GPC3 can only be used as an indicator of poor overall survival in HCC patients.

Among 80 of the 107 (74.6%) patients with initial treatment who underwent hepatectomy, none of the GPC3-negative HCC patients ($n = 16$, 20.0%) died during the follow-up period (Fig. 2b). The mean and median follow-up periods were 3.7 ± 2.1 and 3.7 years respectively. The 1-, 3-, and 5-year survival rates of the GPC3-positive HCC group were 84.4, 62.5, and 32.8% respectively. With regard to the tumor grade of HCC, 9 (11.3%) of the 80 patients with well-differentiated tumors showed significantly better prognosis without any record of deaths, compared with 71 (88.7%) patients with moderately and poorly differentiated HCC (Fig. 2c).

Further, among the 71 initial treatment patients who underwent hepatectomy and were found on histopathological examination to have moderately and poorly differentiated HCC, there were no deaths during the follow-up period in the GPC3-negative HCC group (Fig. 2d). The mean and median follow-up periods were 3.6 ± 2.0 and 3.6 years respectively.

Univariate and multivariate analyses to identify the prognostic variables in HCC patients. To identify the variables of potential prognostic significance in all the patients with HCC, univariate analysis of each variable was carried out in relation to the survival time. The difference in the prognosis was assessed by examining the relative hazard and *P*-value for each variable. The relative importance of each variable was then determined by multivariate Cox proportional hazards model analysis. Univariate analysis with stepwise inclusion of variables in the model revealed that the significant prognostic factors were GPC3 expression status, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, indocyanine green-retention at 15 min (ICG-R15), serum protein induced by vitamin K absence II (PIVKA-II), tumor occurence, number of tumors, resection volume, pathological bile duct involvement, and pathological intrahepatic metastasis (Table 2). However, the multivariate analysis identified only GPC3 expression ($P = 0.034$), intrahepatic metastasis ($P = 0.027$), and multiple tumors ($P = 0.006$) as the independent prognostic factors related to overall survival (Table 2).

Discussion

In this study, we characterized the association between the expression level of GPC3 and the malignancy grade, and the prognostic value of GPC3 in HCC. Higher levels of GPC3 expression were observed in moderately or poorly differentiated tumor cells, which was in agreement with previous reports.[19] Our contingency table analysis showed that the GPC3 expression level was correlated with the tumor differentiation level. In addition, Kaplan–Meier survival analysis revealed that GPC3 expression was significantly linked to a poor prognosis after surgical resection in HCC patients. Moreover, univariate analysis indicated that GPC3 expression is associated with an increased risk of death from HCC, and this risk factor could still be extracted in a multivariate setting. On the other hand, multivariate analysis did not identify the tumor differentiation level as an independent predictive factor of the prognosis. Among the 80 HCC patients who underwent initial surgical treatment, the GPC3-negative patients showed better prognosis than the GPC3-positive patients. Patients with well-differentiated HCC also showed a better prognosis than those with moderately and poorly differentiated HCC. Furthermore, we confirmed that among the previously treated subjects, the GPC3-negative group had a better prognosis than the GPC3-positive group with moderately and poorly differentiated HCC tumors.

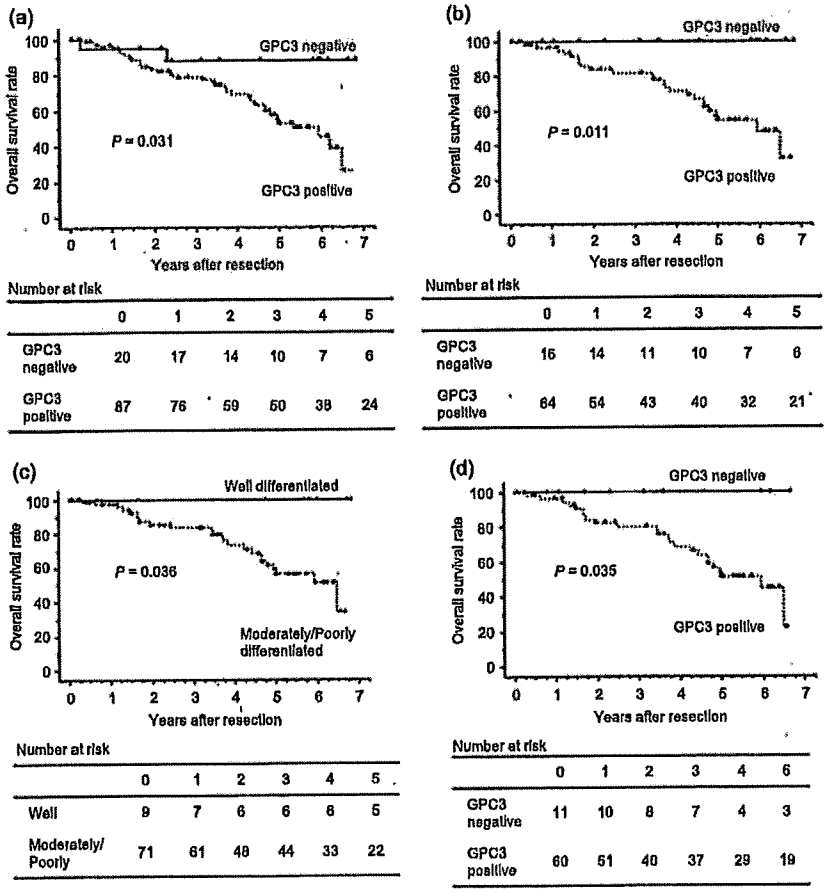

Fig. 2. Overall survival curves for the 107 hepatocellular carcinoma (HCC) patients stratified into those with glypican (GPC)-3-positive and GPC3-negative HCC. (a) Overall survival of patients with GPC3-positive HCC was shorter than those with GPC3-negative HCC ($P = 0.031$). (b) Overall survival curves in 80 of 107 HCC patients with initial treatment who underwent hepatectomy with positive and negative GPC3 expression. Patients with GPC3-positive HCC had a lower 5-year survival than those with GPC3-negative HCC ($P = 0.011$). (c) Overall survival curves in the 71 HCC patients with initial hepatectomy who exhibited well- and moderately and poorly differentiated HCC on histopathological examination. The 5-year survival rate was lower in the moderately and poorly differentiated GPC3-positive HCC than in the corresponding GPC3-negative HCC ($P = 0.036$). (d) Overall survival curves in the 71 initial treatment patients who underwent hepatectomy and exhibited moderately and poorly differentiated HCC on pathological examination with positive and negative GPC3 expression. The 5-year survival rate was lower in the GPC3-positive HCC patients than in the GPC3-negative HCC patients ($P = 0.035$).

