Table III. *Depletion of BCAA, especially valine, decreased MoDC CD83 expression*[a]

	CCM (n = 8)	ΔBCAA (n = 8)	ΔVal (n = 8)	ΔLeu (n = 8)	ΔIle (n = 7)
CD14					
% Positive cell	8.8 ± 6.4	7.2 ± 7.4	6.9 ± 5.7	4.3 ± 4.9	6.1 ± 5.7
MFI	5.1 ± 2.1	53 ± 3.3	4.8 ± 1.9	3.6 ± 0.9	4.7 ± 1.6
CD83					
% Positive cell	36.2 ± 6.5	***18.8 ± 11.8***[b]	***15.5 ± 3.2***[b]	32.0 ± 8.0	34.6 ± 6.1
MFI	10.7 ± 1.6	***6.6 ± 2.3***[b]	***6.0 ± 1.8***[b]	8.4 ± 1.9	11.7 ± 5.4
CD86					
% Positive cell	63.7 ± 11.4	53.5 ± 14.0	59.9 ± 21.0	57.1 ± 10.5	62.6 ± 19.5
MFI	37.3 ± 12.6	32.1 ± 15.4	27.8 ± 12.3	28.8 ± 19.8	31.3 ± 16.4

[a] Both the proportion of positive cells and the MFI are presented for each marker as the mean ± SD of healthy volunteers.
[b] Value of $p < 0.05$ vs MoDC cultured under CCM (one-way ANOVA and Dunnett's post-hoc procedure).

Immunoblotting

On day 6, MoDC were harvested and lysed using CelLyticTM-M Mammalian Cell Lysis/Extraction Reagent (Sigma-Aldrich). The lysed cells were centrifuged for 10 min at 12,000–20,000 × g to pellet the cellular debris. Thereafter, these protein concentrations were determined by a Modified Lowry Protein Assay kit (Pierce). The total 50 μg of protein were loaded onto SDS-PAGE gel and electrotransferred to a polyvinylidene fluoride (Immun-Blot PVDF membrane; Bio-Rad). After washing, the membranes were incubated in 25 ml of blocking buffer for 1 h at room temperature. Immunostaining was performed with rabbit polyclonal primary Ab (mTOR: no. 2972, p70 S6K: no. 9202, phopho-p70 S6K: no. 9205; Cell Signaling Technology), followed by incubation with a secondary Ab conjugated to HRP (Sigma-Aldrich). Immunoreactive proteins were revealed with an ECL reagent (ECL advance; Amersham Biosciences). To confirm the equal protein loading in all samples, the blot was stripped for 30 min in Ab stripping solution (Re-Blot Plus Western Blot Recycling kit; Chemicon International), washed extensively, and relabeled with anti β-actin Ab and secondary HRP Ab (Sigma-Aldrich). Densitometric analysis was performed using Scion Image for Windows.

Aminogram

The concentrations of plasma amino acids from 27 HCV cirrhotic patients were measured by HPLC. Also, these patients were classified according to the Child-Pugh classification.

Statistical analysis

The data were analyzed with ANOVA, and multiple comparisons were performed with Dunnett's post-hoc procedure. When two groups were analyzed, the differences between groups were analyzed by the Wilcoxon t test. When, more than two groups were analyzed, the differences were analyzed by Bonferroni's analysis. All data are expressed as mean SEM. In all analyses, a p value of <0.05 was considered statistically significant. All statistical analyses were performed with standard statistical software (SPSS 13.0 for Windows).

Results

Depletion of extracellular BCAA did not influence the expression of costimulatory molecules on MoDC, but decreased the expression of CD83

First, to investigate whether the depletion of extracellular BCAA influenced the generation of MoDC, we cultured the monocytes for 6 days under CCM and ΔBCAA. At day 5, the stimulants were added. We evaluated the expression of CD14, CD40, CD80, CD83, CD86, and HLA-DR on the surface of MoDC grown under either CCM or ΔBCAA (Fig. 1*A*) by flow cytometry. There was no difference in the percentage of MoDC expressing CD14, CD40, CD80, CD86, and HLA-DR between the two mediums. Negligible levels of CD14 and higher levels of HLA-DR, CD40, CD80, and CD86 indicated that the cells could differentiate into MoDC in both mediums. However, the CD83 expression was decreased under ΔBCAA as confirmed by the single-color staining. Similarly, when stimulated with either CD40L or poly I:C at day 5, the CD83 expression was impaired in ΔBCAA (data not shown). To investigate which amino acid in BCAA especially influenced the MoDC phenotype, we determined the MoDC phenotype (CD14, CD83, or CD86) in ΔVal, ΔLeu, and ΔIle. In CCM, ΔLeu and ΔIle, the MoDC phenotype was similar. However, in ΔVal, the CD83 expression of MoDC was significantly impaired compared with that in CCM. The CD86 expression was not significantly different in any medium (Table III). On microscopic appearance, depletion of

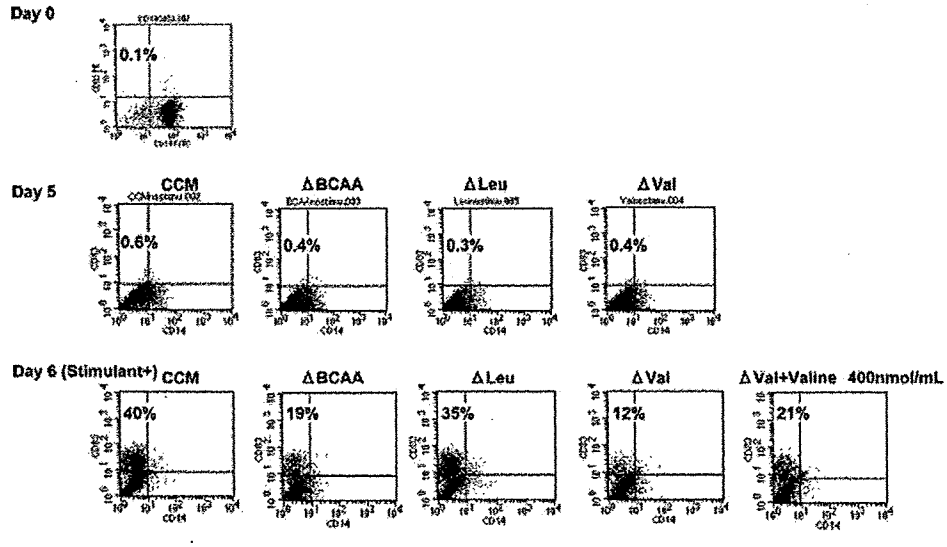

FIGURE 2. BCAA, especially valine, are necessary for MoDC maturation but not for differentiation. Monocytes were cultured under CCM, ΔBCAA, ΔVal, ΔLeu medium as described in Fig. 1. Cells were harvested on days 5 and 6 of culture, stained with different mAbs, and analyzed using flow cytometry. Cells were stained with FITC-labeled anti-CD14 and PE-labeled anti-CD83. For the dot-plot figure, percentages indicate the proportion of cells adopting the DC immunophenotype ($CD14^-/CD83^+$). Results are representative of four experiments from four different donors.

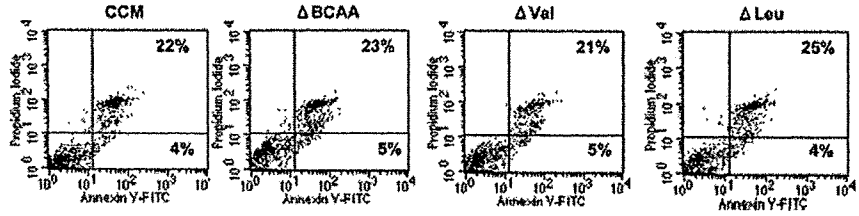

FIGURE 3. Depletion of extracellular BCAA did not influence the MoDC viability. Monocytes were cultured under CCM, ΔBCAA, ΔVal, and ΔLeu medium as described in Fig. 1. Cells were harvested on day 6 of culture. Annexin V^{FITC}/PI staining was performed to determine the cell viability. In the quadrant statistics, PI-negative and annexin V-negative indicated live cells. PI positive (*upper*) indicated necrotic cells. PI negative and annexin V positive (*lower right*) indicated early apoptotic cells. Data shown are representative of four independent experiments with cells from different donors.

BCAA also affected the morphological appearance and behavior of the cells in culture. Monocytes cultured under either CCM, ΔLeu, or ΔIle were adherent with little tendency to form aggregations. On day 6, cells formed large, firmly adherent clusters (Fig. 1*B*), which were typical of mature DCs in vitro. In contrast, monocytes cultured under ΔBCAA and ΔVal formed much smaller clusters. Using a traditional FSC/SSC gate of the FACS plots, we evaluated the mean FSC and SSC values of the MoDC population in each medium. MoDC generated under ΔBCAA or ΔVal expressed lower FSC and SSC values than MoDC generated under CCM.

