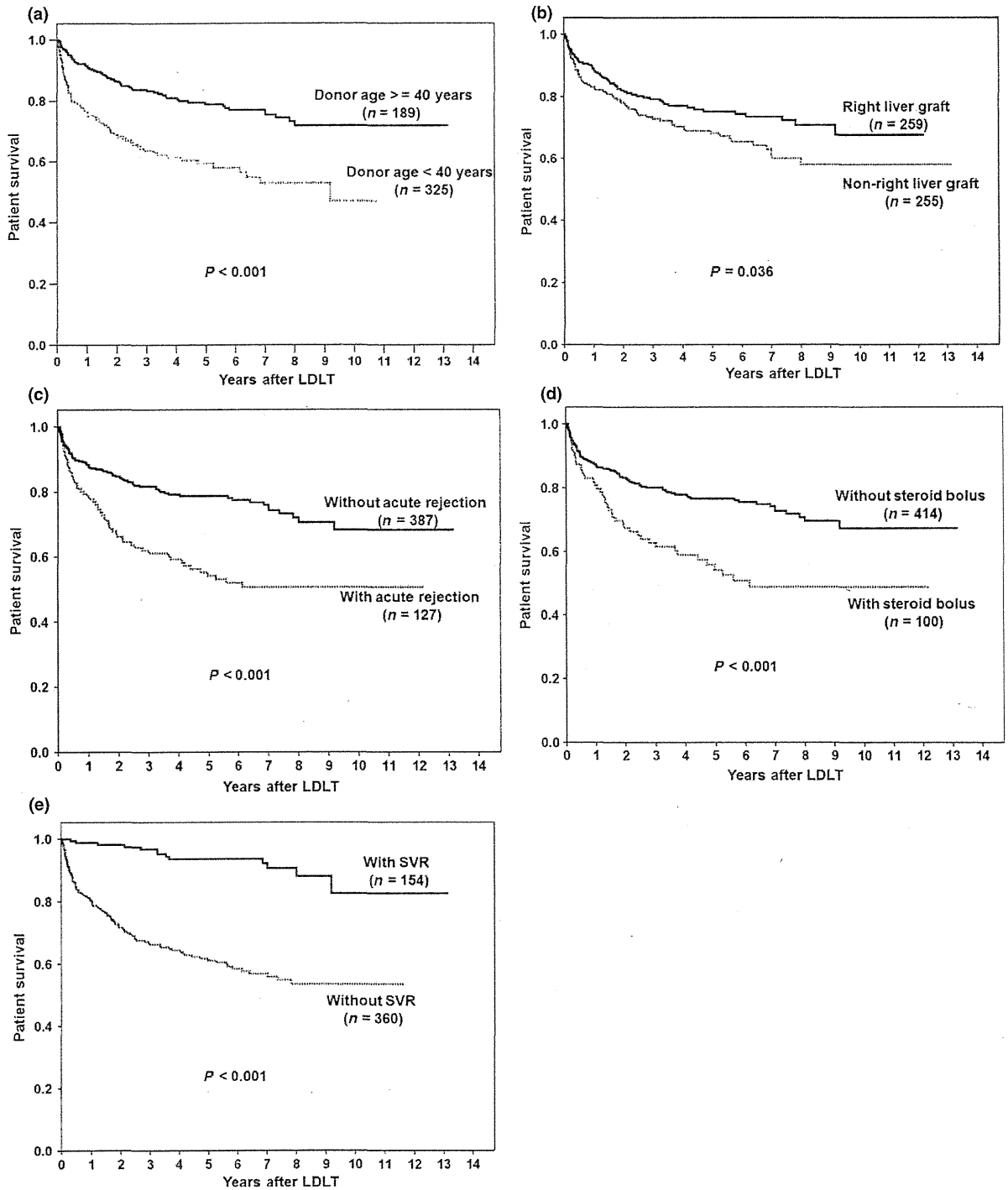

Figure 2 Kaplan–Meier curves stratified by each variable: (a) donor age, (b) graft type, (c) acute rejection, (d) steroid bolus, and (e) sustained virologic response. LDLT, living donor liver transplantation; SVR, sustained virologic response.

increased use of liver grafts from older donors. For HCV-positive recipients, two large retrospective reports from the Scientific Registry of Transplant Recipients and UNOS databases reported that donor age over 40 is an independent predictor of patient death [15,16]. Other accumulating reports [14,17,18] indicate that the grafts from older

Table 4. Factors associated with patient survival among those achieved SVR (n = 154).

