Table 5 (*continued*)

Variable	Factor	N	Coef.	P_{uni}	Coef.	P_{multi}	
	hsa-miR-1275	183	−19.4	9.6E-02			
	HBsAg (IU/l)	184	0.0	1.3E-10			
	HBeAg (IU/l)	184	−0.1	7.4E-18	0.0	8.67E-07	***
	rs8099917 TT	166	−10.6	1.2E-01			
	HBV DNA (IU/ml)	184	−13.9	5.4E-20	−8.7	2.84E-08	***
	AST	184	−0.1	8.4E-02			
	ALT	184	0.0	2.9E-02			
	γ-GTP	178	0.0	6.9E-01			
	Liver fibrosis	170	−1.2	7.8E-01			
	Activity	170	−3.9	4.1E-01			
	Genotype C	144	−11.4	3.3E-01			
ALT (IU/l)	hsa-miR-122	185	17.0	6.3E-01			
	hsa-miR-22	185	337.0	1.0E-06	48.2	1.29E-01	
	hsa-miR-99a	185	−18.8	5.7E-01			
	hsa-miR-720	185	15.5	5.8E-01			
	hsa-miR-125b	185	−1.6	9.6E-01			
	hsa-miR-1275	184	9.0	8.6E-01			
	HBsAg (IU/l)	185	0.0	9.1E-01			
	HBeAg (IU/l)	185	0.1	2.2E-02			
	rs8099917 TT	167	18.2	5.5E-01			
	HBV DNA (IU/ml)	185	25.1	7.4E-04			
	AST	185	1.9	2.6E-66	1.8	2.20E-47	***
	γ-GTP	179	2.0	2.1E-20	0.4	6.05E-04	***
	Liver fibrosis	171	35.8	7.6E-02			
	Activity	171	74.8	4.3E-04	−19.0	4.58E-02	*
	Genotype C	145	30.0	5.6E-01			
AST (IU/l)	hsa-miR-122	185	0.2	9.9E-01			
	hsa-miR-22	185	148.0	8.2E-06			
	hsa-miR-99a	185	−15.1	3.4E-01			
	hsa-miR-720	185	4.1	7.6E-01			
	hsa-miR-125b	185	−7.3	6.6E-01			
	hsa-miR-1275	184	10.3	6.8E-01			
	HBsAg (IU/l)	185	0.0	6.6E-01			
	HBeAg (IU/l)	185	0.0	3.3E-02			
	rs8099917 TT	167	18.3	2.0E-01			
	HBV DNA (IU/ml)	185	12.6	4.2E-04			
	ALT	185	0.4	2.6E-66	0.4	1.05E-59	***
	γ-GTP	179	0.9	8.1E-18			
	Liver fibrosis	171	27.2	4.8E-03			
	Activity	171	48.6	1.5E-06	17.4	1.98E-04	***
	Genotype C	145	4.0	8.7E-01			
γ-GTP (IU/l)	hsa-miR-122	179	−5.3	6.4E-01			
	hsa-miR-22	179	46.4	4.2E-02	−48.0	1.95E-02	*
	hsa-miR-99a	179	−10.1	3.4E-01			
	hsa-miR-720	179	3.9	6.7E-01			
	hsa-miR-125b	179	−9.7	3.8E-01			
	hsa-miR-1275	178	33.9	4.3E-02	43.2	2.70E-03	**
	HBsAg (IU/l)	179	0.0	7.3E-01			
	HBeAg (IU/l)	179	0.0	5.3E-01			
	rs8099917 TT	161	10.9	2.7E-01			
	HBV DNA (IU/ml)	179	3.0	2.3E-01			
	AST	179	0.4	8.1E-18			
	ALT	179	0.2	2.1E-20	0.2	5.35E-19	***
	Liver fibrosis	166	24.1	1.7E-04	15.9	1.59E-03	**
	Activity	166	23.5	7.4E-04			
	Genotype C	140	15.7	3.3E-01			

(*continued on next page*)

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Table 5 (*continued*)