Depletion of extracellular BCAA or valine influenced the maturation but not the differentiation of MoDC

To further investigate at which point in time the amino acids influenced the MoDC phenotype, we cultured monocytes under CCM, ΔBCAA, ΔVal, or ΔLeu, and determined the phenotype of the MoDC before (day 5) and after (day 6) adding LPS and TNF-α by two-color staining (Fig. 2). Most monocytes (>95%), which were freshly isolated from PBMC, expressed the CD14-positive and CD83-negative phenotype. Before adding the stimulants (day

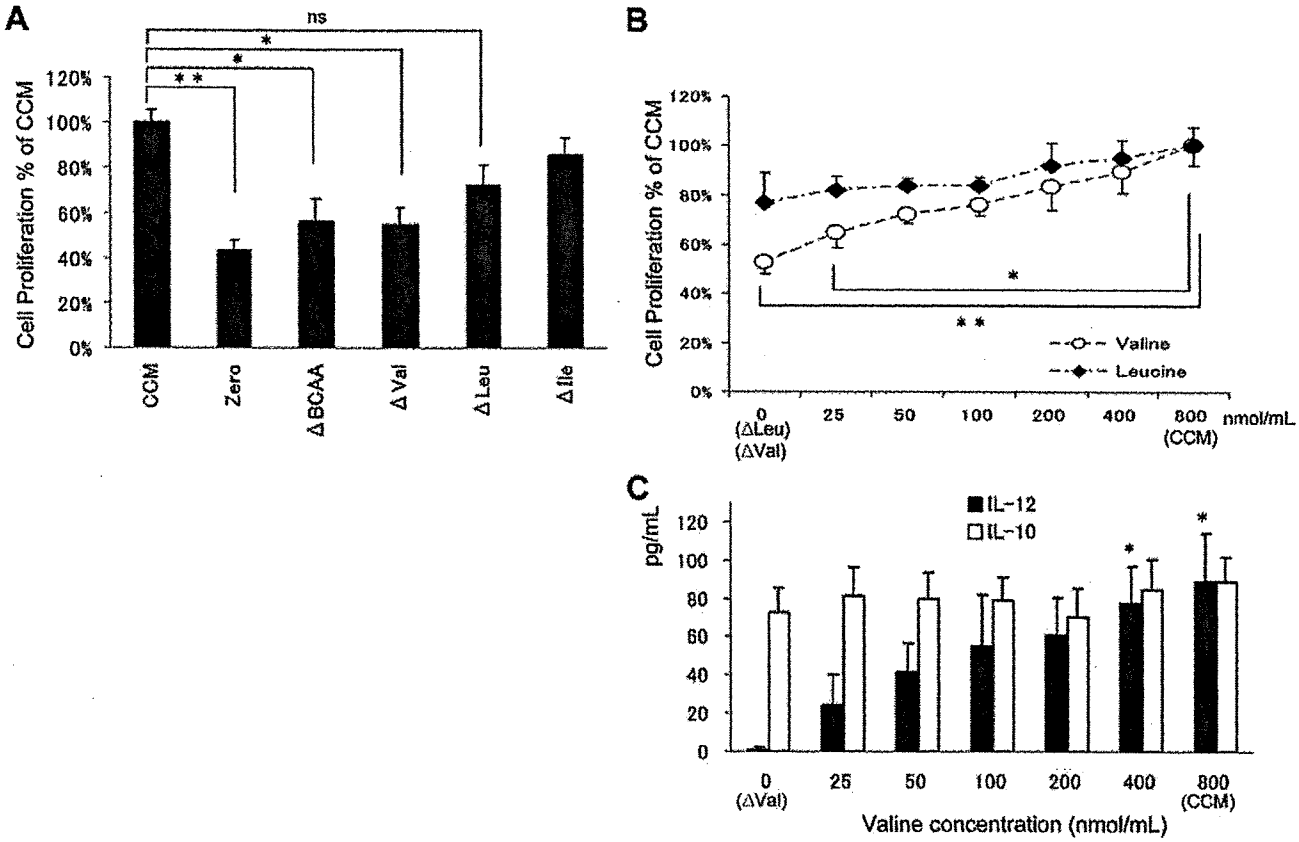

FIGURE 4. BCAA, especially valine, modulated MoDC allostimulatory capacity. *A*, We cultured monocytes under each medium for 5 days (detailed amino acid composition is shown in Table II) in 96-well tissue-culture plates and irradiated MoDC after exposing them to LPS and TNF-α for additional 24 h. The MoDC yielded (5.0×10^4) were cocultured with normal, allogeneic $CD4^+$ T lymphocytes (1×10^5cells/well) under CCM for 4 days and evaluated for their allostimulative capacity by the MLR. *B*, We cultured monocytes in the same way under various mediums that contained 0–800 nM/ml valine or leucine. Zero nanomoles per milliliter of valine or leucine medium are represented by ΔVal or ΔLeu, respectively. CCM contained 800 nM/ml valine and leucine medium. *C*, After 6 days, the supernatants were removed and assayed for the cytokine concentrations. Mean ± (*A*) SEM values from five different donors are shown. *B* and *C*, Mean ± SEM values from four different donors are shown. Statistical significance for all conditions was determined by one-way ANOVA and Bonferroni's post-hoc procedure for *A*, one-way ANOVA and Dunnett's post-hoc procedure for *B* and *C*. **, $p < 0.01$; *, $p < 0.05$ vs CCM (*A* and *B*) or vs ΔVal (*C*).

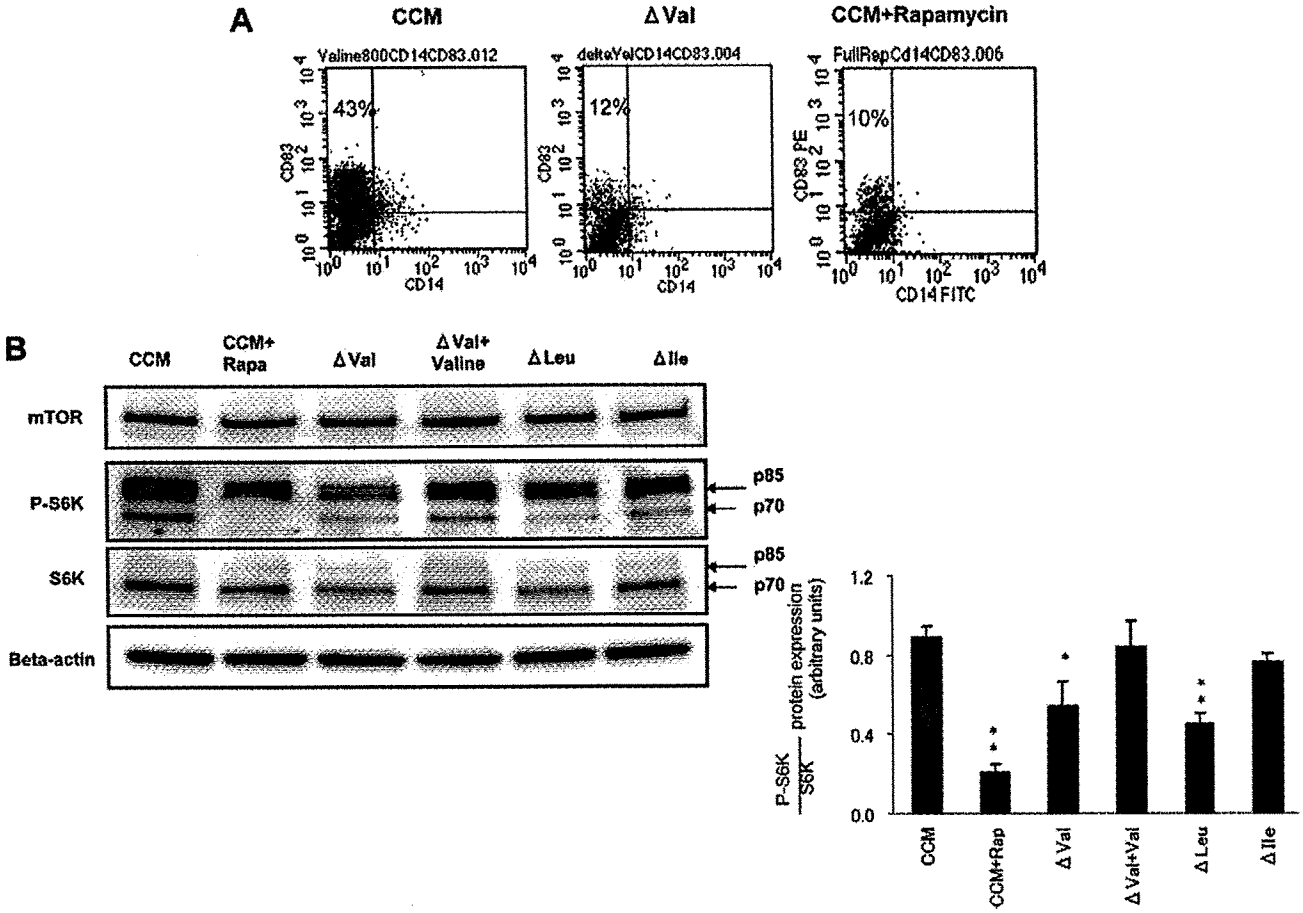

FIGURE 5. Depleting extracellular valine or leucine down-regulated the mTOR/S6K-signaling pathway of MoDC. *A*, We cultured monocytes under CCM for 5 days in 24-well tissue culture plates and exposed them to LPS and TNF-α for an additional 24 h in CCM, ΔVal, and CCM plus rapamycin (1 μM). Representative results from three subjects are shown. *B*, We cultured monocytes under CCM for 5 days and exposed them to LPS and TNF-α for 24 h in either CCM, CCM plus rapamycin (1 μM), ΔVal, ΔLeu, or ΔIle. Also at day 6, we added 400 nM/ml valine to ΔVal. Cells were harvested on day 6 or 7 and lysed. Equal amounts of protein were loaded and the levels of mTOR, p70 S6K, and phosho-p70 S6K were determined by Western blot analysis. Densitometry shows the mean and SEM of the relative levels of phospho-p70 S6K to p70 S6K. Data shown are representative of four independent experiments with cells from different donors. **, $p < 0.01$; *, $p < 0.05$ vs CCM.