Table 2. Prognostic factors for overall survival by univariate and multivariate analyses

Variable	No. patients	Univariate analysis		Multivariate analysis		
		5-year survival rate (%)	*P*-value	RR	95% CI	*P*-value
Age (years) (≥65/<65)	51/56	65.8/53.4	0.531			
Sex (male vs female)	85/22	56.1/72.7	0.403			
HBsAg (positive vs negative)	29/78	51.0/62.3	0.011	1.14	0.31–4.16	0.844
HCV (positive vs negative)	62/45	66.7/46.4	0.004	2.41	0.75–7.69	0.138
ICG R15 (%) (≥15 vs <15)	50/57	70.3/46.8	0.047	0.69	0.31–1.54	0.362
AFP (ng/mL) (≥50 vs <50)	45/62	49.1/65.1	0.132			
PIVKA-II (mAU/mL) (≥700 vs <700)	30/77	35.0/65.6	0.016	1.91	0.730–5.02	0.188
Tumor occurring (first vs recurrence)	80/27	62.8/50.2	0.019	1.83	0.78–4.31	0.167
No. tumors (solitary vs multiple)	75/32	65.7/42.7	0.009	3.53	1.41–8.00	0.006
Resection (trisegmentectomy, lobectomy, or segmentectomy/subsegmentectomy or partial resection)	29/78	36.5/67.1	0.005	1.71	0.52–5.60	0.374
Operation time (min) (>300 vs ≤300)	49/58	43.9/72.3	0.053			
Intraoperative blood loss (mL) (≥1300 vs <1300)	42/65	42.3/68.8	0.097			
Perioperative transfusion (present vs absent)	54/53	49.6/66.5	0.599			
Tumor size (mm) (>50 vs ≤50)	38/69	51.5/62.5	0.154			
Histological differentiation (well vs moderately and poorly)	12/95	77.8/56.4	0.102			
pStage (I vs II/III)	41/66	64.2/56.5	0.071			
Portal vein involvement (present vs absent)	47/60	64.9/58.5	0.369			
Hepatic vein involvement (present vs absent)	10/97	44.4/60.5	0.060			
Bile duct involvement (present vs absent)	12/95	20.0/62.7	0.004	0.94	0.31–2.85	0.912
Intrahepatic metastasis (present vs absent)	24/83	29.0/66.6	0.001	3.57	1.13–10.50	0.027
Non-cancerous lesion (cirrhosis vs non-cirrhosis)	40/67	53.6/61.9	0.232			
GPC3 staining (positive vs negative)	87/20	54.5/87.7	0.025	5.26	1.13–24.39	0.034

AFP, alpha-fetoprotein; CI, confidence interval; HBsAg, hepatitis B's antigen; HCV, hepatitis C virus; ICG-R15, indocyanine green-retention at 15 min; PIVKA-II, protein induced by vitamin K absence II; RR, relative risk; UICC, International Union against Cancer.

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In this study, the patients who were HCV positive, had higher ICG-R15 values, or portal vein involvement showed longer survival times, especially the patients who were HCV-positive or had higher ICG-R15 values, showed statistical significance in the univariate analysis. However, there was no statistical significance in these variables in the multivariate analysis. The reasons for these contradictive results in the univariate analysis are unclear.

In contrast, subgroup analysis did not reveal any significant difference in the disease-free survival rate between the GPC3-positive and GPC3-negative HCC patients (data not shown). The rate of recurrence in patients after surgery was 63.8% within the first 2 years after surgery among the previously treated patients in this study. Tumor recurrence in the GPC3-positive HCC patients occurred earlier than that in the GPC3-negative HCC patients until 9.7 months after the surgery among the patients who had received previous treatment. Two mechanisms of postoperative recurrence of HCC have been suggested: one is intrahepatic metastasis in the residual liver in a metachronous manner, and the other is multicentric hepatocarcinogenesis based on chronic hepatitis.[20–23] Some authors have suggested that early recurrence arises most often from intrahepatic metastases, whereas late recurrence is more likely to be multicentric in origin. Poon *et al.* and Portolani *et al.* reported that tumor factors like neoplastic vascular infiltration, but not host factors, were linked to early recurrence, whereas the risk of late recurrence was dependent on the underlying liver status.[21,22] These results indicate that GPC3 expression may indicate a high risk of intrahepatic recurrence.

Most of the GPC3 expression patterns in HCC cells showed the cytoplasmic pattern. There was no case that showed only the membrane pattern. Almost half of the HCC cases showed the mixed pattern (cytoplasm and membrane) and the other half showed only the cytoplasmic pattern.

There was no statistical significance between the mixed pattern (cytoplasm and membrane) and cytoplasmic pattern ($P = 0.297$) in Kaplan–Meier survival analysis. The functional difference between cytoplasmic GPC3 and membrane GPC3 is unknown, so further investigations are needed to clarify whether the different localization of staining has a different significance.

In addition to the investigation of its role as a prognostic indicator, a phase I clinical trial of a GPC3-derived peptide vaccine for advanced HCC is now underway; GPC3 is an ideal target for this therapy because it is more effective in patients with increased expression of GPC3, which is frequently observed in the later stages of HCC, as shown in the present study. The poor prognosis of patients with GPC3-positive HCC also prompted us to develop a strategy of anticancer immunotherapy,[24,25] that is, we may expect the effect of hepatocarcinogenesis prevention after surgery in patients with GPC3-positive HCC.

In summary, our study evaluated the prognostic significance of GPC3 expression at the protein level in clinical tissue specimens of HCC. The overall survival rate was significantly poorer in patients with elevated GPC3 expression in the tumor than in those with lower levels of GPC3 expression. Further functional characterization of GPC3 may be expected to lead to a better understanding of the molecular mechanisms underlying the development and progression of HCC.