Cox regression analysis	Hazard ratio (95% confidence interval)	P-value
Recipient age: ≥60 years (n = 43) vs. <60 years (n = 111)	1.424 (0.318–2.385)	0.644
Recipient gender: male (n = 100) versus female (n = 54)	4.709 (0.918–24.161)	0.063
Pretransplant antiviral treatment: yes (n = 66) versus no (n = 88)	1.666 (0.350–7.931)	0.522
HCV genotype: 1b (n = 112) versus other types (n = 42)	0.873 (0.203–3.747)	0.855
Co-existence of HCC: yes (n = 54) versus no (n = 100)	0.728 (0.179–2.694)	0.635
MELD score: ≥15 (n = 54) vs. <15 (n = 98)	1.354 (0.578–3.204)	0.785
LDLT cases per year: ≥20 (n = 82) vs. <20 (n = 72)	1.054 (0.458–1.254)	0.854
Calcineurin inhibitor: Tac (n = 94) versus CyA (n = 60)	3.580 (0.736–17.421)	0.114
Mycophenolate mofetil: yes (n = 78) versus no (n = 76)	0.932 (0.456–1.884)	0.781
Steroid withdrawal: yes (n = 40) versus no (n = 114)	0.449 (0.096–2.102)	0.31
Splenectomy: yes (n = 59) versus no (n = 95)	1.402 (0.335–5.873)	0.644
Episode of acute rejection: yes (n = 34) versus no (n = 120)	1.854 (0.216–15.914)	0.574
Steroid bolus injection: yes (n = 26) versus no (n = 128)	0.16 (0.019–1.386)	0.096
Donor age: ≥40 years (n = 43) vs. <40 years (n = 111)	1.18 (0.296–4.698)	0.815
Type of graft: right liver (n = 80) versus non-right liver (n = 74)	2.799 (0.818–9.573)	0.101

HCV, hepatitis C virus; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; MELD, model for end-stage liver disease; Tac, tacrolimus; CsA, cyclosporine; SVR, sustained virologic response.

donors are at greater risk for disease progression and impaired graft/patient survival compared with those from younger donors. Our results are definitely consistent with these reports.

Acute rejection in conjunction with treatment with a steroid bolus is one of the most critical factors to address with respect to HCV recurrence. Historical studies [19,20] have demonstrated that steroid bolus for acute rejection in HCV-positive recipients accelerates the recurrence of hepatitis and decreases patient survival. A recent study reported that HCV-positive recipients who receive high-dose steroid treatment for acute rejection are at increased risk of severe recurrent hepatitis, in which older donor age and an episode of rejection are the two most important predictors of developing fibrosing cholestatic hepatitis [21]. Similarly, our study also revealed that both older donor age and acute rejection are independent predictors for impaired patient outcome among LDLT recipients.

Table 5. Summary of antiviral treatment.

	Total (n = 361)	Treatment for established recurrent hepatitis C (n = 211)	Preemptive treatment (n = 150)
Time since LDLT (months)	3 (0–102)	4 (0.5–102)	1 (0–68)
Treatment duration (months)	15 (0.3–99)	14 (0.3–99)	17 (0.3–55)
Regimen: PEG-INF alfa-2a/RBV	45 (12%)	33 (16%)	12 (8%)
PEG-INF alfa-2b/ RBV	223 (62%)	146 (69%)	77 (51%)
INF alfa-2b	93 (26%)	32 (15%)	61 (41%)
Dose reduction	143 (40%)	85 (40%)	58 (39%)
Discontinuation	150 (42%)	66 (31%)	84 (56%)
Sustained virologic response	154 (43%)	89 (42%)	65 (43%)

LDLT, living donor liver transplantation; PEG-INF, pegylated-interferon; RBV, ribavirin; INF, interferon.

The association between achieving SVR and graft/patient survival after liver transplantation for HCV-positive recipients is a matter of debate [10]. Many studies with standard dual treatment of PEG-INF/RBV for 12 months in a DDLT setting have implied a survival benefit of achieving SVR [8,22], but there has been no evidence to support the recommendation of antiviral treatment for recurrent graft hepatitis C due to the lack of clinical benefit with sufficient long-term observation and the existence of frequent severe adverse effects, as concluded by a recent Cochrane meta-analysis [10]. Recent retrospective cohort studies with a long follow-up duration reported improved patient/graft survival in patients who obtained an SVR after antiviral treatment [23–25]. In accordance with those reports, our retrospective analysis indicated a positive effect of achieving SVR on patient survival. Caution should be taken in interpreting our results; however, as SVR was assessed among the whole cohort, including patients who were not indicated for antiviral treatment, the follow-up period after achieving SVR was rather short, and most importantly, a large variety of antiviral treatment regimens were used in Japan, which will be described later.

A noteworthy finding in the present retrospective analysis is the impaired patient survival in recipients who received a non-right liver graft (left liver in 239 cases and right lateral sector in 16 cases). Recent studies comparing outcomes between LDLT and DDLT in HCV-positive recipients have reported equal or even improved outcomes both in patient/graft survival and in fibrosis progression in the LDLT setting, which could be attributed to the younger donor age and shorter ischemic time of LDLT grafts [13,14,26–29].

Based on these findings, LDLT for HCV-positive recipients is now widely accepted as an established alternative to DDLT, even in Western countries. On the contrary, however, the present finding may raise an alarm for reduced size grafts, as a left or posterior graft is clearly smaller than a right liver graft. Another point to be emphasized here is that all LDLTs investigated in the aforementioned studies comparing LDLT and DDLT were universally performed with right liver grafts. One possible explanation for the inferior outcome of the smaller graft is that the intense hepatocyte proliferation that occurs in smaller partial liver grafts may lead to increased viral translation and replication, as advocated by previous authors [30–32]. However, there are several limitations among these speculations. First, the data of the viral load, which is reported to reach a maximum level between the first and third post-transplant months [33], were not available in this study to demonstrate the higher viral replication in the smaller grafts during this period. Another is that the graft type selection is based on the ratio of the volume of the graft to recipient body weight or standard liver volume in our society, which will lead to the bias in the comparison of the right liver versus non-right liver graft. Despite these limitations, considering that comparable outcomes between left liver graft and right liver graft have been reported by us [34] and others [35] in LDLT recipients as a whole, caution should be taken in selecting the type of graft (left versus right) for HCV-positive recipients. Thus, future LDLT studies are required to investigate whether a smaller partial liver graft (left liver) is potentially inferior compared with a larger graft (right liver) in terms of graft/patient survival and recurrent hepatitis severity among HCV-positive recipients.