5), the MoDC phenotype was CD14 negative and CD83 negative in all medium compositions. These data indicated that the monocytes could differentiate into immature DCs in almost any medium.

Interestingly, after adding the stimulus (day 6), under ΔBCAA and Δvaline, the percentage of $CD14^{-}/CD83^{+}$ mature DCs was lower than that under CCM or ΔLeu. These data indicated that depriving

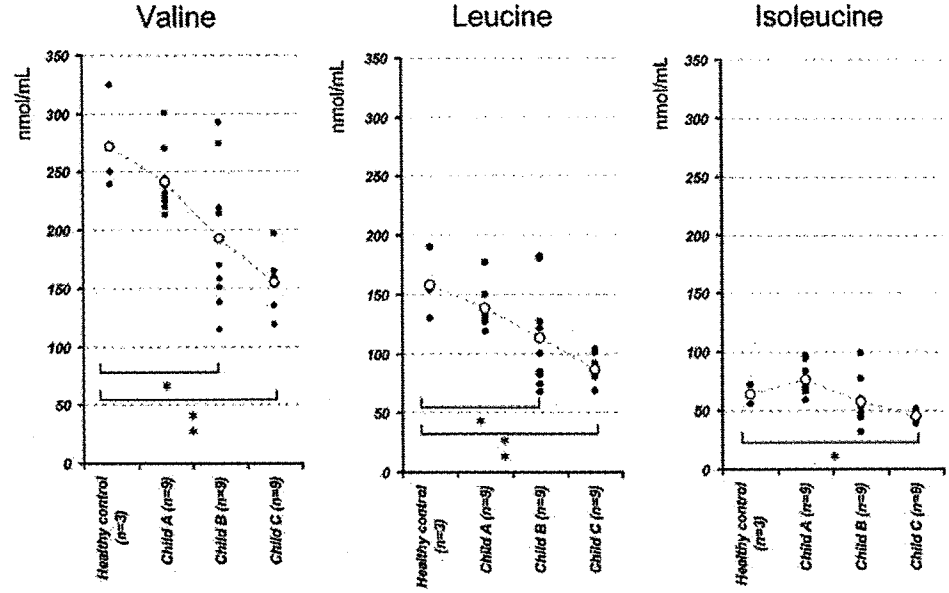

FIGURE 6. The plasma concentrations of BCAA in cirrhotic patients were decreased according to the Child-Pugh grade. Twenty-seven liver cirrhotic patients were classified by Child-Pugh classification. The levels of plasma BCAAs in these patients were measured using HPLC. The dots represent the value from each patient and the circles represent the averages. **, $p < 0.01$, *, $p < 0.05$ vs healthy control.

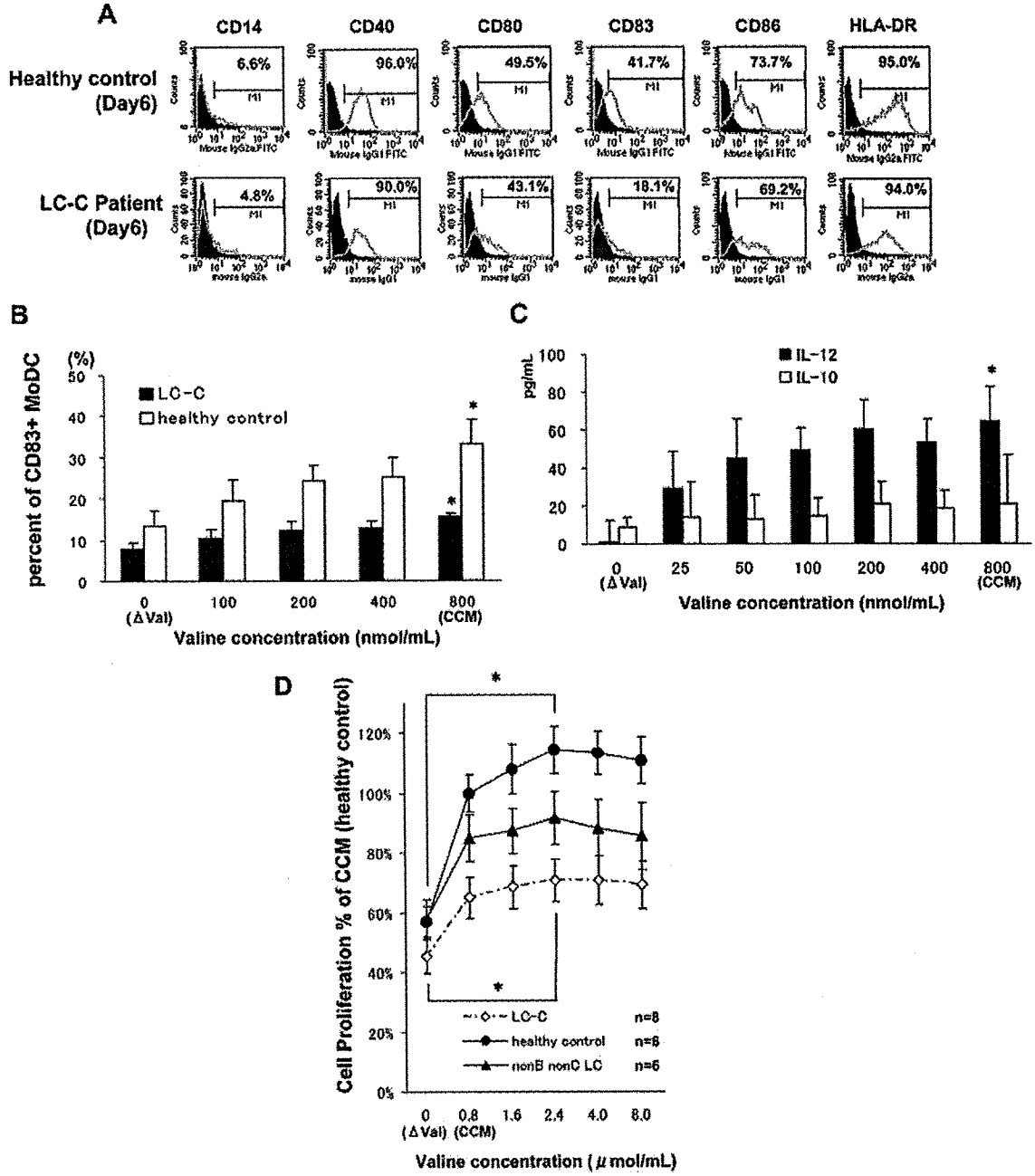

FIGURE 7. Elevating the extracellular valine concentration dose-dependently increased the allostimulatory capacity of MoDC and IL-12 production in cirrhotic patients. *A*, Monocytes isolated from the peripheral blood of HCV liver cirrhotic patients were cultured under CCM and analyzed the phenotypes as described in Fig. 1. For the histogram figure, filled traces represent isotype-matched control Ab staining; open traces indicate a marker-specific Ab; percentages indicate positive cells. Results are representative of five experiments from five different patients. *B*, We cultured monocytes under various mediums that contained 0–800 nM/ml valine and determined the percentage of CD83-positive mature MoDC. *C*, Similarly as in Fig. 4*C*, cytokine productions were measured with ELISA. *D*, We cultured the monocytes under various mediums that contained 0–8.0 μM/ml valine. Zero nanomoles per milliliter of valine is indicated by ΔVal. 0.8 μM/ml valine medium is identical with CCM. Patient number (*B*) 10-13, (*C*) 1-4, (*D*) 5-12, 15-20 in Table I. Statistical significance for all conditions was determined by one-way ANOVA and Dunnett's post-hoc procedure. *, $p < 0.05$ vs ΔVal.

BCAA, especially valine, influenced the maturation of MoDC. After we cultured the monocytes under ΔVal for 5 days, we added 400 nM/ml valine with stimulus to the medium and cultured the cells for an additional 24 h. Then, the percentage of mature DCs was higher than that of ΔVal. We cultured the monocytes under CCM for 5 days, and with an additional 24 h under ΔVal, the percentage of mature DCs was decreased compared with that of CCM (data not shown).