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References

1 Thomas MB, Zhu AX. Hepatocellular carcinoma: the need for progress. *J Clin Oncol* 2005; **23**: 2892–9.
2 Ibrahim S, Roychowdhury A, Hean TK. Risk factors for intrahepatic recurrence after hepatectomy for hepatocellular carcinoma. *Am J Surg* 2007; **194**: 17–22.
3 Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007; **43**: 979–92.
4 Filmus J. The contribution of *in vivo* manipulation of gene expression to the understanding of the function of glypicans. *Glycoconj J* 2002; **19**: 319–23.
5 Filmus J, Capurro M, Rast J. Glypicans. *Genome Biol* 2008; **9**: 224.
6 Capurro MI, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005; **65**: 6245–54.
7 Song HH, Shi W, Xiang YY, Filmus J. The loss of glypican-3 induces alterations in Wnt signaling. *J Biol Chem* 2005; **280**: 2116–25.
8 Stigliano I, Puricelli L, Filmus J, Sogayar MC, Bal de Kier Joffe E, Peters MG. Glypican-3 regulates migration, adhesion and actin cytoskeleton organization in mammary tumor cells through Wnt signaling modulation. *Breast Cancer Res Treat* 2009; **114**: 251–62.
9 Torisu Y, Watanabe A, Nonaka A *et al.* Human homolog of NOTUM, overexpressed in hepatocellular carcinoma, is regulated transcriptionally by beta-catenin/TCF. *Cancer Sci* 2008; **99**: 1139–46.
10 Hippo Y, Watanabe K, Watanabe A *et al.* Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. *Cancer Res* 2004; **64**: 2418–23.
11 Capurro M, Wanless IR, Sherman M *et al.* Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; **125**: 89–97.
12 Nakatsura T, Yoshitake Y, Senju S *et al.* Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003; **306**: 16–25.
13 Nakatsura T, Nishimura Y. Usefulness of the novel oncofetal antigen glypican-3 for diagnosis of hepatocellular carcinoma and melanoma. *Biodrugs* 2005; **19**: 71–7.
14 Ikuta Y, Nakatsura T, Kageshita T *et al.* Highly sensitive detection of melanoma at an early stage based on the increased serum secreted protein acidic and rich in cysteine and glypican-3 levels. *Clin Cancer Res* 2005; **11**: 8079–88.
15 Nakatsura T, Kageshita T, Ito S *et al.* Identification of glypican-3 as a novel tumor marker for melanoma. *Clin Cancer Res* 2004; **10**: 6612–21.
16 Wang XY, Degos F, Dubois S *et al.* Glypican-3 expression in hepatocellular tumors: diagnostic value for preneoplastic lesions and hepatocellular carcinomas. *Hum Pathol* 2006; **37**: 1435–41.
17 Libbrecht L, Severi T, Cassiman D *et al.* Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006; **30**: 1405–11.
18 Di Tommaso L, Franchi G, Park YN *et al.* Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology* 2007; **45**: 725–34.
19 Yamauchi N, Watanabe A, Hishinuma M *et al.* The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005; **18**: 1591–8.
20 Yamamoto J, Kosuge T, Takayama T *et al.* Recurrence of hepatocellular carcinoma after surgery. *Br J Surg* 1996; **83**: 1219–22.
21 Portolani N, Coniglio A, Ghidoni S *et al.* Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. *Ann Surg* 2006; **243**: 229–35.
22 Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer* 2000; **89**: 500–7.
23 Sakon M, Umeshita K, Nagano H *et al.* Clinical significance of hepatic resection in hepatocellular carcinoma: analysis by disease-free survival curves. *Arch Surg* 2000; **135**: 1456–9.
24 Motomura Y, Ikuta Y, Kuronuma T *et al.* HLA-A2 and -A24-restricted glypican-3-derived peptide vaccine induces specific CTLs: preclinical study using mice. *Int J Oncol* 2008; **32**: 985–90.
25 Komori H, Nakatsura T, Senju S *et al.* Identification of HLA-A2- or HLA-A24-restricted CTL epitopes possibly useful for glypican-3-specific immunotherapy of hepatocellular carcinoma. *Clin Cancer Res* 2006; **12**: 2689–97.

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Risk Factors of Surgical Site Infection After Hepatectomy for Liver Cancers

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Abstract
Background Risk factors of surgical site infection (SSI) after hepatectomy under the guideline of Centers for Disease Control and Prevention (CDC) are not well examined.
Methods Hospital records of consecutive patients who underwent hepatectomy without biliary reconstruction for liver cancers were reviewed retrospectively. Prophylactic antibiotics were given to patients just before skin incision and every 3 hours during the operations. Clinicopathological factors were compared between patients who developed SSI and those without it.
Results There were 405 patients identified, and the incidence of SSI was 23 cases (5.8%). In multivariate analysis, intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were significant risk factors of SSI after hepatectomy.
Conclusions Prophylactic antibiotics were necessary only during the operation for most patients who underwent hepatectomy without biliary reconstruction. However, patients with intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years are at risk to develop SSI and might need additional administration of prophylactic antibiotics after surgery.

Introduction

Use of antibiotics is one of the main techniques to prevent surgical site infection (SSI) after surgery. There has been tremendous accumulation of evidence during the last three decades with regard to the optimal methods of its administration [1]. The Centers for Disease Control and Prevention (CDC) recommended in its 1999 guideline to maintain therapeutic levels of prophylactic antibiotic during the operation and, at most, a few hours after closure of incisions [2]. However, it is well known that incidence of SSI is greatly influenced by patients' underlying general status and perioperative factors [3]. Disease and procedure-specific risks and use of prophylactic antibiotics are not well examined, except for colorectal surgery [4, 5], open heart surgery [6], cholecystectomy [7, 8], etc.

It is suggested that hepatectomy suppresses Kupffer cell and T-cell function significantly, which renders patients immunosuppressive [9]. Postoperative infection, including SSI, deteriorates hepatic failure in cases with limited hepatic functional reserve. There is a wide variety in operation time, blood loss, transfusion requirement, etc., depending on the extent of parenchymal resection. Underlying cirrhosis and hypoalbuminemia inhibits normal wound healing [10]. However, perioperative factors that should be considered a significant risk to develop SSI after hepatectomy have not been clear. The purpose of this study was to analyze the risk factors of SSI after hepatectomy with prophylactic antibiotics under CDC guideline and to clarify who might benefit from additional administration of prophylactic antibiotics after operation.

Materials and methods

Patients who underwent hepatectomy for liver cancers from November 2002 to December 2006 at National Cancer Center East Hospital, Kashiwa, Japan, were identified and reviewed retrospectively. Patients who

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underwent hepatectomy without biliary reconstruction regardless of diagnosis were included in the study. Patients who underwent cholecystectomy along with hepatectomy were included in the study, but those who underwent simultaneous procedures, such as colorectal resection or stoma closure, were excluded from the study.