The antiviral treatment for recurrent hepatitis C after LDLT in Japan was also reviewed in the present study. As described elsewhere in detail [11], the antiviral treatment regimen in Japan differs widely from center to center; preemptive treatment versus treatment after confirmation of recurrent disease, starting dose and method of escalation, and the duration of treatment (usually longer than 12 months). Consequently, our data only present an overview of antiviral treatment in Japan, and no definite conclusion can be drawn regarding the actual efficacy of antiviral treatment after LDLT. Moreover, based on the recent prospective, multicenter, randomized study by Bzowej *et al.* [36], European and USA transplant societies do not support the routine use of preemptive antiviral therapy. A review of Western literature regarding the standard 12-month PEG-INF/RBV treatment for established recurrent hepatitis C after DDLT reveals that the median SVR rate is 33% (0–56%) with a dose reduction rate of 70% and a discontinuation rate of 30% [37]. The present result of an SVR rate of 43% with a dose reduction rate of 40% and a discontinuation rate of 42% seems not so different from those of previous literatures; however, as discussed above, the diversity in the methods, the doses, and the duration of treatment in Japan preclude the direct comparison with Western findings.

Conclusion

This retrospective analysis of the largest series of LDLT for HCV-positive recipients in Japan revealed 5- and 10-year survival rates of 72% and 63%, respectively, and that donor age (>40), non-right liver graft, an acute rejection episode, and the absence of SVR are independent predictors of patient survival. Based on the present result, caution should be made in the selection of the left liver graft for HCV-positive recipients; however, the development of more effective antiviral treatment in the near future may facilitate the application of the left liver graft.

Authorship

YM: designed the study. TI: collected data. NA, YS, NK, SE, TF, HO, HN, AT, YK, MS, YK, KY, KS, MM and MT: performed the study. NA and YS: analyzed and wrote the paper.

Funding

This study is funded by the Japanese Ministry of Health, Welfare, and Labor.

References

1. Singal AK, Guturu P, Hmoud B, Kuo YF, Salameh H, Wiesner RH. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Transplantation* 2013; **95**: 755.
2. Adam R, Karam V, Delvart V, *et al.* Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675.
3. Society TJLT. Liver transplantation in Japan. Registry by the Japanese Liver Transplantation Society. *Jpn J Transpl* 2011; **46**: 524.
4. Gane EJ, Portmann BC, Naoumov NV, *et al.* Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med* 1996; **334**: 815.
5. Berenguer M. Natural history of recurrent hepatitis C. *Liver Transpl* 2002; **8**(10 Suppl. 1): S14.
6. Thuluvath PJ, Krok KL, Segev DL, Yoo HY. Trends in post-liver transplant survival in patients with hepatitis C between 1991 and 2001 in the United States. *Liver Transpl* 2007; **13**: 719.
7. Coilly A, Roche B, Dumortier J, *et al.* Safety and efficacy of protease inhibitors to treat hepatitis C after liver

