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Induction of interleukin-8 (CXCL-8) by tumor necrosis factor-α and leukemia inhibitory factor in pancreatic carcinoma cells: Impact of CXCL-8 as an autocrine growth factor

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Abstract. Pancreatic carcinoma is one of the most lethal of the gastrointestinal malignant tumors. Chronic inflammation leads to cancer development and progression. Interleukin-8 (CXCL-8) is a CXC chemokine, which plays an important role in neutrophil chemotaxis and activation. We previously reported that CXCL-8 was produced by a variety of human carcinoma cells and tissues, and that CXCL-8 promoted proliferation in pancreatic carcinoma cells (SUIT-2). In the present study, we analyzed whether various cytokines affect cell proliferation by CXCL-8 expression in pancreas carcinoma cells. All examined pancreatic carcinoma cells expressed CXCL-8 and TNFRII mRNA constitutively in RPMI-1640 medium without FBS. TNF-α, LIF, IL-1ß, IL-6, IL-8, or IFN-ß enhanced the expression of CXCL-8 mRNA, but IL-10 did not in Hs-700T cells. Actinomycin D suppressed and cycloheximide augmented CXCL-8 mRNA which was induced by TNF-α or not. The half-life of CXCL-8 mRNA was 36.5 min by TNF-α and 35.2 min by no stimulation. In our previous study, LIF promoted cell growth in Hs-700T cells. LIF induced CXCL-8 mRNA in a dose- and time-dependent manner. Addition of recombinant CXCL-8 did not induce cell growth of Hs-700T. Anti-CXCL-8 IgG significantly suppressed cell growth. CXCL-8 would act as an autocrine growth factor in Hs-700T cells, which expressed CXCL-8 mRNA highly without stimulation. Curcumin (diferuloylmethane), NF-κB inhibitor, suppressed cell proliferation in Hs-700T cells. These results suggest that CXCL-8 plays a pivotal role in progression of pancreatic cancer, and its expression is influenced by inflammatory cytokines in pancreatic tumor microenvironment.

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Introduction

Pancreatic cancer is an aggressive disease in gastrointestinal malignancy. Surgical resections of tumor are only effective therapy before it has spread outside the pancreas, but have little effect with locally advanced or metastatic disease. Other current therapies, such as chemotherapy, radiation, and immunotherapy, rarely improve the prognosis of patients bearing pancreatic cancer, whereas they can alter the quality of life by controlling the symptoms and complications. Chronic inflammation, including hepatitis, gastritis, and colitis, causes cancer development by genetic alterations and cellular transformations (1). Chronic pancreatitis also increases the risk of developing pancreatic cancer (2,3). Understanding the mechanisms underlying the interaction between chronic inflammation and cancer progression would provide novel insights for therapeutic intervention.

Interleukin-8 (CXCL-8) was initially isolated as neutrophil chemotactic factor by Yoshimura *et al* (4). CXCL-8 is a pleiotropic CXC chemokine, which plays an important role in neutrophil chemotaxis and activation. CXCL-8 is produced by a variety of cells, including leukocytes, endothelial cells, and fibroblasts. CXCL-8 contains the ELR (Glu-Leu-Arg) motif, which promotes angiogenesis by endothelial cell proliferation and MMP expression. The expression of CXCL-8 correlated with tumorgenesis and metastatic potentials in human carcinoma cells. CXCL-8 was expressed in obstructive pancreatitis by which pancreatic tumors can be caused. CXCL-8 could be influenced by inflammatory cytokines in the tumor microenvironment.

We previously reported that CXCL-8 was produced by a variety of human carcinoma cells (5) and tissues and promoted cell proliferation in pancreatic carcinoma cells (SUIT-2) (6,7). In this study, we hypothesized that CXCL-8 produced by pancreatic carcinoma cells increases proliferation in an autocrine manner. To test this hypothesis, expression of CXCL-8 mRNA was assessed for changes after the stimulation of cytokines, especially TNF-α and LIF. Pancreatic carcinoma cells were treated with recombinant CXCL-8 or neutralizing antibody. We demonstrated that pancreatic carcinoma cells produced CXCL-8 in a cytokine network and CXCL-8 influenced cell growth in various conditions and mechanisms.

Materials and methods

Reagents. Human recombinant tumor necrosis factor-α (TNF-α), interleukin-1ß (IL-1ß), leukemia inhibitory factor (LIF), interleukin-6 (IL-6), interleukin-8 (CXCL-8) and interferon-ß (IFN-ß) were purchased from R&D systems (Minneapolis, MN). Anti-CXCL-8 polyclonal rabbit IgG and control rabbit IgG were from Santa Cruz Biotechnology (Santa Cruz, CA). 3-(4,5-dimethylthiazol-2-tl)-2,5-diphenyl-tetrazolium bromide (MTT), actinomycin D (Act D) and cycloheximide (CHX) were from Wako (Tokyo, Japan). Curcumin (diferuloylmethane) was from Sigma (St. Louis, MO). [α-^{32}P]dCTP was from ICN (Costa Mesa, CA). Human G3PDH cDNA were from Clontech (Palo Alto, CA). Phosphate-buffered saline (PBS), RPMI-1640, fetal bovine serum (FBS) and TRIzol reagent were from Life Technologies (Gaithersburg, MD).