Variable	Factor	N	Coef.	P_{uni}	Coef.	P_{multi}	
Liver fibrosis	hsa-miR-122	171	−0.3	6.4E-02			
	hsa-miR-22	171	0.0	9.3E-01			
	hsa-miR-99a	171	−0.3	5.3E-02			
	hsa-miR-720	171	−0.1	4.6E-01			
	hsa-miR-125b	171	−0.2	7.7E-02			
	hsa-miR-1275	170	0.2	2.6E-01			
	HBsAg (IU/l)	171	0.0	8.4E-02			
	HBeAg (IU/l)	171	0.0	6.7E-01			
	rs8099917 TT	160	0.4	1.8E-04			
	HBV DNA (IU/ml)	171	0.0	3.8E-01			
	AST	171	0.0	4.8E-03			
	ALT	171	0.0	7.6E-02			
	γ-GTP	166	0.0	1.7E-04	0.0	3.79E-02	*
	Activity	171	0.6	4.8E-15	0.5	1.35E-09	***
	Genotype C	139	0.4	3.0E-02	0.4	2.63E-02	*
Activity	hsa-miR-122	171	0.2	1.6E-01			
	hsa-miR-22	171	0.4	1.3E-01			
	hsa-miR-99a	171	0.2	1.7E-01			
	hsa-miR-720	171	0.2	1.4E-01			
	hsa-miR-125b	171	0.2	1.1E-01			
	hsa-miR-1275	170	0.1	7.4E-01			
	HBsAg (IU/l)	171	0.0	3.1E-01			
	HBeAg (IU/l)	171	0.0	9.2E-02			
	rs8099917 TT	160	0.9	1.9E-17	0.6	3.80E-13	***
	HBV DNA	171	0.1	4.0E-06	0.1	1.51E-03	**
	AST	171	0.0	1.5E-06	0.0	5.66E-04	***
	ALT	171	0.0	4.3E-04			
	γ-GTP	166	0.0	7.4E-04			
	Liver fibrosis	171	0.5	4.8E-15	0.4	7.00E-11	***
	Genotype C	139	0.0	8.1E-01			

predicted targets and "Cancer," "Hematological Disease," and "Gastrointestinal Disease" networks in HBV patients. To determine if the HBV-associated serum microRNAs shared common transcriptional regulators, upstream transcription factors for each up-regulated microRNA were retrieved from ChIPBase (http://deepbase.sysu.edu.cn/chipbase/ accessed on 14 September 2014).[23] NRSF, JunD, c-Jun transcription have been reported to regulate expression of miR-125b, miR-22, and miR-99a. ZNF11 regulates both miR-125b and miR-99a, and NANOG, E2F4, and HNF4A have been reported to regulate miR-122 and miR-22.

Discussion

This study reports a set of microRNAs that were up- or down-regulated in serum of patients with chronic HBV or HCV compared to healthy subjects. MiR-122 was significantly up-regulated in serum of patients with HBV or HCV, whereas elevated miR-22, miR-99, and miR-125b levels were more characteristic of chronic HBV infection. A number of microRNAs were up-regulated in HBeAg-positive patients compared to HBeAg-negative patients. The HBeAg-associated microRNAs are regulated by a small set of shared transcription factors, including c-Jun, ZNF11, and HNF4A.[23] Expression levels of most HBeAg-associated microRNAs were highly correlated, but individual microRNAs were independently associated with different aspects of HBV infection. MiR-122 was independently associated with HBV DNA, whereas miR-125b was associated with multiple aspects of viral replication, including HBV DNA, HBsAg, and HBeAg, and miR-22 and miR-1275 were independently associated with serum levels of γGTP, a liver enzyme normally associated with alcoholic liver disease or biliary obstruction but which may be elevated in the event of severe viral hepatitis.[24] These results suggest that serum microRNA profiles might serve a diagnostic role in monitoring different aspects of viral infection, although their specific roles in pathogenesis of viral hepatitis remain to be worked out.

The presence of specific serum microRNA profiles associated with chronic HCV or HBV infection suggests involvement of these microRNAs in host-mediated antiviral defense or pathogenesis. Hepatic microRNAs enter the serum via apoptosis or necrosis, or they may be actively secreted within exosomes or viral particles.[14] MiR-122 is abundantly expressed in hepatocytes, and its presence in the serum has been shown to correlate with ALT levels and liver damage.[25,26] MiR-122 strongly suppresses HBV replication both through direct binding to HBV RNA as well as indirectly through cyclin G1-modulated p53 activity.[27–31] MiR-125a-5p, miR-199a-3p and miR-210 also

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inhibit viral replication by directly binding to and suppressing HBV RNA.[30,32,33] MiR-99a is abundantly expressed in the liver and in exosomes and acts as a tumor suppressor by targeting IGF-1R and inducing cell cycle arrest.[16,34] In addition, miR-99 suppresses activity of NF-κB, a transcription factor associated with inflammation and tumorigenesis.[35] In HCC, miR-99a may be severely down-regulated in liver tissue, which is associated with poor prognosis and shorter survival time.[34] As with miR-99a, miR-22 is also abundantly expressed in hepatocytes and exosomes and acts as a tumor suppressor.[16] MiR-22 induces cellular senescence by directly targeting CDKN1A, CDK6, SIRT1, and Sp1 HCC[36,37] and is down-regulated in HBV-related HCC.[37]