transplantation: a multicenter experience. *J Hepatol* 2014; **60**: 78.
8. Berenguer M. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. *J Hepatol* 2008; **49**: 274.
9. van der Meer AJ, Veldt BJ, Feld JJ, *et al.* Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012; **308**: 2584.
10. Gurusamy KS, Tsochatzis E, Davidson BR, Burroughs AK. Antiviral prophylactic intervention for chronic hepatitis C virus in patients undergoing liver transplantation. *Cochrane Database Syst Rev* 2010; **12**: CD006573.
11. Akamatsu N, Sugawara Y. Living-donor liver transplantation and hepatitis C. *HPB Surg* 2013; **2013**: 985972.
12. Tamura S, Sugawara Y, Kokudo N. Living donor liver transplantation for hepatocellular carcinoma: the Japanese experience. *Oncology* 2011; **81**(Suppl. 1): 111.
13. Terrault NA, Shiffman ML, Lok AS, *et al.* Outcomes in hepatitis C virus-infected recipients of living donor versus deceased donor liver transplantation. *Liver Transpl* 2007; **13**: 122.
14. Gallegos-Orozco JF, Yosephy A, Noble B, *et al.* Natural history of post-liver transplantation hepatitis C: a review of factors that may influence its course. *Liver Transpl* 2009; **15**: 1872.
15. Lake JR, Shorr JS, Steffen BJ, Chu AH, Gordon RD, Wiesner RH. Differential effects of donor age in liver transplant recipients infected with hepatitis B, hepatitis C and without viral hepatitis. *Am J Transplant* 2005; **5**: 549.
16. Condron SL, Heneghan MA, Patel K, Dev A, McHutchison JG, Muir AJ. Effect of donor age on survival of liver transplant recipients with hepatitis C virus infection. *Transplantation* 2005; **80**: 145.
17. Maluf DG, Edwards EB, Stravitz RT, Kauffman HM. Impact of the donor risk index on the outcome of hepatitis C virus-positive liver transplant recipients. *Liver Transpl* 2009; **15**: 592.
18. Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. *Gut* 2002; **51**: 248.
19. Charlton M, Seaberg E, Wiesner R, *et al.* Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology* 1998; **28**: 823.
20. Sheiner PA, Schwartz ME, Mor E, *et al.* Severe or multiple rejection episodes are associated with early recurrence of hepatitis C after orthotopic liver transplantation. *Hepatology* 1995; **21**: 30.
21. Verna EC, Abdelmessih R, Salomao MA, Lefkowitch J, Moreira RK, Brown RS Jr. Cholestatic hepatitis C following liver transplantation: an outcome-based histological definition, clinical predictors, and prognosis. *Liver Transpl* 2013; **19**: 78.
22. Firpi RJ, Clark V, Soldevila-Pico C, *et al.* The natural history of hepatitis C cirrhosis after liver transplantation. *Liver Transpl* 2009; **15**: 1063.
23. Berenguer M, Palau A, Aguilera V, Rayon JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. *Am J Transplant* 2008; **8**: 679.
24. Selzner N, Renner EL, Selzner M, *et al.* Antiviral treatment of recurrent hepatitis C after liver transplantation: predictors of response and long-term outcome. *Transplantation* 2009; **88**: 1214.
25. Veldt BJ, Poterucha JJ, Watt KD, *et al.* Impact of pegylated interferon and ribavirin treatment on graft survival in liver transplant patients with recurrent hepatitis C infection. *Am J Transplant* 2008; **8**: 2426.
26. Thuluvath PJ, Yoo HY. Graft and patient survival after adult live donor liver transplantation compared to a matched cohort who received a deceased donor transplantation. *Liver Transpl* 2004; **10**: 1263.
27. Russo MW, Galanko J, Beavers K, Fried MW, Shrestha R. Patient and graft survival in hepatitis C recipients after adult living donor liver transplantation in the United States. *Liver Transpl* 2004; **10**: 340.
28. Selzner N, Girgrah N, Lilly L, *et al.* The difference in the fibrosis progression of recurrent hepatitis C after live donor liver transplantation versus deceased donor liver transplantation is attributable to the difference in donor age. *Liver Transpl* 2008; **14**: 1778.
29. Jain A, Singhal A, Kashyap R, Safadjou S, Ryan CK, Orloff MS. Comparative analysis of hepatitis C recurrence and fibrosis progression between deceased-donor and living-donor liver transplantation: 8-year longitudinal follow-up. *Transplantation* 2011; **92**: 453.
30. Garcia-Retortillo M, Forns X, Llovet JM, *et al.* Hepatitis C recurrence is more severe after living donor compared to cadaveric liver transplantation. *Hepatology* 2004; **40**: 699.
31. Zimmerman MA, Trotter JF. Living donor liver transplantation in patients with hepatitis C. *Liver Transpl* 2003; **9**: S52.
32. Olthoff KM. Hepatic regeneration in living donor liver transplantation. *Liver Transpl* 2003; **9**(10 Suppl. 2): S35.
33. Garcia-Retortillo M, Forns X, Feliu A, *et al.* Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology* 2002; **35**: 680.
34. Akamatsu N, Sugawara Y, Tamura S, Imamura H, Kokudo N, Makuuchi M. Regeneration and function of hemiliver graft: right versus left. *Surgery* 2006; **139**: 765.
35. Soejima Y, Shirabe K, Taketomi A, *et al.* Left lobe living donor liver transplantation in adults. *Am J Transplant* 2012; **12**: 1877.
36. Bzowej N, Nelson DR, Terrault NA, *et al.* PHOENIX: a randomized controlled trial of peginterferon alfa-2a plus ribavirin as a prophylactic treatment after liver transplantation for hepatitis C virus. *Liver Transpl* 2011; **17**: 528.
37. Akamatsu N, Sugawara Y. Liver transplantation and hepatitis C. *Int J Hepatol* 2012; **2012**: 686135.

手術治療により長期生存が得られた肝細胞癌リンパ節転移・胆管内腫瘍栓の1例

柿坂 達彦[*1] 神山 俊哉[*1] 横尾 英樹[*1] 折茂 達也[*1] 若山 顕治[*1]
敦賀 陽介[*1] 蒲池 浩文[*1] 畑中佳奈子[*2] 武冨 紹信[*1]

〔*Jpn J Cancer Chemother* 40(12): 1831-1833, *November*, 2013〕

Long-Term Survival of a Patient with Metachronous Lymph Node Metastasis and Bile Duct Tumor Thrombus due to Hepatocellular Carcinoma Successfully Treated with Repeated Surgery: Tatsuhiko Kakisaka[*1], Toshiya Kamiyama[*1], Hideki Yokoo[*1], Tatsuya Orimo[*1], Kenji Wakayama[*1], Yosuke Tsuruga[*1], Hirofumi Kamachi[*1], Kanako Hatanaka[*2] and Akinobu Taketomi[*1] (*[1]*Dept. of Gastroenterological Surgery I, Hokkaido University Graduate School of Medicine,* *[2]*Dept. of Surgical Pathology, Hokkaido University Hospital*)