The extent of hepatectomy was evaluated according to the disease progression, liver function, and general condition of patients [11]. Tumor progression and resectability was assessed by imaging studies, such as contrast enhanced computed tomography (CT) scans, magnetic resonance imaging (MRI), hepatic arterial angiography, ultrasound, and chest x-ray. Liver function was assessed by liver biochemistry test, Child-Pugh grade [12], and the indocyanine green retention rate at 15 minutes [13]. All patients were reviewed before surgery at weekly conferences by hepatic surgeons, medical oncologists, and interventional radiologists to discuss whether the planned procedures were appropriate. Hepatic resection was performed under intraoperative ultrasonographic guidance by the pean fracture method with or without inflow occlusion (Pringle's maneuver). Anatomic hepatectomy was performed whenever possible, whereas partial resection was performed in consideration of limited liver functional reserve or anatomic location of the tumor. During parenchymal resection, all blood vessels and bile ducts were ligated whenever possible with 2-0 or 3-0 braided silk or vessel clip. One or two closed drains were inserted at the end of operation in the right subphrenic space or wherever close to the resected liver parenchyma. Drains were removed when no rebleeding or bile leakage was observed on postoperative day (POD) 3 or 4.

SSI was defined as a condition in which purulent discharge was observed from any incision or space that was manipulated during an operation within 30 days after the operation with or without microbiological evidence as in the guideline issued by CDC [2], and it was identified retrospectively by reviewing clinical records of patients who underwent hepatectomy. Remote site infection was defined as a condition in which fever and leukocytosis were present with bacteria in sputum, urine, catheter-tip, blood, or other body fluid/space, or according to the physician's judgment regardless of microbiological evidence.

Patients were usually given two doses of cefazolin as prophylactic antibiotics. One gram of cefazolin was administered to patients within 30 minutes before skin incision and another dose 3 hours later. When the operation lasted more than 3 hours, additional doses were given every 3 hours thereafter during the operation. No antibiotics were given after incisions were closed if patients had already received two doses of cefazolin.

All data were compiled in a database for analysis (Microsoft Excel and SPSS 11.0 J for Windows). Differences between numerical variables were tested with Mann-Whitney U test and those between categorical variables were tested with χ^2 statistics. Multivariate analysis was performed with logistic regression test. $p < 0.05$ was deemed significant.

Results

During the period of study, 405 patients underwent hepatectomy without biliary reconstruction for primary or secondary liver cancers at National Cancer Center East Hospital, Kashiwa, Japan. Of these 405 patients, 23 patients (5.8%) developed SSI (incisional, 20; organ/space, 3). Incisional SSIs were treated by opening incisions and organ/space SSIs were treated by drainage under ultrasound guidance. The patient characteristics and demographic variables are listed in Table 1. No differences in these basic characteristics, except age, were observed between patients with SSI and those without it. Mean age of patients with SSI was 68.2 years and was statistically older than those without SSI. A cutoff value of aged 65 years had the highest statistical power ($p = 0.016$). Patients' ASA score, comorbidities, and underlying liver pathology were statistically similar between the two groups.

Culture results of infecting organisms included *Bacteroides faragilis* (n = 3), *Staphylococcus aureus* (n = 2), *Klebsiera oxytoca* (n = 1), *Serratia marcescens* (n = 1), *Escherichia coli* (n = 1), *Streptoccoccus anginosus* (n = 1), *Streptococcus constellatus* (n = 1), *Enterobacter cloacae* (n = 1), *Citrobacter braakii* (n = 1), *Citrobacter freundii* (n = 1), Corynebacterium species (n = 1), and Candida species (n = 1).

The perioperative variables are listed in Table 2. Operation time, red blood cell (RBC) transfusion requirement, RBC transfusion volume, and intraoperative bowel injury were statistically different between the two groups. Blood loss did not reach statistical significance, but cutoff value of 2000 ml had the significant power to predict SSI ($p = 0.003$). Multivariate analysis of those variables found that intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were the significant risk factors to develop SSI after hepatectomy without biliary reconstruction (Table 3). Rates of SSI increased dramatically with the number of risk factors present (Fig. 1). Patients with two or more risk factors were statistically more likely to develop SSI than those with none or only one risk factor.

During the same period, three patients died within 30 days from the operations. One patient died from pulmonary embolism on POD 3, another died from brain stroke on POD 3, and the other died from esophageal varix rupture on POD 9. Incidence of remote site infection was

Table 1 Patient characteristics and demographic variables for patients with SSI compared with those without it

	SSI (−) (N = 382)	SSI (+) (N = 23)	*P* value
Age (yr)[a]	63.7 ± 0.5	68.2 ± 2	*0.034*
≥65[b]	194 (50.9)	18 (78.3)	*0.016*
<65	188 (49.1)	5 (21.7)	
Gender[b]			0.809
Male	285 (74.6)	18 (78.3)	
Female	97 (25.4)	5 (21.7)	
Body mass index (kg/m^2)[a]	23.8 ± 0.6	23.6 ± 0.7	0.583
Diabetes mellitus[b]	75 (19.6)	1 (4.5)	0.095
ASA score[b]			0.488
1	111 (29.5)	7 (30.4)	
2	243 (64.6)	16 (69.6)	
3	22 (5.9)		
Diagnosis[b]			0.566
HCC	239 (62.6)	13 (56.5)	
Metastases	126 (33)	8 (34.8)	
Others	16 (4.5)	2 (8.7)	
Viral hepatitis serology[b]			0.858
HBV	51 (14)	3 (13)	
HCV	141 (38.7)	8 (34.8)	
HBV and HCV	7 (1.9)		
Liver parenchyma[b]			0.758
Chronic hepatitis	105 (29.6)	9 (39.1)	
Liver cirrhosis	93 (26.2)	5 (21.7)	
Child class[b]			0.634
A	355 (94.4)	21 (91.3)	
B	21 (5.6)	2 (8.7)	
ICG15R[a]	14.6 ± 0.4	15.5 ± 1.6	0.571

[a] Mann-Whitney *U* test

[b] χ^2 test

Data are numbers with percentages in parentheses or means ± standard error of the mean

ASA American society of anesthesiology, *HCC* hepatocellular carcinoma, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *ICG15R* indocyanin green 15 min retention rate

11 (2.5%) (pneumonia (n = 6), urinary tract infection (n = 1), catheter infection (n = 1), epididymitis (n = 1), unknown origin (n = 2)). Other morbidities included bile leak (n = 9), retractable ascites (n = 6), ileus (n = 4), transient renal insufficiency (n = 4), rebleeding (n = 3), pleural effusion (n = 3), skin rash (n = 2), poor oral intake (n = 2), delirium (n = 1), transient heart failure (n = 1), pulmonary embolism (n = 1), upper gastrointestinal bleeding (n = 1), wound dehiscence (n = 1). There were four reoperations for three rebleedings and one wound dehiscence.