Two serum microRNAs investigated in this study (miR-1246 and miR-1275) are part of a set of 13 mitomiRs that have been reported to be significantly enriched in the mitochondrial RNA fraction.[38] Mitochondria play a central role in oxidative stress and apoptosis and are targeted by the HBV X (HBx) protein and the HCV p7 protein.[39] Most mitomiRs, including miR-1246 and miR-1275, are predicted to target COX1, ND5, or other components of the respiratory chain.[38] In this study miR-1275 was significantly up-regulated in patients with HBV and was independently associated with γGTP level, whereas miR-1246 was marginally up-regulated in patients with HCV. MiR-720 has been reported to target the oncogene TWIST1 involved in tumor metastasis in breast cancer,[40] but its status as a microRNA has been challenged due to a possible mis-annotation of what may be a tRNA fragment instead.[41]

An unexpected result of this study is that serum levels of a number of microRNAs were elevated in HBeAg-positive patients compared to HBeAg-negative patients, even though expression levels of both HBeAg-positive and negative patients were both higher than in healthy subjects. The role of the HBe antigen in HBV infection remains unclear, as it is not required for infection but may serve an immunomodulatory role and contribute to chronic infection through vertical transmission by crossing the placenta. However, the HBV precore region that codes for the HBe antigen is highly conserved among hepadnaviruses, which also infect avian hosts lacking a placenta, suggesting that the protein has a more fundamental function. The precore protein contains a signal peptide, causing it to be secreted.[42] However, up to 30% of the protein is retained in the cytoplasm.[43] While secreted HBeAg may have an immunosuppressive role, intracellular HBeAg instead promotes inflammation.[44] However, HBeAg has been shown to inhibit Toll-like receptor signaling and suppress NF-kB and interferon-beta promoter activity.[45] HBeAg also inhibits IL-6 production by blocking activation of RIPK2-mediated activation of NF-kB.[46] Therefore HBeAg may have a complex roles in both intracellular and extracellular immune modulation.

Seroconversion of HBeAg-positive patients to HBe antibody (HBeAb)-positive patients is usually accompanied by a stop codon mutation within the precore open reading frame.[47] This region has been identified as a mutation hotspot for APOBEC3G, an interferon-stimulated deaminase that inhibits HBV replication by hyper-editing of single-stranded HBV DNA[22] as well as by directly blocking reverse transcription.[48] While hypermutation is deleterious to the virus, a small fraction may acquire mutations conferring a selective advantage.[22] Warner et al. proposed a frequency-dependent selection model positing that while HBeAg suppresses the immune response, HBeAg-negative strains may have an initial competitive advantage by benefitting from HBeAg-mediated immune suppression conferred by HBeAg-positive strains while expending fewer of its resources.[49] However, as the frequency of the HBeAg-positive strain falls, the immune system begins to mount a defense against HBeAg-negative viruses, leading to seroconversion.

It is not clear why serum microRNA levels of several microRNAs, including miR-122, miR-22, miR-125, and miR-99a, tended to be higher in HBeAg-positive individuals compared to HBeAg-negative individuals and are higher in HBV-infected individuals compared to healthy subjects. However, Winther et al. reported similar results in children with chronic hepatitis B and found that plasma levels of a subset of microRNAs decreased significantly in one child before and after HBe seroconversion.[50] We have previously shown that both HBc and HBs proteins co-localize and physically interact with AGO2 in hepatocytes and that siRNA ablation of AGO2 suppressed HBV DNA and HBsAg production,[10] suggesting that components of the RNA silencing machinery are recruited during HBV replication. HSP90 has been reported to act as a chaperone during RNA loading of Argonaute proteins[51] and is also essential in catalyzing HBV reverse transcription and capsid formation by interacting with the pregenomic RNA encapsidation signal, reverse transcriptase, and the core protein.[52] Interestingly, APOBEC3G has been shown to interfere with microRNA regulation by disrupting assembly of the miRNA-inducing silencing complex (miRISC).[53] APOBEC3G itself is also incorporated into nucleocapsids by directly binding to the core protein.[54] While microRNA-mediated gene silencing is associated with accumulation in P-bodies, microRNAs may also be sorted into multivesicular bodies by ESCRT proteins and secreted as exosomes.[55] MiR-122, miR-125b, miR-199a, miR-210, and possibly other microRNAs bind directly to targets within the HBV genome. MiR-199a and miR-210 have been shown to suppress HBsAg production in cell culture. However, HBV has been shown to enhance autophagy without a corresponding increase in protein degradation by HBsAg-mediated activation of the unfolded protein response, and disruption of autophagy inhibits HBV production.[56] Although it is not clear how or if HBeAg is involved in this process, it is possible that the loss of non-secreted intracellular HBeAg or a conformational change in precore RNA resulting from precore mutations interferes with viral control of autophagy or suppression of innate immune signaling. This loss of control over the intracellular environment might result in suppressed viral replication and decreased secretion of exosome-associated microRNAs.