Summary

A 64-year-old man with hepatocellular carcinoma located in the left lateral lobe and segment 5 was referred to our hospital for surgical treatment. We performed left lateral sectionectomy and segmentectomy 5. The pathological diagnosis was moderately to poorly differentiated hepatocellular carcinoma, and the pathological stage was stage III. Eight months later, intrahepatic recurrence in segment 1 and lymph node metastasis in the hepatoduodenal ligament occurred. Partial resection of segment 1 was performed, and the metastatic lymph node was surgically removed. Twenty four months after the first operation, lymph node metastases along the lesser curvature and retropancreatic space were extirpated. Lymph node metastases along the common hepatic artery were removed 76 months after the first operation. The patient developed jaundice 88 months after the initial surgery, and the bile duct tumor thrombus derived from intrahepatic recurrence in segment 1 caused obstructive jaundice. After percutaneous transhepatic biliary drainage, we performed median sectionectomy and bile duct tumor thrombus removal without bile duct resection. At his 8-year follow-up visit after the primary operation, the patient was healthy and did not show any signs of recurrence. Lymph node metastasis and bile duct tumor thrombus are rare patterns of hepatocellular carcinoma recurrence, and aggressive surgery can result in long-term survival when complete resection is anticipated. Key words: Hepatocellular carcinoma, Lymph node metastasis, Bile duct tumor thrombus

要旨 症例は64歳，男性。S2/3 5.0 cm，S5 4.3 cmの肝細胞癌（HCC）を発症し，肝外側区切除，S5亜区域切除術を施行。病理結果は，mod to por，fc（+），fc-inf（+），sf（+），s0，vp0，vv0，va0，b0，im（+），sm（−），T3N0M0，stage IIIであった。8か月後，S1 1再発，#12リンパ節転移を認め，肝S1 1部分切除，リンパ節摘出術施行。初回手術から2年後，#3，#13リンパ節に転移を認め，リンパ節摘出術を施行。初回手術より6年4か月後，#8，#12リンパ節に転移を認め，リンパ節摘出術を施行。初回手術より7年4か月後，黄疸を発症し，精査でS4/1r再発，B1rから総胆管・右肝管に及ぶ胆管内腫瘍栓を認めた。経皮経肝胆管ドレナージによる減黄後に肝内側区切除，胆管内腫瘍栓摘出術を施行。現在再発なく経過し，初回手術から8年の長期生存が得られている。リンパ節転移，胆管内腫瘍栓はまれであるが，病変を完全切除することで長期予後を期待できるため，切除可能な病変な場合，手術治療を検討すべきである。

はじめに

肝細胞癌（HCC）のリンパ節転移・胆管内腫瘍栓といった再発形式はまれで予後不良な病態であり，治療法に検討を要する。今回，HCC切除後にリンパ節転移・胆管内腫瘍栓を発症したが，繰り返す手術治療により長期生存が得られた1例を経験したので報告する。

I. 症 例

患者: 64歳，男性。

主訴: 特になし。

家族歴: 兄がHCC。

*[1] 北海道大学大学院医学研究科・消化器外科学分野I
*[2] 北海道大学病院・病理部

連絡先: 〒060-8638 北海道札幌市北区北15条西7 北海道大学大学院医学研究科・消化器外科学分野I
柿坂 達彦

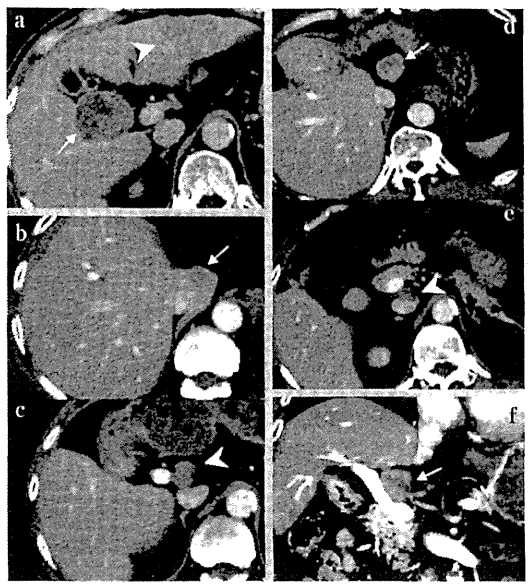

Fig. 1 a: First operation. Abdominal computed tomography (CT) showing two tumors in left lateral lobe (arrowhead) and segment 5 (arrow).
b, c: Second operation. Abdominal CT illustrating intrahepatic recurrence in segment 1 (b: arrow) and lymph node metastasis in the hepatoduodenal ligament (c: arrowhead).
d, e: Third operation. Abdominal CT showing lymph node metastases along the lesser curvature (d: arrow) and retropancreatic space (e: arrowhead).
f: Fourth operation. Abdominal CT illustrating lymph node metastases along the common hepatic artery (arrow).

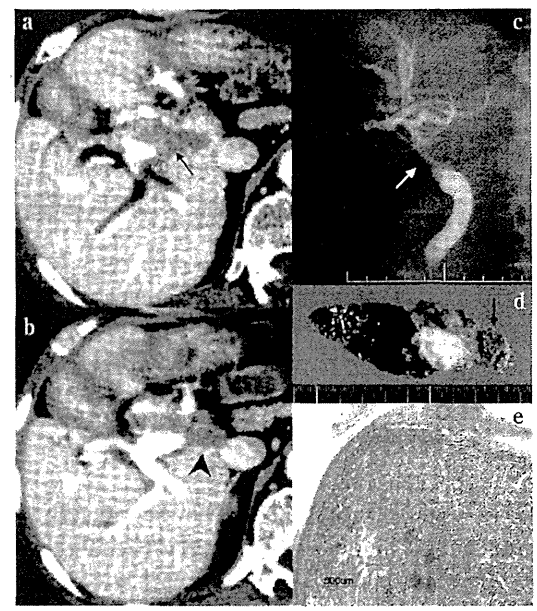

Fig. 2 Fifth operation
a, b: Abdominal CT showing bile duct tumor thrombus (a: arrow) and intrahepatic recurrence in segment 1 (b: arrowhead).
c: Percutaneus transhepatic cholangiography illustrating tumor thrombus in common bilehepatic duct (arrow).
d: Cut surface of the resected specimen showing intrahepatic recurrence (arrow).
e: Histologically, bile duct tumor thrombus was composed of poorly differentiated hepatocellular carcinoma (hematoxylin and eosin stain).