Depletion of extracellular BCAA does not influence MoDC viability

To elucidate the possibility that impaired MoDC maturation under ΔBCAA or ΔVal was caused by decreased cell viability, we evaluated the cell recovery, yield of DCs, and viability on day 6 in each medium. The cell recovery and yield of DCs was not significantly different between any medium. The percentage of cells recovered

was 54 ± 8.4 (±SD), 49 ± 12.1, 49 ± 11.3, and 50 ± 8.9 for the CCM, ΔBCAA, ΔVal, and ΔLeu, respectively. The DC yield percentages were 57 ± 9.2, 56 ± 10.4, 58 ± 8.4, and 57 ± 8.1 for CCM, ΔBCAA, ΔVal, and ΔLeu, respectively. The viability of MoDC on day 6 was determined by annexin V/PI staining. The percentages of necrotic cells, living cells, and early apoptotic cells were not different under any medium (Fig. 3).

BCAA, especially valine, modulated MoDC allostimulatory capacity and cytokine production

Based on the result that MoDC maturation was suppressed by the lack of extracellular BCAA, especially valine, we hypothesized that the concentration of extracellular BCAA could influence the function of MoDC. To investigate this hypothesis, we cultured monocytes for 6 days under CCM, Zero, ΔVal, ΔLeu, ΔIle, and ΔBCAA. The MoDC (5.0×10^4) were cocultured with normal allogeneic $CD4^+$ lymphocytes under CCM and evaluated for their allostimulative capacity by the MLR. Expectedly, the allostimulatory capacity of MoDC cultured under ΔBCAA and ΔVal was significantly impaired ($p = 0.017$, $p = 0.012$, Bonferroni's analysis, respectively), although there was no significant difference among ΔLeu, ΔIle, and CCM (Fig. 4*A*). There was no statistically difference between ΔVal and ΔLeu ($p = 1.000$, Bonferroni's analysis). Furthermore, to examine whether the addition of valine enhanced the function of MoDC, we cultured monocytes under various mediums that contained 0–800 nM/ml valine or leucine, and evaluated the allostimulatory capacity of the MoDC. The addition of valine increased the allostimulatory capacity of MoDC in a dose-dependent manner. However, the concentration of leucine did not influence the pharmacological effect (Fig. 4*B*). Cytokines play key roles in determining the strength and the phenotypes of the T cell response. Thus, on day 6, we measured the cytokine production from MoDC. The addition of valine increased the IL-12 production of MoDC in a dose-dependent fashion. Interestingly, the IL-10 production was not influenced by the concentration of valine (Fig. 4*C*).

Depletion of extracellular valine down-regulated the mTOR/S6K-signaling pathway of MoDC

The mTOR-signaling pathway, one of the most representative pathways, is known as a major effecter of cell growth and proliferation via the regulation of protein synthesis. A previous study showed that the removal of extracellular amino acids, especially leucine, inhibited the ability of mTOR to signal to p70 S6 kinase (15–17). We hypothesized that BCAA modulate the mTOR/S6K-signaling pathway of MoDC and influences the maturation markers. First, to investigate whether the mTOR/S6K inhibitor rapamycin could influence the MoDC phenotype, we cultured monocytes under CCM for 5 days. At day 5, LPS and TNF-α were added to the medium with or without rapamycin (1 μM). Under CCM with rapamycin, the percentage of $CD14^-/CD83^+$ mature DCs was lower than under CCM without rapamycin (Fig 5*A*). These data indicated that rapamycin could suppress the maturation of MoDCs similarly as when depriving them of BCAA or valine. Also, we recovered MoDCs cultured under CCM for 5 days, and matured these MoDCs by stimulant in either CCM, CCM added with rapamycin, ΔVal, ΔLeu, or ΔIle for 24 h. On day 6, we determined the expression of mTOR, p70 S6K, and phospho-p70 S6K by immunoblotting. MoDC expressed similar levels of mTOR, p70 S6K, and β-actin among all mediums. MoDCs cultured in ΔVal and ΔLeu expressed significantly lower levels of phospho-p70 S6K than those cultured in CCM (Fig. 5*B*). Expression of phospho-p70 S6K expression by MoDC in ΔVal was recovered by adding 400 nM/ml valine to medium during stimulation.

Table IV. *Comparison of the phenotypic characteristics of MoDC derived from HCV cirrhotic patients and healthy volunteer*[a]

	CD14		CD40		CD80		CD83		CD86		HLA-DR	
	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI	%	MFI
Healthy control ($n = 5$)	8.8 ± 5.0	5.1 ± 1.4	92.7 ± 4.5	38.0 ± 13.5	65.9 ± 19.0	30.1 ± 23.0	41.7 ± 2.7	11.9 ± 1.2	71.0 ± 9.0	54.9 ± 33.5	93.1 ± 4.1	143.6 ± 20.4
LC-C patient ($n = 5$)	10.8 ± 9.1	4.5 ± 1.5	94.6 ± 2.9	21.1 ± 1.9*	51.0 ± 12.0	10.8 ± 3.8	16.1 ± 4.7**	5.5 ± 1.9**	65.2 ± 5.9	25.0 ± 7.9	92.3 ± 4.6	71.6 ± 26.9**

[a] Both the proportion of positive cells and the MFI are presented for each marker as the mean ± SD of healthy controls or LC-C patients. LC-C patient means HCV cirrhotic patients. Patient number 10-14, *, $p < 0.05$, **, $p < 0.01$.

Elevating the extracellular valine concentration improved the allostimulatory capacity and IL-12 production dose-dependently in MoDC from HCV cirrhotic patients

The functions of DCs are impaired in patients with chronic hepatitis C (28–30). As in the in vivo study, we measured the concentrations of BCAAs in the peripheral blood of 27 HCV cirrhotic patients by HPLC. We confirmed that the plasma concentrations of BCAAs were decreased along with the Child-Pugh grade (Fig. 6). In Child-Pugh B or C patients, the concentrations of BCAA, especially valine, were significantly decreased compared with those of healthy subjects. As in the in vitro study, we evaluated the function of MoDC from HCV cirrhotic patients (Table I). First, we determined the phenotype of MoDC from the patients and controls (Fig. 7*A*). There was no difference regarding the percentage or mean fluorescence intensity (MFI) of MoDC expressing CD14, CD80, and CD86 between healthy volunteer and patients. However, the CD40, CD83, and HLA-DR expression by MoDC from the patients was significantly decreased compared with healthy volunteers (Table IV). Second, as shown in Fig. 4, we cultured monocytes under the medium that contained 0–800 nM/ml valine and evaluated the expression of CD83, cytokine production, and allostimulatory capacity by MoDC. The addition of valine dose-dependently increased the percentage of $CD83^+/CD14^-$ mature MoDC in both healthy volunteers and patients (Fig. 7*B*). Regarding the cytokine production, the addition of valine increased the IL-12 production by MoDC in a dose-dependent manner, although the IL-10 production was not influenced by the concentration of valine in the culture medium (Fig. 7*C*). These tendencies were similar to those in healthy controls. Regarding the allostimulatory capacity, the values were maximum under 2.4 μM/ml valine, and those of cirrhotic patients were lower than those of healthy controls (Fig. 7*D*). In contrast, the allostimulatory capacities of MoDC from nonviral cirrhotic patients were at the intermediate level between healthy controls and HCV cirrhotic patients (Fig. 7*D*).

Discussion

In this study, we showed that BCAAs, especially valine, influenced the function of MoDCs. Cultures of human MoDCs are typically made in medium containing human or FCS supplements. Medium that contains serum varies in its concentration of amino acids according to each lot number, which can influence the phenotypes of the cells and their functional properties. Thus, the current study evaluated the concentrations of the amino acids strictly (details in *Materials and Methods*).

First, we found that depletion of extracellular BCAA did not influence the expression of costimulatory molecules (CD40, CD80, and CD86) on MoDC, but decreased the CD83 expression. Human CD83 is a 45-kDa glycoprotein which belongs to the Ig superfamily. CD83 is expressed on MoDCs after stimulation with inflammatory cytokines (26), and CD83 is considered as a maturation marker. Although the function of CD83 is still unknown, inhibition of CD83 expression by interfering with a specific RNA-exporting pathway leads to a dramatic reduction of the DC-mediated T cell stimulation (32). This study supports our hypothesis that the impairment of the allostimulatory capacity of MoDC cultured in medium deprived of valine was caused by the lower expression of CD83. We also found that during the generation of MoDC, depletion of extracellular BCAA, especially valine, did not influence the differentiation but impaired the maturation of MoDC. Moreover, this phenomenon was accompanied by a suppression of the mTOR/S6K-signaling pathways. Monti et al. (22) recently reported that rapamycin-treated DCs were less capable of up-regulating CD83 after exposure to CD40L. This observation partially supports our result, although the relation between mTOR/S6K signaling and CD83 expression should be evaluated in future studies. Although the depletion of leucine is believed to suppress the mTOR/S6K pathway in general, our study demonstrated that the depletion of valine also suppressed P-S6K. This conflicting observation could have resulted from differences in the cell sources evaluated.