The millions of people chronically infected with HBV or HCV pose a serious public health challenge. While cirrhosis and HCC may develop over a span of decades, HCC is often not detected until late in development, resulting in poor prognosis and leaving few treatment options. Sensitive, non-invasive methods able to detect subtle changes in disease state are needed for early identification of individuals at increased risk. Serum microRNAs may improve

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early detection by providing an indirect means to monitor changes in gene and microRNA expression in the liver.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at doi:10.1016/j.jinf.2014.10.017.

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Original Article

Role of 3-D conformal radiotherapy for major portal vein tumor thrombosis combined with hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

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Aim: To evaluate the response, survival and safety on 3-D conformal radiotherapy (3D-CRT) for major portal vein tumor thrombosis (PVTT) combined with hepatic arterial infusion chemotherapy (HAIC) for advanced hepatocellular carcinoma (HCC).

Methods: In this retrospective study, 83 advanced HCC patients treated with HAIC who met the following criteria were enrolled: (i) PVTT of the main trunk or first branch of the portal vein; (ii) no extrahepatic metastasis; (iii) Child–Pugh score of 5–7; (iv) performance status of 0 or 1; and (v) no history of sorafenib treatment. The response, overall survival (OS), time to treatment failure (TTF), post-progression survival (PPS) and safety were compared between HAIC combined with 3D-CRT for PVTT (RT group, $n = 41$) and HAIC alone (non-RT group, $n = 42$).

Results: The objective response of PVTT was significantly higher in the RT group (56.1%) than in the non-RT group (33.3%), while that of intrahepatic tumor and OS were not significantly different between groups. Median OS, TTF and PPS were significantly longer in the RT group than in the non-RT group (8.6 and 5.0 months, 5.0 and 2.7 months, and 5.3 and 1.5 months, respectively) among intrahepatic tumor non-responders to HAIC, whereas those were not significantly different between groups among intrahepatic tumor responders to HAIC. By multivariate analysis, the combination of 3D-CRT with HAIC was an independent contributing factor for OS (hazard ratio, 3.2; 95% confidence interval, 1.692–6.021; $P < 0.001$) among intrahepatic HCC non-responders to HAIC.

Conclusion: 3D-CRT for PVTT combined with HAIC could provide survival benefit to non-responder to HAIC.

Key words: hepatic arterial infusion chemotherapy, hepatocellular carcinoma, portal vein tumor thrombosis, radiotherapy

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers and causes of cancer death worldwide.[1–3] Although the survival of patients with HCC has gradually improved following the development of new diagnostic techniques and advancements in therapeutic modalities, such as surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection, transcatheter arterial chemoembolization (TACE), radiotherapy (RT), hepatic arterial infusion chemotherapy (HAIC) and the oral multikinase inhibitor sorafenib,[4–11] the prognosis of patients with advanced HCC remains poor.

In recent phase III trials, the efficacy of sorafenib in advanced HCC was demonstrated in terms of overall survival (OS) and time to progression (TTP) compared with placebo.[10,11] These studies reported that median

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overall survival (MST) was 10.7 and 6.5 months, respectively, and the median time to radiological progression was 5.5 and 2.8 months, respectively, in the sorafenib group. While sorafenib seems to have survival benefits, the objective response rate is less than 3.3%. Furthermore, the presence of macroscopic vascular invasion (MVI) still remains a poor prognostic factor.[10,11] Also, the efficacy and safety of sorafenib for patients with Child–Pugh score B has yet to be demonstrated.[12,13]

Recent advances in implantable drug delivery systems have facilitated repeated arterial infusion of chemotherapeutic agents. HAIC increases local tissue drug concentrations and consequently reduces the side-effects of anticancer agents. Several groups reported favorable results with low-dose cisplatin plus 5-fluorouracil (5-FU; FP therapy) for advanced HCC, especially those with portal vein tumor thrombosis (PVTT) in the first branch (Vp3) or in the main trunk (Vp4).[9,14] Recent studies have also reported the survival benefits (response rate, ~30–50%) of the combination therapy of intra-arterial 5-FU with interferon (IFN)-α (5-FU/IFN) for advanced HCC with Vp3/4.[15–18] While the cumulative survival rates of patients stratified by response to HAIC was significantly higher in responders than others, those were limited in non-responders.