Discussion

Our study clearly demonstrated the risk factors of SSI after hepatectomy with prophylactic antibiotics under the CDC guideline. Intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were the significant risk factors. Although both alimentary tract surgery and hepatobiliary surgery are classified as clean-contaminated [14], biliary tract without calculus is normally sterile contrary to the alimentary tract, which has high bacterial densities [15, 16]. Intraoperative bowel injury is suspected to contaminate surgical field of hepatectomy without biliary reconstruction and to increase the risk of SSI. Blood loss reduces the concentration of antibiotics and is found to be a risk factor of SSI [17, 18]; 1500 ml to 2000 ml of blood loss is the suggested threshold to administer additional doses of cefazolin to maintain a concentration higher than the minimum inhibitory concentration for the common infecting organisms [19, 20]. Our threshold of 2000 ml of blood loss is compatible with previous findings. Elderly patients also are reported to be susceptible to SSI [18, 21]. Because aging involves complex physiologic changes, it is difficult to clarify a definitive mechanism of the vulnerability of elderly patients. Reduction in immune function is one suggested mechanism [10].

Rates of SSI increased dramatically with the number of the three risk factors present (Fig. 1). According to the National Nosocomical Infections Surveillance (NNIS) report, rates of SSI after hepatopancreaticobiliary complex

Table 2 Perioperative variables for patients with SSI compared with those without it

	SSI (–) (N = 382)	SSI (+) (N = 23)	*P* value
Operation time (min)[a]	210 ± 19	269 ± 23	*0.021*
≥300[b]	68 (17.8)	9 (39.1)	*0.017*
<300	313 (82.2)	14 (60.9)	
Pringle time (min)[a]	63.3 ± 2.1	75.9 ± 9.7	0.259
None[b]	26 (7.3)	0 (0)	0.23
>0	331 (92.7)	20 (100)	
Repeat resection[b]	110 (28.8)	4 (17.4)	0.338
Blood loss (ml)[a]	1070 ± 69	1928 ± 470	0.068
≥2000[b]	50 (13.2)	9 (39.1)	*0.003*
<2000	332 (86.8)	14 (60.9)	
RBC transfusion (ml)[a]	177 ± 29	537 ± 192	*0.003*
None[b]	297 (78.2)	12 (52.2)	*0.009*
>0	83 (21.8)	11 (47.8)	
Intraoperative bowel injury[b]	3 (0.8)	4 (17.4)	*<0.001*
Bile leak[b]	7 (1.8)	2 (22.2)	0.087
Resected segments (Couinaud)[b]			0.96
<2	285 (74.8)	16 (69.6)	
2–3	42 (11)	3 (13)	
≥4	54 (14.2)	4 (17.4)	
Resected weight (g)[a]	221 ± 19	269 ± 77	0.281
Largest tumor size (cm)[a]	3.8 ± 0.2	3.7 ± 0.4	0.253
NNIS index[b]			0.184
0	293 (76.9)	14 (60.9)	
1	86 (22.6)	9 (39.1)	
2	2 (0.5)		
Postoperative length of stay[a]	10.2 ± 0.2	23.7 ± 5.7	*<0.001*

[a] Mann-Whitney *U* test

[b] χ^2 test

Data are numbers with percentages in parentheses or means ± standard error of the mean

RBC red blood cell, *NNIS* national nosocomical infection surveillance

Table 3 Multivariate analysis of SSI risk factors

	P value	Odds ratio (95% confidence intervals)
Age ≥65 yr	*0.027*	3.4 (1.15–10.05)
Blood loss ≥2000 ml	*0.004*	4.4 (1.63–11.91)
Intraoperative bowel injury	*<0.001*	20.08 (4–100.8)
RBC transfusion	0.62	1.51 (0.31–7.42)
Operation time >300 min	0.67	1.35 (0.34–5.32)

SSI risk factors identified by univariate analysis were compared by multivariate analysis (logistic regression test)

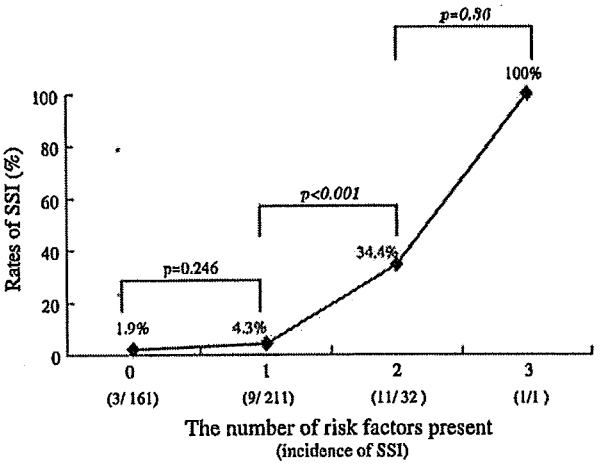

Fig. 1 Rates of SSI increased with the number of risk factors present. Rates of SSI were not statistically different between patients with one risk factor and those without any factors. However, patients with two or more risk factors developed SSI at a significantly higher rate than those with none or only one risk factor

surgery range from 3.24–7.04% [22]. Other reported rates of SSI after hepatectomy range from 4.6–25.2% [23, 24]. Compared with those previously reported rates, the rates of SSI for patients with none or only one risk factor, 1.9% and 4.3% respectively, are considered allowable. Prophylactic antibiotics for hepatectomy without biliary reconstruction are necessary only during operations for patients with none or only one risk factor. However, patients with two or more risk factors developed SSI at statistically higher rates. Fujita et al. [4] reported that two additional doses of postoperative antibiotics reduced the incidence of incisional SSI from 14.2% to 4.3% compared with single-dose preoperative administration in elective colorectal surgery

[4]. Additional administration of postoperative antibiotics maintains therapeutic levels for longer hours and reduces the incidence of SSI more effectively for patients at higher risk. Although there have been no published data concerning the effectiveness of postoperative administration of antibiotics in hepatectomy, Fig. 1 illustrates that patients with two or more risk factors may receive some additional doses of postoperative antibiotics as in colorectal surgery. Appropriate doses of additional antibiotics are matters to be discussed.