We next showed that the addition of valine increased the IL-12 production by MoDC in a dose-dependent manner, and that the IL-10 production was not influenced by the concentration of valine in either healthy controls or patients. IL-12 is an IL that is naturally produced by macrophages, B-lymphoblastoid cells, and DCs in response to antigenic stimulation. It is involved in the differentiation of naive T cells into Th1 cells, which is important in the resistance to foreign pathogens. IL-10 is naturally produced by monocytes and type 2 Th cells. It is believed to have important suppressive functions on immune responses and also may be involved in the maintenance of tolerance. Our results raised the possibility that elevating the extracellular valine concentration could modulate Th1/Th2 differentiation in both healthy subjects and patients. To examine this possibility, it was necessary to coculture MoDC and naive CD4 T cells, and determine the phenotype of T cells and cytokine production. In addition, we found that depriving extracellular valine decreased the IL-12 production by MoDC with impaired mTOR/S6K signaling. In a previous study, active S6K1 suppressed the PI3K-Akt pathway by inactivating the insulin receptor substrate (33), whereas PI3K negatively regulated IL-12 synthesis by DCs (34). These results permit us to speculate that valine influences IL-12 production by MoDC through the PI3K/mTOR/S6K pathway.

In this study, we found that an increased concentration of valine could recover the impaired function of DCs in cirrhotic patients. However, the degree of this improvement was very modest, which lead to the speculation that persistent HCV infection itself could suppress the function of DCs in such patients. The MoDCs from hepatitis C patients have been previously reported by several studies (28–30), although their results regarding allostimulatory capacity or phenotype had been conflicting. Our results demonstrated the decreased allostimulatory capacity and decreased expression of CD40, CD83, and HLA-DR. In our series, the medium was serum-free medium and the patients' backgrounds were all cirrhotics. This is similar to study by Auffermann-Gretzinger (28) in which serum-free medium was used and had a more dominant cirrhotic population. It seemed that the expression of CD83 is decreased in either serum-free medium or progressed liver disease including cirrhosis. However, the allostimulatory capacities of HCV-infected cirrhotic patients tended to be lower compared with those of non-B, non-C cirrhotics. This impaired DC function could be possibly due to HCV infection itself, although we have not proved this hypothesis in the current study. We also found that the increase of extracellular valine could increase the phenotype (CD83 expression), allostimulatory capacity and cytokine production in HCV cirrhotic patients. The allostimulatory functions of DCs were maximum at a considerably higher than physiological concentration in both normal subjects and patients. However, the concentrations of valine in either the liver, portal blood flow, or lymph nodes could be higher than that in the peripheral blood. This issue should be evaluated in future studies. Furthermore, the changes of this allostimulatory capacity were most apparent at ranges near the physiological concentration in peripheral blood.

Recently, Osugi et al. (35) showed several differences between MoDC and the myeloid DCs present in vivo. In contrast, monocytes could differentiate into DCs in vivo (36–38). Further evaluations using circulating DCs will be needed to clarify this issue.

As previously described in a review (8), BCAA are essential for the synthesis of proteins required for cellular proliferation. However, little information is available regarding the immunologic effect of variations in the concentrations of BCAA at ranges that might occur physiologically or pathophysiologically. In this study, we have demonstrated that 1) depriving extracellular BCAA for 6 days does not influence the viability of MoDC, 2) depriving extracellular isoleucine did not decrease the allostimulatory capacity of MoDC, 3) CD40, CD80, CD86, and HLA-DR molecules were equally expressed in both CCM and ΔBCAA medium, 4) IL-10 production was not influenced by the extracellular valine concentration, 5) mTOR signaling was associated with decreased DC function in valine or leucine depletion.

These data suggest that BCAA are important for cell function through a nutrient-sensitive signaling pathway rather than through acting as substrates for various metabolic pathways and cell structures. Also, it is preferable to measure the intracellular concentration of amino acids, although their uptake was reported to be either sodium dependent or independent (39). Finally, we need to clarify why the depletion of valine itself caused a more potent inhibition of the allostimulatory capacity compared with depletion of all three components of BCAA.

In clinical situations, the administration of BCAA was reported to increase the number of peripheral lymphocytes and improve opportunistic infections or immune functions (9, 10). In advanced cirrhosis, long-term nutritional supplementation with oral BCAA has been shown to be useful to prevent progressive hepatic failure and to improve surrogate markers and the perceived health status (7). Our data concerning immune functions provide the rationale for future nutrition therapy, which could be beneficial to patients with cirrhosis.

Disclosures

The authors have no financial conflict of interest.

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ORIGINAL RESEARCH

Retrospective evaluation of tumor-mass-reduction therapy for the prognosis of recurrent hepatocellular carcinoma

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Abstract Although hepatocellular carcinoma (HCC) is the liver cancer that requires repeated treatments because of a high tendency for recurrence, few data have been available about whether repeated treatments, including those to reduce tumor mass, are effective in prolonging survival. We retrospectively analyzed the effectiveness of tumor-mass-reduction therapy for the prognosis of patients with recurrent HCC. To analyze the effectiveness of various modalities of therapies with a single criterion, we defined a tumor-mass-reduction grade (TMRG), which was retrospectively evaluated by dynamic CT or MRI. Grade A: no evident HCC remains untreated; Grade B1: more than 50% of lesions are treated; and Grade B2: less than 50% of lesions are treated. Subjects were stratified by Child-Pugh classification and the number of admissions for HCC treatment. In those classified as Child-Pugh A, a better survival rate was obtained, depending on the degree of TMRG from the first to the fifth admission ($P < .01$), suggesting that these patients are endurable for repeated therapies and benefit from the many sessions of treatment. In those classified as Child-Pugh B, on the second to the fifth admissions, survival rates showed statistical difference depending on the TMRG ($P < .01$), which may suggest that only a few sessions of treatment are meaningful. In those classified as Child-Pugh C, any number of mass-reduction treatment sessions did not improve the survival rate. In conclusion, repeated tumor-mass-reduction therapies for recurrent HCC are most beneficial in Child-Pugh A patients. Patients with Child-Pugh B who experience several recurrence episodes and any patients with Child-Pugh C may benefit more from modalities other than tumor-mass-reduction therapies.

Keywords Hepatocellular carcinoma · Tumor-mass-reduction · Child-Pugh classification · Hospitalization

Abbreviations

AFP	Alpha-fetoprotein
CT	Computed tomography
CTAP	CT during arterioportography
CTHA	CT during hepatic arteriography
DCP	des-gamma carboxy prothrombin
HCC	Hepatocellular carcinoma
MRI	Magnet resonance imaging

Introduction

Hepatocellular carcinoma (HCC) is the most common malignant tumor in the liver, with high recurrence rates, either as intrahepatic metastasis or multicentric carcinogenesis [1–3]. In Japan, more than 90% of HCCs occur from chronic liver diseases caused by hepatitis B or hepatitis C virus infection. Development of local ablation methods such as radiofrequency ablation [4] and surgical resection [5] has remarkably reduced the rates of local recurrence, mainly adjacent to treated lesions. However, despite these therapeutic developments, HCC recurs frequently, because of multicentric carcinogenesis arising from an already cirrhotic liver and also because of insufficient treatment, consequently requiring repeated therapy.

However, a more complete local ablation may not be enough: for better outcome, the residual hepatic function

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should be maintained in a better state, because it can be the other factor determining the prognosis [6, 7]. For example, CLIP score, a new scoring system proposed by the Cancer of the Liver Italian Program, shows that both the residual hepatic function as evaluated by Child-Pugh score and tumor factors, including tumor morphology, AFP levels, and portal vein thrombosis, determine the prognosis [8–10]. From this viewpoint, a dilemma exists; treatments performed for HCC may influence, or even damage, liver function to some extent, thus worsening the prognosis than that without any treatments.

In spite of the fact that majority of HCC patients need repeated treatments for recurrent diseases, the survival benefit of such treatments, especially medical interventions just to reduce tumor development, remains uncertain. Moreover, many of the studies reported so far are somewhat impractical because in the clinical management of HCC, we choose to combine various methods of treatments to obtain the best results, and most studies address the contribution of only a single modality or combined therapies of a few modalities for prognosis [4, 11–14]. This lack of data is partly due to the existence of a variety of therapeutic options in the treatment of HCC, which complicates analysis, and the ethical consideration when dealing with patients whose lives are in jeopardy on account of malignant diseases.

However, regardless of the difficulty of analysis, we need to obtain data to answer this unresolved question: Are repeated treatments for HCC effective for the improvement of prognosis? To answer this question, we retrospectively analyzed 878 HCC patients in total admitted to Tohoku University Hospital from 1989 to 2003. As discussed in this article, our results support the idea that to treat recurrent HCC, we need to consider the number of times the patients were hospitalized and residual liver function.