Meanwhile, advances in 3D-CRT have allowed the delivery of radiation doses to the tumor and minimized the radiation dose to normal tissue. As a result of improvement of the antitumor effect and minimization of damage to normal tissue, this modality makes a possible local irradiation for PVTT.[8,19] In spite of the development of new chemotherapies and radiotherapies, the prognosis of HCC patients with Vp3/4 remains poor. Considering the poor outcome of monotherapy for locally advanced solid tumors, the combination of chemotherapy and RT may result in a higher response rate. In fact, recent studies reported the efficacy of combination therapy of arterial infusion chemotherapy and 3D-CRT in HCC patients with PVTT.[20,21] Although these reports demonstrated higher response rates and safety for this combination therapy, the survival benefit remains uncertain due to an insufficient number of cases and lack of subanalyses of survival benefit according to tumor response to HAIC. In the retrospective cohort study, we investigated tumor control, survival benefit and safety by comparing the combination therapy (3D-CRT for PVTT [Vp3/4] along with HAIC) and HAIC alone.

METHODS

Patients

Study design and eligibility

FROM JUNE 2000 to March 2013, 325 patients with unresectable advanced HCC were treated with HAIC in our hospital. In this study, the following enrollment criteria were applied: (i) HCC with Vp3 or Vp4; (ii) without extrahepatic metastasis; (iii) Child–Pugh score of 5–7; (iv) performance status (PS) of 0 or 1; (v) no history of sorafenib treatment; and (vi) at least a 4-week rest period of no treatment since any previous treatment for HCC. Eighty-three patients who met the criteria were enrolled in this retrospective cohort study. Forty-one patients treated with HAIC combined with 3D-CRT for PVTT were defined as the RT group and the remaining 42 patients treated with HAIC alone were defined as the non-RT group. In the RT group, patients received 3D-CRT concomitantly with the first course of HAIC. The use of RT varied according to the study period. HAIC alone was mainly in use between June 2000 and March 2004, whereas HAIC combined with 3D-CRT was mainly in use between April 2004 and March 2013. The baseline characteristics of these patients are summarized in Table 1. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Institutional Review Board of Hiroshima University. Written informed consent was obtained from each patient after detailed explanation about the therapy.

Therapeutic protocol

HAIC

Patients with advanced HCC underwent arterial infusions of anticancer agents via the injection port. Two drug regimens were used for HAIC: intra-arterial low-dose cisplatin (CDDP; Nihonkayaku, Tokyo, Japan) combined with FP (5-FU; Kyowa Hakko, Tokyo); or intra-arterial 5-FU with s.c. IFN combination therapy (5-FU/IFN). One course of chemotherapy lasted 2 weeks. In both regimens, 5-FU (330 mg/m^2 per day) was administrated over 24 h using a mechanical infusion pump from days 1 to 5 of the first and second weeks. In addition to 5-FU, FP chemotherapy included daily intra-arterial CDDP (20 mg/m^2 per day; Randa [Nippon Kayaku, Tokyo, Japan]) on day 1 and 8. The IFN used in the 5-FU/IFN regimen was recombinant IFN-α-2b (Intron A; MSD, Osaka, Japan) at 3×10^6 U (3 MU), or natural IFN-α (OIF; Otsuka Pharmaceuticals, Tokyo, Japan) at 5×10^6 U (5 MU), was administrated i.m. on