There were five infecting organisms that were resistant to cefazolin: *Bacteroides fragilis, Enterobacter, cloacae, Serratia marcescens,* Corynebacterium species, and Citrobacter species. Because some patients lack microbiologic data, a definitive conclusion about the optimum choice of prophylactic antibiotics was not possible. However, it is evident that cefazolin alone was effective for most patients who underwent hepatectomy without biliary reconstruction. Two of the seven patients with intraoperative bowel injury developed SSI with *Bacteroides fragilis.* Because likely pathogens in alimentary tract surgery are gram-negative bacilli and anaerobes [2], postoperative antibiotics with anaerobic coverage might be more effective for patients with intraoperative bowel injury.

Postoperative infections, especially organ/space SSI, sometimes deteriorate hepatic function and may cause mortalities. We experienced 23 SSIs and 11 remote site infections, but none of the patients died from those infections. We speculate that our strict evaluation of extent of hepatectomy using CT volumetry and liver function test precluded some excessive hepatic resection and saved postoperative hepatic function. Postoperative infection is more likely to occur in patients with hepatic dysfunction [25]. Our relatively low rate of major hepatectomy in consideration of hepatic functional reserve might be related to the fewer incidence of SSI.

RBC transfusion requirement and operation time were significant risk factors of SSI in univariate analysis, but not in multivariate analysis. Transfusion has immunosuppressive effects on postoperative patients via reductions in natural killer cell number and cytotoxic T-cell function [26, 27] and is reported to be a risk factor of SSI in colorectal surgery [28, 29]. However, controversy exists concerning the causal relationship between transfusion and SSI [30], and a recent meta-analysis denies the association between transfusion and postoperative infection [31]. Our result is consistent with the meta-analysis. Operation time is another reported risk factor of SSI [18]. Cefazolin exhibits time-dependent decrease in serum and tissue concentration, and additional administrations are recommended every 3 or 4 hours during operation to maintain therapeutic levels of cefazolin [2]. Because all of our patients received a second dose of cefazolin at 3 hours from incision, serum and tissue concentration of cefazolin was expected to exceed therapeutic levels during the whole time of operations for most patients. Influence of operation time on the incidence of SSI was suspected to be minimized with additional dose of cefazolin at 3 hours from incision.

Abdominal drainage after elective hepatectomy is controversial. Some randomized, controlled trials (RCTs) reported increased incidence of SSI and other morbidities associated with abdominal drainage and denied the routine placement of drainage catheters [32, 33]. However, the routine drainage group in those RCTs had drainage catheters placed for at least 5 to 9 days, which was unnecessarily long. We almost routinely placed drainage catheters but removed them on POD 3/4 or earlier if postoperative bleeding and bile leakage were denied. Early removal of prophylactic drains prevents intra-abdominal infections [34]. We do not consider that abdominal drainage causes more infections if drains are removed on POD 3/4 or earlier.

Our study has several limitations. First, SSI was detected indirectly by retrospectively reviewing patient records and laboratory data. It has been suggested to be a less accurate method than prospective direct observation of surgical sites [2]. Some SSI might be possibly undetected because of inappropriate patient records. However, indirect case-finding by reviewing daily records and laboratory data is the most widespread method of surveillance in the medical literature. Its reported sensitivity is as high as 83.8–92.3% compared with prospective direct finding of SSI [35]. Since then, we do not consider that our surveillance method precludes the importance of our findings. Second, it is a single-center study. Our department is one of the highest volume centers in Japan and performs 250 hepatopancreaticobiliary cancer surgeries in a year. Also, we do not perform operations on patients with end-stage renal disease on dialysis due to inadequacies of dialysis facilities. Our relatively low rate of SSI incidence may be attributable to the high volume of cases and to the patient selection.

Conclusions

Our study demonstrated that prophylactic antibiotics were necessary only during operations and, at most, a few hours after closure of incisions in most of the patients who underwent hepatectomy without biliary reconstruction. However, patients with intraoperative bowel injury, blood loss >2000 ml, and age older than 65 years were at risk for developing SSI. Patients with two or more risk factors may receive additional doses of postoperative antibiotics to prevent SSI more effectively.