Materials and methods

Study population

We enrolled 386 HCC patients who were admitted to Tohoku University Hospital from December 1989 to December 2003. Initially 320 patients were admitted to this hospital for their first treatment of HCC, and then 66 patients were newly referred to this hospital for the treatment of recurrent HCC. Most of patients experienced more than one hospitalization (median: 2.0; range: 1–14) owing to recurrence, so the total number of subjects who were admitted to the hospital for the treatment of HCC were 878. The diagnosis of HCC was performed by the combination of dynamic CT (or dynamic MRI), and tumor markers (AFP and DCP). Majority of patients who were diagnosed or suspected as having HCC were rendered to hepatic subtraction angiography (DSA), often to angio-CT (CTHA and CTAP [15, 16] for a definite diagnosis. If a lesion was difficult to be diagnosed as HCC by methods described above, it was further evaluated histologically (16 cases). Any subjects whose hepatic tumors are diagnosed as other than HCC or who have extrahepatic metastases at the entry were excluded from this study. The modalities of treatment include transcatheter arterial embolization (TAE) [14, 17–19], transcatheter arterial infusion (TAI) chemotherapy [20–24], percutaneus ethanol injection (PEI) [25], percutaneus microwave coagulation therapy (PMCT) [11], radiofrequency ablation (RFA) [26–28], radiation therapy (RT) [29–32], and hepatic resection [5]. No antiviral drugs were given as an adjuvant therapy to any patient in this study. Our principle in HCC treatment was to obtain an as sufficient control of HCC lesions (represented as necrosis or shrinkage of lesions) as possible and limiting the liver damage accompanying treatments as much as possible. To satisfy this principle, we chose either of these treatments or several modalities in combination. If we could not achieve complete necrosis because of the therapeutically difficult location, multiple distribution, poor liver function, or a poor general condition, we performed tumor-mass-reduction therapies as suboptimum treatments to reduce tumor growth, assuming that even such palliative treatments could prolong survival. The endpoint of a series of combination therapy in one hospitalization was determined when all lesions were regarded to be completely treated, when further curative treatments were difficult to perform owing to technical difficulty, poor liver function, distant metastasis, any serious complications, or poor performance status. Thereafter, these subjects were discharged from the hospital, and took a medical examination including dynamic CT, dynamic MRI, abdominal ultrasonography, or a blood test at least every 3 months as an outpatient at the hospital. If new lesions emerged or insufficiently controlled lesions developed during the follow-up periods, these subjects were hospitalized again for the detailed medical examination described above and received repeated treatments with the same principle.

Study design

In general, evaluation was made retrospectively according to the dynamic CT or dynamic MRI performed at least 1 month later from the last treatment in the previous hospitalization. However, if possible to follow up, we referred to any dynamic CT or MRI performed at any subsequent points of time to detect local recurrence after an interval of several months. Also, if available, we referred to an angio-CT performed during subsequent hospitalization for

confirmation of suspected lesions. With those concepts, we defined a set of criteria for grading tumor-mass-reduction as follows:

Grade A: No evident cancerous lesion remains untreated after a series of treatments in one admission. In other words, complete necrosis of all HCC lesions is regarded to be obtained in this group, by evaluating all cross sections in dynamic CT or MRI.

Grade B: Cancerous lesions remain untreated after a series of treatments in one admission. This grade is categorized further into two subgroups, according to the percentage of the treated volume compared to the pretreated volume. However, if appropriate, the largest cross section of a lesion is preferably evaluated, because in many cases the largest cross section could be regarded to represent the whole lesion.

Grade B1: More than 50% of the lesions are estimated to be treated.

Grade B2: Less than 50% of the lesions are estimated to be treated.

If more than one HCC lesion exists, this categorization is made by calculating the sum of all lesions. The schematic demonstration of TMRG is illustrated in Fig. 1. For instance, when a lesion was treated without an evident viable lesion and no recurrent lesion appeared by any retrospective evaluation, it was categorized as Grade A (the best result in our classification). However, if a patient with HCC was seemingly treated completely at discharge despite microlesions being actually left untreated failing detection, they were categorized as Grade B1 if these unchecked lesions adjacent to the treated lesions became manifest later. Thus only lesions that emerged adjacent to the treated lesions were evaluated to be the local recurrence due to insufficient therapy. Moreover, when a lesion could not be completely treated owing to some reasons, but residual viable lesion could be evaluated as less than 50% of pretreated lesions, it was also categorized as Grade B1 (not completely, but relatively well treated). When more than 50% of pretreated lesions could not be treated, it was categorized as Grade B2 (the worst result in our classification).

Statistical analysis

For the analysis of baseline characteristics, the Kolmogorov–Smirnov normality test was performed, and continuous variables that show normal distribution were expressed as mean ± SD, then compared using the Student *t*-test. Continuous variables that do not show normal distribution were expressed as median, then compared by the Mann–Whitney *U* test. Categorical variables were compared with the use of the χ^2 test. For these analyses, P values <0.05 were considered statistically significant. The survival of each tumor-mass-reduction grade (TMRG) was calculated by the Kaplan–Meier method and the differences between the curves were evaluated with the log-rank test. P values <0.01 were used for statistical significance. All statistical analyses were performed using StatView ver5.0.

Results

Baseline characteristics of subjects

A total of 320 subjects received therapy for the initial occurrence of HCC (Table 1a). The average age at the initial therapy was 63.3 ± 9.3 (range, 28–81). Overall, 386 patients were enrolled from December 1989 to December 2003, and the total number of subjects we analyzed in this study was 878 (M:F = 594:284; mean age: 64.6 ± 8.8) (Table 1b). Among 386 patients, 198 patients died up to December 2003 owing to the development of HCC (n = 135), hepatic failure (n = 35), variceal bleeding (n = 11), insufficiency of other organs (n = 16), or unknown causes (n = 10). The etiology of background liver disease was 69.5% of HCV infection, 17.0% of HBV infection, 5.2% of HCV and HBV superinfection, 3.2% of alcoholic liver injury, and 5.1% of other causes (Table 1b). The distribution of residual liver function was Child-Pugh A 68.6%, Child-Pugh B 29.5%, and Child-Pugh C 1.9% (Table 1b). Median value for AFP was 61.6 ng/dl (range, 0–10^6), and for DCP was 26.0 AU/l (range, 0–193,000) (Table 1b). Median tumor size was 25.0 mm (range, 5–190). Forty-four percent of patients had solitary tumor, 30.3% two or three tumors, 7.4% four or five tumors, and 18.4% more than five tumors (Table 1). Between subjects at the initial treatment and the total subjects during the whole course of treatment, age, tumor size, and survival showed statistical significance, although other parameters were statistically insignificant (Table 1a, b). Therapeutic modalities we selected for recurrent HCC included 27 patterns (Table 2). The intervals of period between hospitalizations were almost in inverse proportion to the number of times for hospitalization (Fig. 2), indicating that the speed for recurrence was gradually accelerated.

The evaluation of prognosis of initial treatments for the first occurrence of HCC

When subjects who received the initial treatment for the first occurrence of HCC were stratified using the CLIP score (Fig. 3a, b), each group of score showed better survival with the previous report in Japan or Italy [8–10], suggesting that our strategy for HCC treatments was up to

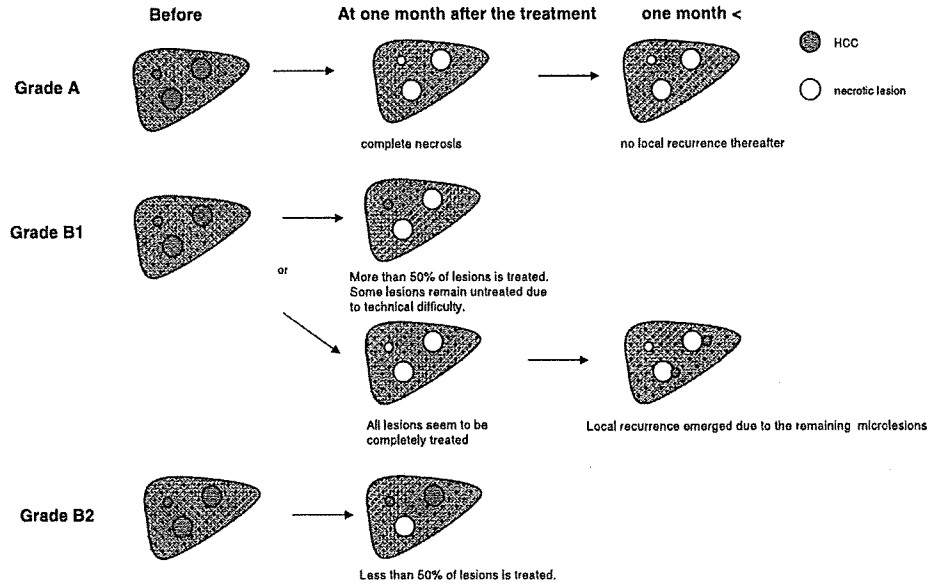

Fig. 1 Schema of the concept of tumor-mass-reduction-grade used in the current study

the standard. Furthermore, when these patients were stratified with the mass-reduction grade, each mass-reduction group was discriminated well from each other with statistical significance ($P < 0.0001$) (Fig. 4). The 1-year survival rates of Grade A, B1, and B2 patients were 98.4, 90.0, and 42.6%, respectively; the 3-year survival rates of Grades A, B1, and B2 were 76.6, 56.6, and 16.0%, respectively; and the 5-year survival rates of Grades A, B1, and B2 were 52.7, 23.7, and 10.0%, respectively (Fig. 3). These analyses of the initial treatment show that this grading system can function for evaluating the relationship between the prognosis and the grade of mass reduction.