Table 1 Comparison of clinical profile between the RT group and non-RT group

Variables	RT group ($n = 41$)	Non-RT group ($n = 42$)	P
Age (years)	66 (35–84)	64.5 (40–85)	0.891
Sex (male/female)	40/1	36/6	0.052
EOCG PS (0/1)	37/4	35/7	0.353
Etiology (HBV/HCV/NBNC/alcohol)	12/18/6/5	10/27/4/1	0.140
Child–Pugh score (5/6/7)	19/14/8	14/18/10	0.480
Rate of tumor occupation in the liver (≤50%/>50%)	28/13	27/15	0.699
Vp (3/4)	18/23	29/13	0.021
Vv (0/2/3)	38/2/1	38/2/2	0.718
Alb (g/dL)	3.5 (3.1–5.0)	3.5 (2.6–4.6)	0.602
Total bilirubin (mg/dL)	1.0 (0.4–3.0)	1.0 (0.5–2.9)	0.631
Prothrombin time activity (%)	86 (58–119)	85.5 (56–117)	0.938
Platelet count ($\times 10^4/\mu L$)	12.4 (3.8–25.1)	11.6 (4.6–88.8)	0.816
AFP (ng/mL)	464 (5–200000)	1742 (12.4–1895000)	0.135
DCP (mAU/mL)	3991 (32–287180)	2275 (11–722140)	0.503
Regimen (5-FU/IFN/low-dose FP)	25/16	23/19	0.567

Categorical data are represented as numbers of patients, and continuous data is represented as median and range.
5-FU, 5-fluorouracil; AFP, α-fetoprotein; Alb, albumin; DCP, des-c-carboxy prothrombin; EOCG PS, Eastern Cooperative Oncology Group performance status; FP, cisplatin plus 5-fluorouracil therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, non-B, non-C; RT, radiotherapy; Vp3, tumor thrombus in the first branch of the portal vein; Vp4, tumor thrombus in the trunk of the portal vein; Vv2, tumor thrombus in the right, middle or left hepatic vein trunk, posterior inferior hepatic vein trunk or short hepatic vein; Vv3, tumor thrombus in inferior vena cava.

days 1, 3 and 5 of each week (total dose, 18 and 30 MU, respectively). We have previously reported that there was no significant difference between using recombinant IFN-α-2b and natural IFN-alpha with regard to survival rates when 5-FU/IFN was used for the treatment of advanced HCC.[18] In principle, HAIC was repeated several times during the treatment as much as possible until we considered that it was impossible for patients to continue HAIC based on the following criteria: (i) PS changed to 3 or 4; (ii) adverse events were estimated as grade 4 by Common Technology Criteria for Adverse Events (CTCAE) version 4.0; and (iii) patient requested termination of treatment.

3D-CRT

Among 83 patients, 41 received 3D-CRT, a high-energy photon beam irradiation using 18, 10 or 6 MV, delivered by Linear accelerators (CLINAC 2300 C/D or CLINAC iX; Varian Medical Systems, Palo Alto, CA, USA), at the Department of Radiation Oncology in our hospital. Respiratory motion was coordinated by voluntary breath-holding at the end-expiratory phase. For simulations, contrast enhanced computed tomography (CT) scans (Lightspeed QX/I; GE Medical Systems, Waukesha, WI, USA) were performed by giving an injection of non-ionic iodinated contrast material (100 mL at a rate of 1 mL/s). This planning CT volume data was transferred to a 3-D treatment planning system (Pinnacle3 version 9.0; Phillips Medical Systems, Fitchburg, WI, USA). Gross tumor volume (GTV) was defined as only the PVTT. The clinical target volume margin was usually defined as 0–5 mm around the GTV. A planning target volume (PTV) margin of 5–8 mm, including the respiratory motion reproducibility and setup error, was usually added. Three to five coplanar ports were selected in all patients, including beam direction that avoided the critical organs, if possible. The prescribed doses and fractionations were 30–45 Gy in 10–15 fractions, and were evaluated at the isocenter. The dose constraints of the critical organs were as follows: the percentages of uninvolved liver volume (total liver – PTV) exceeding 20 Gy (V20) was 25% or less, the maximum dose of spinal cord 30 Gy or less.

Assessment of treatment efficacy

The treatment response to HAIC was assessed by contrast-enhanced CT at 4 weeks after completion of each course of the treatment, and then every 2–3 months. The response was defined according to the Response Evaluation Criteria in Solid Tumors version 1.1.[22] A complete response (CR) was defined as disappearance of all target/non-target lesions, no appearance

of any other lesion, and normalization of α-fetoprotein and des-γ-carboxy prothrombin. CR was confirmed at 4 weeks after the first evaluation of CR. A partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of target lesions with the baseline sum of that as the reference. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of target lesions. Stable disease (SD) was defined as meeting neither the PR nor PD criteria. In addition, we evaluated the treatment response of PVTT and that of intrahepatic tumor by measuring the longest diameter.