References

1. Nichols RL (1996) Surgical infections: prevention and treatment-1965 to 1995. Am J Surg 172:68–74
2. Mangram AJ, Horan TC, Pearson ML et al (1999) Guideline for prevention of surgical site infection, 1999. Infect Control Hosp Epidemiol 20:247–278
3. Culver DH, Horan TC, Gaynes RP et al (1991) Surgical wound infection rates by wound class, operative procedure, and patient risk index. Am J Med 91(3B):152S–157S
4. Fujita S, Saito N, Yamada T et al (2007) Randomized, multicenter trial of antibiotic prophylaxis in elective colorectal surgery. Arch Surg 142:657–661
5. Tang R, Chen HH, Wang YL et al (2001) Risk factors for surgical site infection after elective resection of the colon and rectum: a single-center prospective study of 2, 809 consecutive patients. Ann Surg 234:181–189
6. Harbarth S, Samore MH, Lichtenberg D et al (2000) Prolonged antibiotic prophylaxis after cardiovascular surgery and its effect on surgical site infections and antimicrobial resistance. Circulation 101:2916–2921
7. Dobay KJ, Freier DT, Albear P (1999) The absent role of prophylactic antibiotics in low-risk patients undergoing laparoscopic cholecystectomy. Am Surg 65:226–228
8. Higgins A, London J, Charland S et al (1999) Prophylactic antibiotics for elective laparoscopic cholecystectomy: are they necessary? Arch Surg 134:611–613
9. Karpoff HM, Tung C, Ng B et al (1996) Interferon gamma protects against hepatic tumor growth in rats by increasing Kupffer cell tumoricidal activity. Hepatology 24:374–379
10. Silverstein JH (2002) Physiologic changes associated with aging. In: O'Leary JP (ed) The physiologic basis of surgery, 2nd edn. Lippincott Williams & Wilkins, Philadelphia, pp 705–711
11. Makuuchi M, Kosuge T, Takayama T et al (1993) Surgery for small liver cancers. Semin Surg Oncol 9:298–304
12. Pugh RN, Murray-Lyon IM, Dawson JL et al (1973) Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60:646–649
13. Lau H, Man K, Fan ST et al (1997) Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. Br J Surg 84:1255–1259
14. Talbot TR, Kaiser AB (2005) Postoperative infections and antimicrobial prophylaxis. In: Mandell GL (ed) Principles and practice of infectious diseases, 6th edn. Elsevier, Philadelphia
15. Fry DE (2002) Surgical infection. In: O'Leary JP (ed) The physiologic basis of surgery, 3rd edn. Lippincott Williams & Wilkins, Philadelphia, pp 212–258
16. Ahrendt SA, Pitt HA (2001) Biliary tract. In: Townsend CMJ (ed) Textbook of surgery. The biological basis of modern surgical practice, 16th edn. Saunders, Philadelphia, pp 1076–1111
17. Griffiths J, Demianczuk N, Cordoviz M et al (2005) Surgical site infection following elective caesarian section: a case-control study of postdischarge surveillance. J Obstet Gynaecol Can 27:340–344
18. Yamamoto S, Kanamaru S, Kunishima Y et al (2005) Perioperative antimicrobial prophylaxis in urology: a multi-center prospective study. J Chemother 17:189–197
19. Swoboda SM, Merz C, Kostuik J et al (1996) Does intraoperative blood loss affect antibiotic serum and tissue concentrations? Arch Surg 131:1165–1171
20. Meter JJ, Polly DWJ, Brueckner RP et al (1996) Effect of intraoperative blood loss on the serum level of cefazolin in patients managed with total hip arthroplasty. A prospective, controlled study. J Bone Joint Surg Am 78:1201–1205
21. Yoshida J, Shinohara M, Ishikawa M et al (2006) Surgical site infection in general and thoracic surgery: surveillance of 2663 cases in a Japanese Teaching Hospital. Surg Today 36:114–118
22. Gaynes RP, Culver DH, Horan TC et al (2001) Surgical site infection (SSI) rates in the United States, 1992–1998: The National Nosocomial Infections Surveillance System Basic SSI Risk Index. Clin Infect Dis 33(2):S69–S77
23. Togo S, Matsuo K, Tanaka K et al (2007) Perioperative infection control and its effectiveness in hepatectomy patients. J Gastroenterol Hepatol 22:1942–1948
24. Wu CC, Yeh DC, Lin MC et al (1998) Prospective randomized trial of systemic antibiotics in patients undergoing liver resection. Br J Surg 85:489–493
25. Schindl MJ, Redhead DN, Fearon KC et al (2005) The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. Gut 54:289–296
26. Gascon P, Zoumbos NC, Young NS (1984) Immunological abnormalities in patients receiving multiple blood transfusions. Ann Intern Med 100:173–177
27. Kaplan J, Samaik S, Gitlin J et al (1984) Diminished helper/suppressor lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions. Blood 64:308–310
28. Vamvakas EC, Carven JH, Hibberd PL (1996) Blood transfusion and infection after colorectal cancer surgery. Transfusion 36:1000–1008
29. Mynster T, Christensen IF, Moesgaard F et al (2000) Effects of the combination of blood transfusion and postoperative infectious complications on prognosis after surgery for colorectal cancer. Br J Surg 87:1553–1562
30. Vamvakas EC, Carven JH (1998) Transfusion of white-cell-containing allogeneic blood components and postoperative wound infection: effect of confounding factors. Transfus Med 8:29–36
31. Vamvakas EC (2007) White-blood-cell-containing allogeneic blood transfusion and postoperative infection or mortality: an updated meta-analysis. Vox Sang 92:224–232
32. Fong Y, Brennan M, Brown K et al (1996) Drainage is unnecessary after elective liver resection. Am J Surg 171:158–162
33. Liu CL, Fan ST, Lo CM et al (2004) Abdominal drainage after hepatic resection is contraindicated in patients with chronic liver disease. Ann Surg 239:194–201
34. Kawai M, Tani M, Terasawa H et al (2006) Early removal of prophylactic drains reduces the risk of intra-abdominal infections in patients with pancreatic head resection. Ann Surg 244:1–7
35. Cardo DM, Falk PS, Mayhall CG (1993) Validation of surgical wound surveillance. Infect Control Hop Epidemiol 14:211–215

Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer

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Abstract. Primary liver cancers are classified into three types based on their morphology and cytogenetic characteristics hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and combined hepatocellular and cholangiocarcinoma (CHC). It is often difficult to distinguish these liver tumors. Glypican-3 (GPC3) is serological and histochemical marker of hepatocellular carcinoma. In order to separate these three types of liver cancers, we analyzed the GPC3 expression in 85 liver resection specimens, including 46 HCCs, 28 ICCs and 11 CHCs. GPC3 immunohistochemical staining was used to distinguish HCC from ICC by comparing with the conventional biomarker, α-fetoprotein (AFP). The immunostaining of GPC3 was identified in 78.3% (36/46) of HCCs, 60% (9/15) of well differentiated, 88.9% (16/18) of moderately differentiated and 84.6% (11/13) of poorly differentiated HCCs. It was negative in the ICCs. We confirmed that GPC3 expression is specific to HCC component (8/11, 72.7%) but few samples also showed weakly in ICC component (2/11, 18.2%) of CHC sections among 11 cases compared with HCC biomarkers including AFP and hepatocyto paraffin 1 (HepPar1), and ICC biomarkers cytokeratin (CK) 7 and CK19. Three cases in which the macroscopic features resembled ICC did not express GPC3 even in the pathological HCC component. Most (10/11, 91%) of the pathological cholangiocarcinoma components in CHC showed positive staining for CK7 and CK19. The results of this study suggest that GPC3 is a biomarker that is sensitive and specific to HCC component of CHC, and CK7 and CK19 are markers for pathological cholangiocarcinoma component of CHC.

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Abbreviations: HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; CHC, combined hepatocellular and chorangiocarcinoma; GPC3, glypican-3; AFP, α-fetoprotein; HepPar1, hepatocyto paraffin 1; CK, cytokeratin; CC, cholangiocarcinoma; cp, component

Key words: hepatocellular carcinoma, intrahepatic cholangiocarcinoma, combined hepatocellular and chorangiocarcinoma, glypican-3, CK7, CK19, immunohistochemical analysis

Introduction

Liver cancer is one of the common malignancies that are rapidly increasing throughout the world. Primary liver cancers are classified into three types based on their morphology and cytogenetic characteristics, hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and combined hepatocellular and cholangiocarcinoma (CHC). HCC is hepatocyto-origin, and ICC is from the epithelium of the intrahepatic bile duct. CHC is a rare type of liver cancer with features of both hepatocellular and biliary differentiation (1-3). The pathological structure of CHC is composed of hepatocellular element showing bile production, an intercellular bile canaliculi or trabecular growth pattern and cholangiocellular component showing mucin production or gland formation.