既往歴: 脳梗塞，高血圧，糖尿病，B型慢性肝炎。

治療経過: 2005年，近医で施行された血液検査で肝機能異常を指摘され，腹部CTで肝腫瘍を認めたため，手術目的で当科紹介となった。左不全麻痺以外は特に身体所見に異常なし。$ICGR_{15}$ 4.5%，Child-Pugh分類A（5点），liver damage A，AFP 6,696.5 ng/mL，AFP-L3 13.7%，PIVKA-II 29,743 mAU/mLであった。腹部造影CT上，S2/3に5.0×3.5 cm大，S5に4.3×3.7 cm大の腫瘍を認め，動脈相で早期濃染，平衡相でwashoutを呈し，HCCの診断となった（Fig. 1a）。2005年4月，肝外側区切除，S5亜区域切除，胆嚢摘出術を施行した。病理結果はS2/3が中分化（mod）～低分化型（por）HCC，S5がpor，ともにfc（+），fc-inf（+），sf（+），s0，vp0，vv0，va0，b0，im（+），sm（−）でT3N0M0，stage III，背景肝はf2の診断であった。

2006年，S1 1に1.8×1.7 cmの肝内再発，2.5 cm大の#12aリンパ節転移を認め（Fig. 1b, c），1月，S1 1部分切除，リンパ節摘出術を施行した。病理所見は，St-C，単純結節型，mod to por，fc（−），sf（+），s0，vp0，vv0，va0，b0，sm（−），リンパ節はporの組織型であった。

2007年，3.0 cm大の#3リンパ節転移，1.8 cm大の#13リンパ節転移を認め（Fig. 1d, e），4月，リンパ節摘出術を施行した。#3はmod to por，#13はporの病理所見であった。

2011年，#8a，#8p，#12aが一塊となった4.0 cm大のリンパ節転移を認め（Fig. 1f），8月，リンパ節摘出術を施行した。病理結果はmod to porであった。

2012年8月，T-Bil 17.1 mg/dLと黄疸を認め当科入院となった。造影CTでS4/1rに1.3 cm大の腫瘍とそこから総胆管・右肝管に及ぶ胆管内腫瘍栓を認めた（Fig. 2a, b）。B6より経皮経肝胆管ドレナージを施行し（Fig. 2c），T-Bilが1.9 mg/dLまで減黄されたところで11月に肝内側区切除，胆管内腫瘍栓摘出術を施行した。肝内側区と主病巣であるS1rを切除し，左肝管断端から腫瘍栓を摘出した。胆管断端は連続縫合で閉鎖した。病

理結果はSt-C，por，ig，fc（－），sf（－），s0，vp0，vv0，va0，b4，p0，im（－）（Fig. 2d）で，胆管内腫瘍栓もporの所見であった（Fig. 2e）。術後，減黄に時間を要したが保存的に軽快し，術後74日目に退院となった。

合計五度の手術を施行し，初回手術から8年，初回再発から7年5か月経過した現在，再発なく外来で経過観察中である。

Ⅱ. 考　察

Xiaohongらは，523例のHCCに対してリンパ節郭清を伴う肝切除を施行し，7.45%の頻度でリンパ節転移を認めたと報告している[1]。同時性リンパ節転移陽性例の手術治療による5年生存率は22～29.5%，生存期間中央値（MST）は28か月と報告されており[1,2]，放射線治療（MST 8か月）[3]，sorafenib投与（MST 6.5か月）[4]と比較し良好な予後を期待できる。

HCCの胆管侵襲・腫瘍栓は手術症例中3.4%の頻度で，左右肝管より十二指腸側への進展は0.9%である[5]。Liuらは胆管内腫瘍栓で再発する場合，初回の手術や局所療法の断端が不十分なためと報告している[6]。生存率の改善のためには，手術による病巣の完全切除が必要で[7-9]，手術治療の5年生存率は24～33%と報告されている[9-12]。腫瘍栓が胆管に直接浸潤していることは少なく，胆管から剝離・摘出可能なことが多いため，剝離困難例以外を除いて肝外胆管切除は必要ないと考える[8,12,13]。また，胆管内腫瘍栓に対する肝外胆管切除は予後に影響しないとする報告が多い[11-13]。