Adverse events were evaluated according to the CTCAE version 4.0. Radiation-induced liver disease (RILD) is separated into "classic" and "non-classic" RILD. Classic RILD, typically occurring between 2 weeks and 3 months after treatment, involves anicteric hepatomegaly and ascites, the elevation of alkaline phosphatase levels to at least a twofold increase over the upper limit of normal or of pretreatment value in the absence of tumor progression. This end-point can occur in patients with good liver function. Non-classic RILD, typically occurring between 1 week and 3 months after treatment, involves the elevation of alkaline phosphatase levels to more than fivefold the upper limit of normal or CTCAE grade 4 levels in patients with baseline values more than fivefold the upper limit of normal within 3 months after completion of RT, or a decline in liver function (measured by a worsening of Child–Pugh score by ≥2), in the absence of classic RILD. The end-point was described in patients with poor liver function.[23]

Additional therapy

After estimating the response to therapy, we provided various additional therapies such as operation, RFA or TACE for patients with PS of 0–1 when applicable. Patients who attained PR subsequently received HAIC repeatedly. When advanced HCC was downstaged to a single tumor of 50 mm or less in diameter or 1–3 tumors of 30 mm or less in diameter by repeated HAIC, RFA or operation was considered. Patients with SD or PD received TACE with cisplatin–lipiodol suspension. Patients with CR were observed during the clinical course periodically without additional therapy.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test, logistic regression test and χ^2-test when appropriate. We evaluated OS, time to treatment failure (TTF) and post-progression survival (PPS). The cumulative survival rate was calculated from the initial date of the therapy. TTF was calculated from the initial date of HAIC treatment including additional therapies. PPS was calculated from the date of confirmation of PD. These parameters were assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log–rank test. Multivariate analysis of predictors of OS were assessed by Cox proportional hazard model. $P < 0.05$ was considered statistically significant. All aforementioned analyses were performed using SPSS software (version 11; SPSS, Chicago, IL, USA).

RESULTS

Baseline patient characteristics

THE BASELINE CHARACTERISTICS of patients in the RT and non-RT groups are shown in Table 1. No differences were found in age, sex, PS, etiology, Child–Pugh grade, tumor volume, grade of hepatic vein invasion, HAIC regimen, levels of albumin, total bilirubin, prothrombin time activity, platelet count, α-fetoprotein and protein induced by vitamin K absence/antagonist-II between groups. Patients with Vp4 were larger in the RT group (56.1%) than in the non-RT group (31.0%) ($P = 0.021$). The median radiation dose was 39 Gy (range, 30–45 Gy) delivered in 13 fractions (range, 10–15).

Treatment response

The maximum treatment response to HAIC of intrahepatic HCC during treatment courses were as follows. CR, PR, SD and PD were seen in two (5%), 10 (24%), 16 (39%) and 13 patients (32%) in the RT group, and four (9%), 13 (31%), 12 (29%) and 13 (31%) in the non-RT group, respectively. No statistically significant differences were found in objective response rates (CR and PR) of intrahepatic HCC to HAIC between the groups ($P = 0.284$).

The maximum treatment response of PVTT during treatment courses were as follows. CR, PR, SD and PD were seen in six (15%), 17 (41%), 13 (32%) and five patients (12%) in the RT group, and five (12%), nine (21%), 15 (36%) and 13 (31%) in the non-RT group, respectively. The objective response rates of PVTT were 56.1% and 33.3% in the RT group and non-RT group, respectively. This was significantly higher in the RT group than the non-RT group ($P = 0.013$).

OS and significant factors in OS

The MST of the RT group was 12.1 months and that of non-RT group was 7.2 months. The MST was not significantly different between the two groups ($P = 0.3087$, Fig. 1a). Univariate analysis identified platelet count ($<15 \times 10^4/\mu L$) ($P < 0.001$), the treatment response of intrahepatic HCC ($P < 0.001$) and the treatment response of PVTT ($P < 0.001$) as significant factors of OS. Multivariate analysis identified the treatment response of intrahepatic HCC (hazard ratio [HR], 3.3; 95% confidence interval [CI], 1.801–6.114; $P < 0.001$) and the treatment response of PVTT (HR, 2.6; 95% CI, 1.425–4.562; $P = 0.002$) as significant and independent factors of OS among all 83 patients (Table 2).