The relationship between prognosis and tumor-mass-reduction therapy for recurrent HCC

On the basis of the evaluation performed above, we used this system for the analysis of recurrent HCC. The number of hospitalizations for treatment of HCC ranged from 1 to 14. We separated the subjects into three groups using Child-Pugh classifications A, B, and C, and then analyzed each category of patients according to the number of hospitalizations to receive treatment for HCC. Since the number of subjects who were categorized into strata of more than the eighth admission was too small to analyze, we analyzed the strata of admission from the first to the eighth admission. As Table 3 shows, in Child-Pugh A, different grades of mass reduction brought significant differences in survival ($P < 0.01$) from the first to the fifth admission, suggesting that patients with Child-Pugh A are endurable for repeated therapies and benefited from many sessions of treatments without reducing their prognosis. In Child-Pugh B, our analysis showed somewhat confusing data, that is, biphasic statistical significance at the second and fifth hospitalizations ($P < 0.01$). In Child-Pugh C, no HCC treatment, regardless of curative or just tumor-mass-reduction treatments, brought any statistical differences in improving survival at any point of time, indicating that reduction therapy is consistently meaningless in such patients.

Discussion

Preceding this study, our clinical observation in treatments of HCC had given us an impression that treatments for recurrent HCC might not be always effective in view of prognosis. Although some data were available regarding the effectiveness of the initial therapy for HCC, effectiveness of a single therapy or therapies in combination with a limited number of modalities [4, 11–13, 26, 33], little data have been available regarding if repeated treatments for recurrent HCC and combined treatments for the better control of HCC are effective in improving prognosis. Furthermore, we observed that mere numbers and distribution of HCC or residual liver function as evaluated by Child-Pugh score etc. might not determine the outcome of HCC therapy. We speculated that "repeated sessions of HCC treatments" per se might worsen prognosis or induce recurrence, although the accurate mechanism for the aggravation of prognosis by this "repeating" was nebulous. Actually, in our data, the intervals between recurrences got shorter and shorter, while the events of recurrence increased (Fig. 2). One possible mechanism could be that during each session of

Table 1 Baseline characteristics of subjects

		(a) $n = 320$	(b) $n = 878$	P value
Age (mean ± SD)		63.3 ± 9.3	64.6 ± 8.8	0.025*
Gender	M/F	211/109	594/284	0.952
Etiology (%)	HCV	71.9	69.5	0.101
	HBV	19.0	17.0	
	HBV + HCV	4.5	5.2	
	Alcohol	2.9	3.2	
	Others	1.6	5.1	
Number of treatments (median)			2.0 (1–14)	
Tumor size (mm) (median)		30.0 (8–190)	25.0 (5–190)	0.001*
Number of tumors (%)	1	59.1	44.0	0.061
	2, 3	29.2	30.3	
	4, 5	5.0	7.4	
	> 5	6.6	18.4	
Vascular invasion	Yes/No	26/294	74/804	0.983
Child-Pugh class (%)	A	69.7	68.6	0.875
	B	28.8	29.5	
	C	1.6	1.9	
Median survival time (months)		56.8 (2.5–157.1)	37.4 (1.0–157.1)	<0.0001*
Alpha-fetoprotein (ng/dl) (median)		46.6 (0–10^6)	61.6 (0–10^6)	0.059
DCP (AU/L) (median)		19.0 (0–193,000)	26.0 (0–193,000)	0.140
Total bilirubin (mg/dl)		1.3 ± 0.6	1.3 ± 1.1	0.187
Serum albumin (mg/dl)		3.6 ± 0.6	3.6 ± 0.6	0.922
Prothrombin activity (%)		79.1 ± 16.5	79.6 ± 30.9	0.757
ICG R15 (%)		26.1 ± 14.5	27.4 ± 15.3	0.194
Aspartate aminotransaminase (IU/dl)		79.7 ± 45.5	76.8 ± 45.0	0.331
Alanine aminotransferase (IU/dl)		71.8 ± 46.2	66.4 ± 45.8	0.070
Platelet count (10^4/mm^3)		11.2 ± 5.9	10.5 ± 5.8	0.155
Alkaline phosphatase (IU/dl)		291 ± 177	307 ± 172	0.147

Note: (a) Subjects who received the initial treatments for the first occurrence of HCC. (b) Total subjects. Values are mean ± standard deviation

Abbreviations: DCP: des-gamma carboxy prothrombin; ICG R15: retention of indocyanine green at 15 min

*$P < 0.05$

therapy not only were the HCC lesions necrotized, but the surrounding liver tissues were also injured, cumulatively damaging liver functions. Although this possibility may be applicable in some cases, it does not always seem to be true, because our analysis showed that although in some cases Child-Pugh classification changed, indicating deterioration, in many cases, the scores did not change during a course of repeated treatments. Only 16.5% of subjects underwent changes in their grading. For example, among 223 patients who were graded as Child-Pugh A at the first treatment, 44 subjects underwent deterioration to Grade B or C, at the following therapy. Eight among 90 Child-Pugh B patients at the first treatment underwent deterioration to Grade C at the next occasion (data not shown). Another possibility is that the therapeutic stimuli may induce transformation of HCC, making it more malignant and more resistant to therapies. Although this speculation may not be always true, it may explain some cases, because a study suggested that anoxia caused by TAE induced Bcl-2 expression, which changed HCC cells more tolerant to apoptosis [34]. The other possibility is that during HCC treatments, multiple microlesions or precancerous lesions of HCC may be left untreated failing detection that later develop into intractable lesions [1–3, 20]. In such cases, the probability of multicentric carcinogenesis may be increased if the period from the initial onset of HCC becomes longer; the probability of intrahepatic metastasis of HCC with poorer differentiation may be increased if each session of treatment leaves viable cancer cells undetectable by medical examination. Whatever mechanism is true, the data of repeated treatments for HCC is urgently needed.

However, to address this subject, we confronted a challenging situation. To begin with, we found that randomized controlled trials (RCTs) were quite difficult to conduct in our setting owing to ethical reasons. Those patients whose life expectancy is limited on account of malignant diseases do not dare to risk reducing their chances of survival by participating in such studies, because medical care is affordable for almost all patients in

Table 2 Modalities of treatments performed for HCC

	n (%)
Surgical resection	36 (4.1)
PEI	113 (12.9)
PEI, PMCT	16 (1.8)
PEI, PMCT, RFA	2 (0.2)
PEI, PMCT, TAE	1 (0.1)
PEI, RFA	19 (2.2)
PEI, RFA, RT	1 (0.1)
PEI, RFA, TAE	9 (1.0)
PEI, RT	1 (0.1)
PEI, TAE	134 (15.3)
PEI, TAE, RT	1 (0.1)
PEI, TAI	6 (0.7)
PMCT	6 (0.7)
PMCT, RFA	1 (0.1)
PMCT, RFA, TAE	1 (0.1)
PMCT, TAE	8 (0.9)
PMCT, TAI	1 (0.1)
RFA	111 (12.6)
RFA, TAE	24 (2.7)
RFA, TAI	2 (0.2)
RT	5 (0.6)
RT, TAE	9 (1.0)
RT, TAE, TAI	4 (0.5)
RT, TAI	10 (1.1)
TAE	294 (33.5)
TAE, TAI	5 (0.6)
TAI	58 (6.6)
Total	878 (100)

Abbreviations: PEI, percutaneous ethanol injection; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation; TAE, transcatheter arterial embolization; TAI, transcatheter arterial infusion; RT, radiation therapy

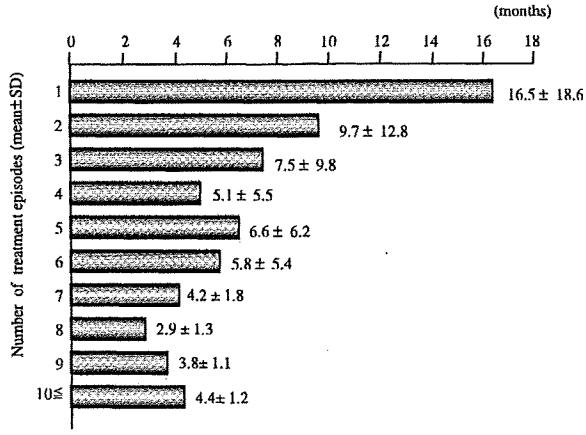

Fig. 2 Mean periods of time until the next hospitalization for treatments of HCC

Japan. Therefore, although RCTs would have been the most reliable way to assess this subject, we had to find a way to analyze the effectiveness of HCC treatments in the absence of controls.

Second, most studies that have attempted to analyze treatments for HCC have been limited to the analysis of only a single modality [11–13, 33]. However, in practice, we treat HCC with modalities prudently combined because it can usually better control HCC than does a single modality. Thus, in a situation where data for survival are lacking, our tentative goal has been to control HCC lesions as completely as possible using available modalities, with the expectation that such treatments improve survival.

Finally, because HCC is a cancer that frequently tends to recur and needs to be treated repeatedly, our practical interest was not only the influence of the initial treatment upon prognosis but also the total influence of repeated therapy. Most previous reports, however, deal with the prognosis when a modality is selected as an initial treatment. Since actually an identical modality is seldom selected consistently for one patient and various therapies are selected for recurrent lesions, analysis becomes too complicated.