リンパ節転移，胆管内腫瘍栓はまれであるが，病変を完全切除することで長期予後を期待できるため耐術能があり，切除可能な病変の場合，手術治療を検討すべきである。

文　献

1) Xiaohong S, Huikai L, Feng W, *et al*: Clinical significance of lymph node metastasis in patients undergoing partial hepatectomy for hepatocellular carcinoma. *World J Surg* **34**(5): 1028-1033, 2010.
2) Hasegawa K, Makuuchi M, Kokudo N, *et al*: Impact of histologically confirmed Lymph node metastases on patient survival after surgical resection for hepatocellular carcinoma: report of a Japanese nationwide survey. *Ann Surg*: 2013 Mar 25.[Epub ahead print]
3) Chen YX, Zeng ZC, Fan J, *et al*: Defining prognostic factors of survival after external beam radiotherapy treatment of hepatocellular carcinoma with lymph node metastases. *Clin Transl Oncol*: 10.1007/s12094-012-0997-6, 2013.
4) Cheng AL, Kang YK, Chen Z, *et al*: Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* **10**(1): 25-34, 2009.
5) 工藤正俊，有井滋樹，猪飼伊和夫・他：第18回全国原発性肝癌追跡調査報告（2004～2005）（日本肝癌研究会追跡調査委員会）．肝臓　**51**(8): 460-484, 2010.
6) Liu QY, Lai DM, Liu C, *et al*: A special recurrent pattern in small hepatocellular carcinoma after treatment: bile duct tumor thrombus formation. *World J Gastroenterol* **17**(43): 4817-4824, 2011.
7) 土屋泰夫，佐野佳彦，中村利夫・他：胆管内発育型肝細胞癌の1例　本邦報告例の臨床的検討．日消外会誌　**32**(9): 2258-2262, 1999.
8) Peng BG, Liang LJ, Li SQ, *et al*: Surgical treatment of hepatocellular carcinoma with bile duct tumor thrombi. *World J Gastroenterol* **11**(25): 3966-3969, 2005.
9) Xiangji L, Weifeng T, Bin Y, *et al*: Surgery of hepatocellular carcinoma complicated with cancer thrombi in bile duct: efficacy for criteria for different therapy modalities. *Langenbecks Arch Surg* **394**(6): 1033-1039, 2009.
10) Esaki M, Shimada K, Sano T, *et al*: Surgical results for hepatocellular carcinoma with bile duct invasion: a clinicopathologic comparison between macroscopic and microscopic tumor thrombus. *J Surg Oncol* **90**(4): 226-232, 2005.
11) Moon DB, Hwang S, Wang HJ, *et al*: Surgical outcomes of hepatocellular carcinoma with bile duct tumor thrombus: a Korean multicenter study. *World J Surg* **37**(2): 443-451, 2013.
12) Shiomi M, Kamiya J, Nagino M, *et al*: Hepatocellular carcinoma with biliary tumor thrombi: aggressive operative approach after appropriate preoperative management. *Surgery* **129**(6): 692-698, 2001.
13) Satoh S, Ikai I, Honda G, *et al*: Clinicopathologic evaluation of hepatocellular carcinoma with bile duct thrombi. *Surgery* **128**(5): 779-783, 2000.

本論文の要旨は第35回日本癌局所療法研究会において発表した。

20142301IA（2/2）

厚生労働科学研究費補助金

肝炎等克服緊急対策研究事業

次世代シーケンシング・ゲノムワイド関連解析を用いた C型肝炎治療に伴う肝病態進展軽快、肝発癌に関わる宿主因子の解析

平成２６年度　総括・分担研究報告書

研究代表者　坂本　直哉

平成２７（２０１５）年　５月

２／２冊

Carcinogenesis vol.35 no.2 pp.272–281, 2014
doi:10.1093/carcin/bgt343
Advance Access publication October 15, 2013

Heat shock factor 1 accelerates hepatocellular carcinoma development by activating nuclear factor-κB/mitogen-activated protein kinase

Makoto Chuma*, Naoya Sakamoto, Akira Nakai[1], Shuhei Hige, Mitsuru Nakanishi, Mitsuteru Natsuizaka, Goki Suda, Takuya Sho, Kanako Hatanaka[2], Yoshihiro Matsuno[2], Hideki Yokoo[3], Toshiya Kamiyama[3], Akinobu Taketomi[3], Gen Fujii[4], Kosuke Tashiro[5], Yoko Hikiba[6], Mitsuaki Fujimoto[1], Masahiro Asaka and Shin Maeda[7]

Department of Gastroenterology and Hepatology, Hokkaido University, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan, [1]Department of Biochemistry and Molecular Biology, Yamaguchi University, Ube, Japan, [2]Department of Pathology and [3]Department of Gastroenterological Surgery I, Hokkaido University, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan, [4]Division of Cancer Prevention, National Cancer Center Research Institute, Tokyo, Japan, [5]Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka, Japan, [6]Division of Gastroenterology, Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan and [7]Department of Gastroenterology, Yokohama City University, Yokohama, Japan

*To whom correspondence should be addressed. Tel: +81 11-716-1611; Fax: +81 11-706-7867;
Email: mchuuma@med.hokudai.ac.jp