Because of their rapid growth rate and the lack of accurate ways of diagnosis in the early stages, the prognosis and the survival rate for liver cancer patients remain poor. Currently, ultrasound sonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and histopathological examination for tumor biopsy are used for diagnosis. However, distinguishing the three different primary liver tumors is often a challenging task in diagnosis, for which immunohistochemical analysis for specific antigens is a helpful tool: α-fetoprotein (AFP) and hepatocyto paraffin 1 (HepPar1) for HCC (4-8) and cytokeratin (CK) 7 and CK19 for ICC (9-11).

Glypican-3 (GPC3) was discovered as a potential serological and histochemical marker whose expression is specific for HCC (12-16). GPC3 belongs to glypican family that is a group of heparan sulfate proteoglycans linked to the outer surface of cell membrane through a glycosylphosphatidylinositol anchor (17). In mammals, six members of GPCs have been reported, GPC1 to GPC6. GPCs are released from the cell surface by a lipase called Notum to regulate the signaling of Wnts, Hedgehogs, fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) (18-25). Depending on the cellular context, their function can be stimulatory or inhibitory activity, or signaling. The expression of GPC3 is detected in placenta and fetal liver, but not in other normal organs. During hepatic carcinogenesis, GPC3 have been reported to reappear in HCC and to be released into serum (12,13,15,26). Its expression is also detected in melanoma (27-29). The functions of GPC3 in cancer cells are still unclear.

In this study, we examined whether immunohistochemical analysis for GPC3 can be used to distinguish HCC from ICC, if so, how effectively GPC3 can be detected, compared to other biomarkers that are conventionally used. We demonstrate that distinguishing HCC from ICC by detecting the expression of GPC3 enables more accurate diagnosis.

Materials and methods

Case selection. We selected 85 cases of liver tumors from the surgical pathology files from 1992 to 2006 of National Cancer Center Hospital East, Kashiwa, Chiba, Japan. The cases included 46 primary HCCs, 28 ICCs, and 11 CHCs that underwent hepatectomy. All identifiers were eliminated to protect patients' identities. Size of the tumor and any clinicopathologic factors (age, sex and grade of tumor) were matched between HCC and ICC. The 46 cases of HCCs occurred in 33 men and 13 women with a mean of age at 65.3 years (range, 44-80 years). HCC was subclassified into well (n=15), moderately (n=18), and poorly (n=13) differentiated types according to the World Health Organization classification criteria. The 28 cases of ICC consisted of 18 men and 10 women. Their mean age was 65.7 years (range, 51-82 years). All 28 resected cases of ICC were confirmed by hematoxylin-eosin (H.E.) staining.

The 11 cases of CHC included 7 men and 4 women with a mean age of 62.5 years (range, 47-76 years). All CHCs were pathologically confirmed after surgery.

Tissue samples. Liver tissue sections were retrieved from the files of the Department of Pathology in our institution. All liver specimens were prepared from surgically resected tumors and adjacent parenchyma. They were fixed in 10% formalin and paraffinized for routine histological examination.

Immunohistochemical staining procedure. Six-micrometer-thick sections were made from the paraffin-embedded blocks. Subsequently the sections were deparaffinized in xylene and rehydrated through ethanol to water. Endogenous peroxidase activity was blocked using 3% H_2O_2 in methanol for 20 min. For antigen retrieval, Sections were heated in 10 mM citrate buffer (pH 6.0) with microwave for 15 min in a water bath at 95°C. Only for CK7 immunostaining, sections were digested by Proteinase K (DakoCytomation, Carpenteria, CA) for 5 min at room temprature. Slides were then allowed to cool down. The prediluted primary antibodies, monoclonal anti-GPC3 (dilution 1:300, 1G12; Biomosaics, Inc., Burlington, VT), anti-AFP (dilution 1:400, DakoCytomation), anti-HepPar1 (dilution 1:100, DakoCytomation), anti-CK7 (dilution 1:100, DakoCytomation), and CK19 (dilution 1:200, DakoCytomation) were added to cover each slide, and the slides were incubated for 2 h at room temperature. Slides were washed 3 times in phosphate-buffered saline (PBS)/Tween for 5 min each. Mouse Envision Polymer (DakoCytomation) was used as a secondary antibody for 30 min at room temperature followed by washes in PBS/Tween 3 times for 5 min each. Diaminobenzidine chromagen (DakoCytomation) was added to each slide and incubated for 2 min. Slides were washed in distilled water, counterstained with hematoxylin and dehydrated in xylene. To analyze GPC3 expression, the immunohistochemical results were classified according to the number of positive cells as follows: -, negative (<10%); ±, weakly positive (10-30%); + positive (>30%). To validate the data in GPC3 as a marker for HCC, parallel staining for AFP of 46 cases were further analyzed. For 11 CHC cases, AFP, HepPar1, CK7 and CK19 were stained and compared with GPC3 staining pattern.

The slides were examined independently by 3 observers (Shirakawa H, Kuronuma T and Nakatsura T) and then collectively by 2 more pathologists (Hasebe T and Nakano M).

Statistical analysis. Differences in proportion were tested by the χ^2 test. Differences in the means of each subgroup were tested using the Student's t-test. P-value of <0.05 was considered statistically significant.

Results

GPC3 was present in 80% of HCC and negative in ICC. In order to examine the levels and pattern of GPC3 expression, 46 cases of HCC and 28 cases of ICC were immunohistochemically analyzed. GPC3 was detected in 36 cases (78%) of HCC (Fig. 1a), and no expression of GPC3 was found in any of the ICC patients (Fig. 1b). The GPC3 staining was diffused throughout (Fig. 1c) or localized in a granular pattern in the cytoplasm (Fig. 1d). In other cases, GPC3 was observed at the plasma membrane (Fig. 1e). Previously GPC3 is shown to bind to the cell membrane (16), however, those cases with membranous GPC3 had staining in the cytoplasm as well, but there was no case of GPC3 located only at the plasma membrane. When sensitivity of GPC3 was evaluated, 36 cases (78%) were positive for GPC3 when only 16 cases (35%; P<0.0001) were stained for AFP in HCC suggesting that GPC3 is more sensitive than AFP. Thus, GPC3 was confirmed to be specific and sensitive to HCC compared to AFP.

GPC3 expression increased in moderately and poorly differentiated HCC. In terms of GPC3 expression and tumor