Next, we compared the OS according to the treatment response of intrahepatic HCC. No significant differences of baseline characteristics between the RT and non-RT groups were found in both intrahepatic HCC responders and non-responders to HAIC (Table 3). While MST was not significantly different between the RT group (30.2 months) and non-RT group (23.3 months) in intrahepatic HCC responders ($P = 0.7181$, Fig. 1b), that was significantly longer in the RT group (8.6 months) than in the non-RT group (5.0 months) in intrahepatic HCC non-responders ($P = 0.0002$, Fig. 1c). Among intrahepatic HCC non-responders, univariate analysis identified five significant factors for OS: (i) male sex ($P = 0.0382$); (ii) Child–Pugh score (5 or 6) ($P = 0.0116$); (iii) platelet count ($<15 \times 10^4/\mu L$) ($P = 0.0366$); (iv) combination with 3D-CRT ($P = 0.0002$); and (v) PVTT treatment response ($P = 0.0164$). By multivariate analysis, Child–Pugh score (5 or 6) (HR, 2.1; 95% CI, 1.061–4.265; $P = 0.033$) and combination with 3D-CRT (HR, 3.2; 95% CI, 1.692–6.021; $P < 0.001$) were significant and independent factors for OS in intrahepatic HCC non-responders (Table 4).

TTF

Additionally, we estimated the TTF, defined as the time from the initial date of HAIC to the final date of HCC treatment including additional therapies for HCC. The median TTF of the RT group was 6.3 months and that of the non-RT group was 4.3 months. The median TTF was not significantly different between the two groups ($P = 0.1116$, Fig. 2a). Median TTF was not significantly different between the RT and non-RT groups (26.6 and 12.6 months, respectively; $P = 0.2998$) among intrahepatic HCC responders (Fig. 2b). On the other hand, this was significantly longer in the RT group than in the

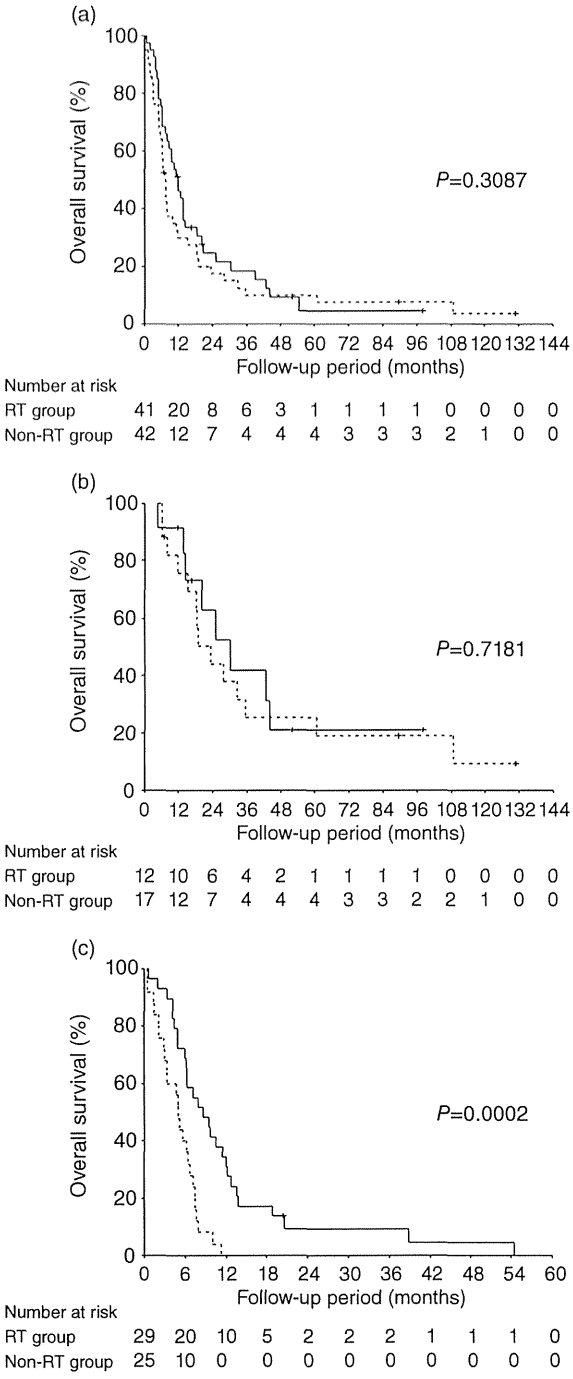

Figure 1 Overall survival. (a) Comparison of overall survival between the radiotherapy (RT) group and non-RT group. (b) Comparison of overall survival between the RT group and non-RT group among intrahepatic tumor responder to hepatic arterial infusion chemotherapy (HAIC). (c) Comparison of overall survival between the RT group and non-RT group among intrahepatic tumor responders to HAIC non-responders. —, RT group; -----, non-RT group.