Heat shock factor 1 (HSF1), a major transactivator of stress responses, has been implicated in carcinogenesis in various organs. However, little is known about the biological functions of HSF1 in the development of hepatocellular carcinoma (HCC). To clarify the functional role of HSF1 in HCC, we established HSF1-knockdown (HSF1 KD) KYN2 HCC cells by stably expressing either small hairpin RNA (shRNA) against HSF1 (i.e. HSF1 KD) or control shRNA (HSF1 control). Tumorigenicity was significantly reduced in orthotopic mice with HSF1 KD cells compared with those with HSF1 control cells. Reduced tumorigenesis in HSF1 KD cells appeared attributable to increased apoptosis and decreased proliferation. Tumor necrosis factor-α-induced apoptosis was increased in HSF1 KD cells and HSF1$^{-/-}$ mouse hepatocytes compared with controls. Decreased expression of IκB kinase γ, a positive regulator of nuclear factor-κB, was also observed in HSF1 KD cells and HSF1$^{-/-}$ mouse hepatocytes. Furthermore, expression of bcl-2-associated athanogene domain 3 (BAG3) was dramatically reduced in HSF1 KD cells and HSF1$^{-/-}$ mouse hepatocytes. We also found that epidermal growth factor-stimulated mitogen-activated protein kinase signaling was impaired in HSF1 KD cells. Clinicopathological analysis demonstrated frequent overexpression of HSF1 in human HCCs. Significant correlations between HSF1 and BAG3 protein levels and prognosis were also observed. In summary, these results identify a mechanistic link between HSF1 and liver tumorigenesis and may provide as a potential molecular target for the development of anti-HCC therapies.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and the third leading cause of cancer death worldwide (1). Despite marked advances in diagnostic and therapeutic techniques, prognosis remains unsatisfactory for HCC patients (2,3). An understanding of HCC carcinogenesis at the molecular level is thus urgently needed in order to identify novel molecular targets for the development of more effective therapies.

Heat shock factor 1 (HSF1) is the main regulator of the heat shock response, which is involved in protecting cells and organisms from heat, ischemia, inflammation, oxidative stress and other noxious conditions (4,5). Under various forms of physiological stress, HSF1 drives the production of heat shock proteins (HSPs), such as HSP27, HSP70 and HSP90, which act as protein chaperones (5,6). The functions of HSF1 are not limited to increasing the expression of chaperones; HSF1 also modulates the expression of hundreds of genes other than chaperones that are critical for survival under an array of potentially lethal stressors (6–8). As a result, HSF1 influences fundamental cellular processes such as cell cycle control, protein translation, glucose metabolism and proliferation (7–12). In human tumors, constitutive expression of Hsp27, Hsp70 and Hsp90 at high levels predicts poor prognosis and resistance to therapy (13–15). These effects are often attributable to HSF1-dependent mechanisms (16). Thus, as a master regulator of cellular processes, the roles of HSF1 in carcinogenesis and tumor progression are now emerging. Several recent investigations using mouse models have suggested that HSF1 is involved in carcinogenesis (9,17). In clinical samples, HSF1 is often constitutively expressed at high levels in a variety of tumors, including breast cancer (7,18), pancreatic cancer (19), prostate carcinoma (20) and oral squamous cell carcinoma (21).

Hepatocarcinogenesis is a multistep process, in the majority of cases slowly developing within a well-defined etiology of viral infection and chronic alcohol abuse, leading to the chronic hepatitis and cirrhosis that are regarded as preneoplastic stages (22). A great number of factors, receptors and downstream elements of signaling cascades regulate proliferation and apoptosis. Dysregulation of the balance between cell proliferation and apoptosis thus plays a critical role in hepatocarcinogenesis (23,24). Two of the major pathways of cell proliferation and apoptosis are nuclear factor kappa B (NF-κB) signaling and mitogen-activated protein kinase (MAPK) signaling. NF-κB transcription factors are critical regulators of genes involved in inflammation and the suppression of apoptosis. NF-κB has been shown to be instrumental for tumor promotion in colitis-associated cancer and inflammation-associated liver cancer (25,26). Activation of the extracellular signal-regulated kinase (ERK)/MAPK pathway regulates many important cellular processes, such as proliferation, differentiation, angiogenesis, survival and cell adhesion (27). Importantly, the ERK/MAPK pathway is constitutively activated in HCC (28).

The present study investigated the biological influences of HSF1 in HCC cell proliferation and apoptosis involving the NF-κB and MAPK signal pathways. We found that HSF1 deficiency significantly diminished NF-κB and MAPK activation in primary hepatocytes and HCC cells, so HSF1 deficiency inhibited the development of HCC. Furthermore, clinicopathological analysis demonstrated a significant correlation between HSF1 protein level and prognosis. Our results suggest HSF1 as a promising molecular target for the development of anti-HCC therapeutics.

Abbreviations: BAG3, bcl-2-associated athanogene domain 3; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FACS, fluorescence-activated cell sorting; HCC, hepatocellular carcinoma; HSF1, heat shock factor 1; HSF1 KD, HSF1 knockdown; HSP, heat shock protein; IKKγ, IκB kinase gamma; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mRNA, messenger RNA; NF-κB, nuclear factor kappa B; PCNA, proliferating cell nuclear antigen; SCID, severe combined immune-deficient mice; shRNA, small hairpin RNA; TNF-α, tumor necrosis factor alpha; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling; WT, wild type.

Materials and methods

Cell cultures and reagents

Human HCC cell lines HepG2, PLC/PRF/5, HLE and HLF were obtained from the American Type Culture Collection. Huh7 was obtained from the Japanese Collection of Research Bioresources Cell Bank (Ibaraki, Japan). KIM-1 and KYN2 were kindly provided by Dr Hirohisa Yano (Department of Pathology, Kurume University, Kurume, Japan). Li7 was kindly provided by Dr Yae Kanai (Division of Molecular Pathology, National Cancer Center Research Institute,