Considering these situations, we took a step for the analysis with the following concepts: (1) We analyzed not only a single modality, but also treatments sequentially combined to obtain the best control, performed during one hospitalization. Such treatments include seven kinds of monotherapy and 21 kinds of combination therapy (Table 2). (2) We analyzed not only an initial treatment but every repeated treatment performed at each hospitalization. (3) We defined our original classification for evaluation of HCC treatments in order to apply it commonly for all single or combined treatments. To be evaluated as "a sufficient therapeutic effect," enough safety margin will be usually necessary for PEI, RFA, etc.; sufficient deposit of lipiodol completely covering the margin of tumors for TAE; and complete disappearance of tumors for chemotherapy. However, when aiming to evaluate a variety of therapeutic patterns, it was not feasible to apply these criteria individually. Therefore, by making the best use of retrospective analysis, we simply judged the effectiveness of therapies by mainly focusing on recurrent lesions retrospectively using our original evaluation system described above.

In this study, we showed that both Child-Pugh classification and number of times of hospitalization for HCC therapy determine the effectiveness of mass reduction therapy on the prognosis of HCC. This observation is also true in view of tumor markers because regardless of different Child-Pugh grades, majority of patients underwent decreases of AFP and DCP levels. Furthermore, additional decrease of AFP and DCP levels tend to be acquired by the

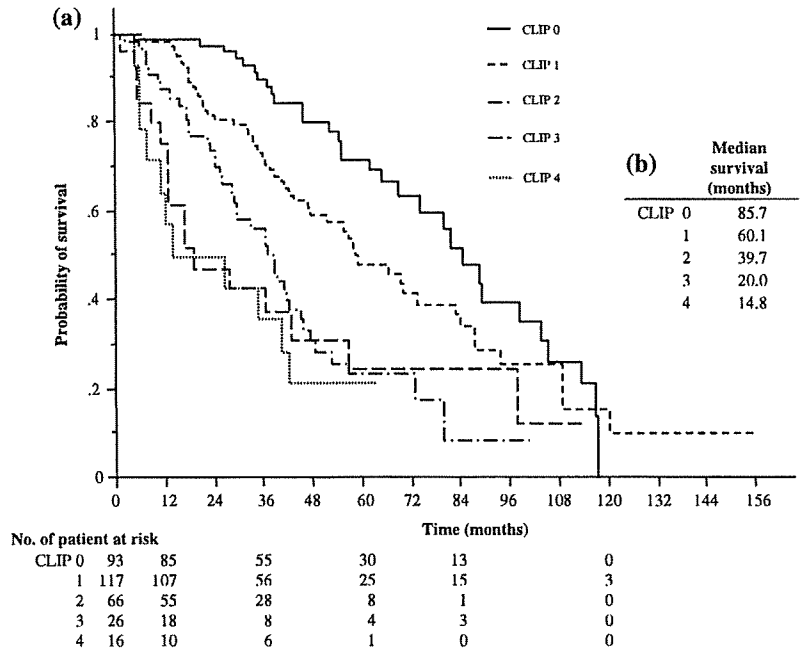

Fig. 3 (a) Survival curves for different CLIP scores in patients who have undergone initial treatments for the first occurrence of HCC. Data for two were excluded because of missing values of AFP. (b) Median survival of each CLIP score

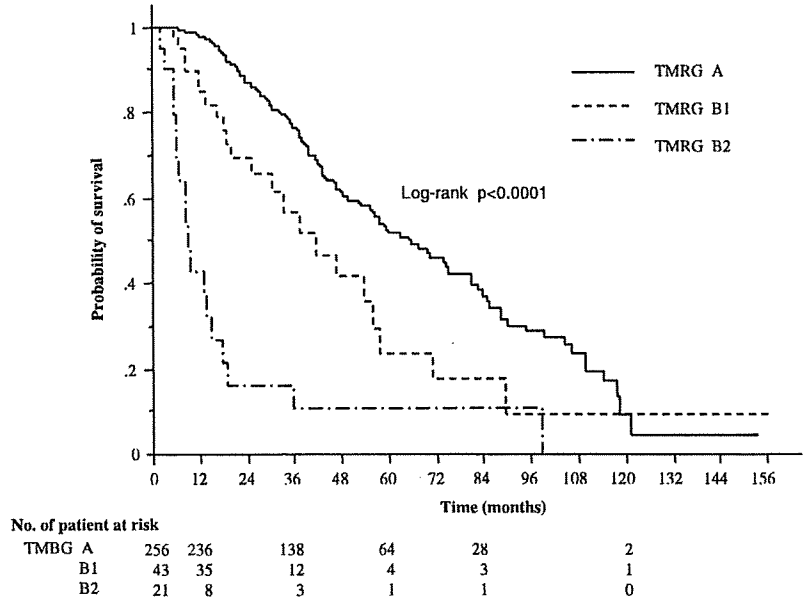

Fig. 4 Survival curves for three different grades of tumor-mass-reduction in patients who received initial treatments for HCC (n = 320; log-rank test, P values <0.01 were considered statistically significant)

better TMRG (data not shown). In Child-Pugh A, up to the fifth hospitalization, better prognosis was achieved in proportion to grades of mass reduction, but in subsequent hospital admissions, attempts for mass reduction did not make any difference in the prognosis. In Child-Pugh B, rather confused results were obtained; only at the second and fifth admission, TMRGs showed statistical significance in prognosis. The reason that there was no statistical difference on the first admission of Child-Pugh B patients may be that a good survival rate was achieved even by the B2 grade of treatment (Table 2). In the majority of these patients, Child-Pugh grade was maintained at the same level without worsening into C after therapy. This observation may indicate, that good survival can be achieved by therapy that does not damage liver function even if the TMRG is not so satisfactory. On the other hand, although the precise reason why Child-Pugh B patients on the fifth admission showed statistical significance is unclear, it may be explained simply by the lack of analyzed subject.

We emphasize that the number of times for hospitalization indicated in this study is neither universal nor absolute, so some differences will exist from institute to

Table 3 Association between TMRG and survival stratified by Child-Pugh classification and the frequency of treatments

		Child-Pugh A			Child-Pugh B			Child-Pugh C		
No.	TMRG	*n*	MS	*P* value	*n*	MS	*P* value	*n*	MS	*P* value
1	A	177	75.1	<0.0001*	76	57.7	0.065	3	37.3	0.1159
	B1	30	46.5		12	25.5		1	20.0	
	B2	16	8.6		4	36.1		1	13.9	
2	A	112	54.4	0.0020*	43	34.4	<0.0001*	2	19.2	0.1573
	B1	26	24.7		11	20.2		1	10.7	
	B2	3	9.6		3	3.0				
3	A	62	42.9	<0.0001*	25	30.1	0.0737	2	2.2	0.1387
	B1	18	26.1		9	25.5		1	9.9	
	B2	7	6.2		4	7.8		3	4.9	
4	A	36	42.6	0.0020*	13	34.2	0.0772	0		0.8084
	B1	14	14.1		10	12.9		2	4.9	
	B2	2	9.5		1	12.0		1	6.4	
5	A	21	39.6	<0.0001*	11	23.7	<0.0001*			
	B1	11	31.8		8	13.8				
	B2	6	7.4		1	5.5				
6	A	14	39.6	0.1795	3	15.6	0.0125			
	B1	6	33.2		7	14.2				
	B2	2	6.7		2	5.8				
7	A	8	26.1	0.8072	0		0.9189			
	B1	8	19.6		6	9.2				
	B2	2	9.5		1	13.0				
8	A	3	32.6	0.7820	0		0.3173			
	B1	3	18.2		1	4.7				
	B2	2	18.2		1	4.2				

Abbreviations: No., number of hospitalization; TMRG, tumor-mass-reduction grade; MS, median survival (months)

institute. Also we admit that our study fundamentally has limits in terms of analytical methods. One limit is that this study is a nonrandomized retrospective analysis. The other limit is that we do not have any data of natural history. Without the data of no treatment, we cannot exactly decide, which grade of mass reduction can improve prognosis compared to that of no treatment or just a symptomatic treatment. Regardless of these limits, however, an unchangeable advantage of this analytical method is, that if there is no statistical difference between the various grades, it can be demonstrated that any attempts for mass reduction will fail to improve prognosis.

Despite these relations or limits, our analysis seems to be presenting important suggestions. In Child-Pugh A, many occasions of hospitalization for HCC therapy seem to be endurable (in our institute, they were up to five) and can be of benefit for prognostic improvement by more curative treatments; in Child-Pugh B, only initial few occasions of hospitalization for HCC therapy may be beneficial; in Child-Pugh C, any therapeutic attempts are useless. For the better prognosis of HCC patients with Child-Pugh B, it may be crucial to administer new modalities including liver transplantation as early as possible, while the same strategy is also relevant for any HCC patients with Child-Pugh C. Comparing with our data, which addressed repeated treatments by various modalities in combination, further studies are warranted, including the analysis of survival benefit due to liver transplantation.

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肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究

平成19年度～平成21年度　総合研究報告書

研究代表者　　熊田　博光

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Ⅲ. 平成20年度 研究成果の刊行に関する一覧表

Ⅳ. 研究成果の刊行に関する一覧表

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