Cell lines and cell culture. Carcinoma cell lines of the pancreas (BxPc-3, Hs-700T and Hs-766T, AsPc-1, PANC-1, Capan-1 and Capan-2) were purchased from the American Type Cell Culture (ATCC). SUIT-2 was maintained in our laboratory. All cell lines were cultured in RPMI-1640, supplemented with 10% FBS, penicillin (100 units/ml) and streptomycin (100 mg/ml) at 37°C in a humidified 5% CO_2 to 95% air atmosphere. The cells were starved overnight before isolation of mRNA.

Northern blot analysis. When carcinoma cells were harvested at 90% confluence, cells were washed with PBS. Cells were further incubated for 24 h in the serum-free medium until the experiment. Cells were stimulated with the reagents for indicated times. Total RNA of carcinoma cells was extracted by the guanidine thiocyanate-phenol-chloroform method as previously described (8). Northern blot analysis was performed as previously described (8). Membranes were hybridized with various ^{32}P-labeled probes including CXCL-8 (kindly provided by Dr Teizo Yoshimura, NIH, NCI-Frederick, USA) for Northern blot analysis. G3PDH was purchased from Clontech. The results were expressed as a ratio to G3PDH.

RT-PCR analysis. RT-PCR analysis was performed as described previously (8). The following primers were used for PCR: TNFR II (Rp75) sense primer, 5'-GTGGAATG GACTACTCCAAGG-3'; TNFR II (Rp75) antisense primer, 5'-TCCTTCCCACCTTCATCTGT-3'; G3PDH sense primer, 5'-GAAATCCCATCACCATCTTCC-3'; G3PDH antisense primer, 5'-CCAGGGGTCTTACTCCTTGG-3'. The PCR fragments were analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. PCR-assisted mRNA amplification was repeated twice for at least two separately prepared cDNA samples for each experiment. Data was representative in at least three different experiments.

Cell proliferation assay. Carcinoma cells were washed by PBS, and suspended at 1x10^5 cells/ml in medium (RPMI-1640 + 2%FBS). Cells were transferred in triplicate to the 96-well microtitre plates containing diluted recombinant human TNF-α, LIF or CXCL-8. Plates were incubated for indicated periods. To evaluate the proliferation of pancreatic carcinoma cells, we performed MTT assay as described (9).

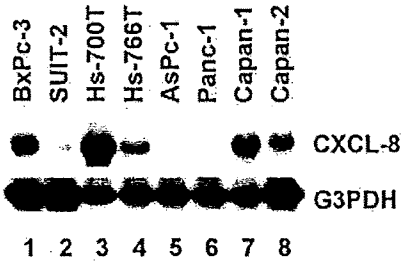

Figure 1. Pancreatic carcinoma cells constitutively expressed CXCL-8 mRNA. Carcinoma cells were cultured at 70-80% confluent in a 25-cm^2 flask. They were then incubated with serum-starved medium for 24 h, and mRNA was isolated. Approximately 10 μg per lane total cellular RNA was used for Northern blot analysis.

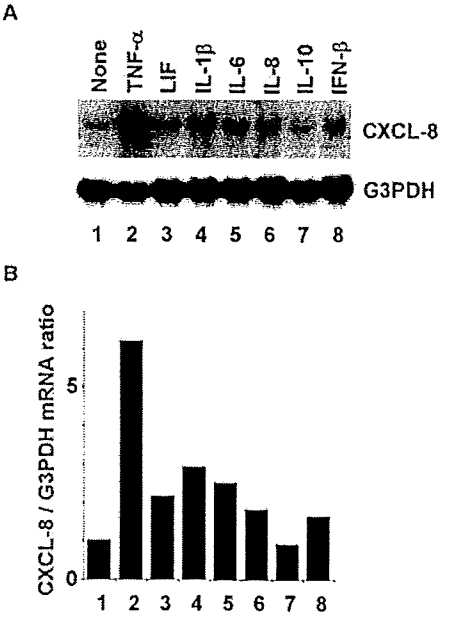

Figure 2. Regulation of CXCL-8 mRNA expression in Hs-700T cells. (A) Carcinoma cells were incubated in medium RPMI-1640 without FBS for 24 h. Then, cells were incubated with or without TNF-α (10 ng/ml), LIF (10 ng/ml), IL-1ß (10 ng/ml), IL-6 (10 ng/ml), CXCL-8 (10 ng/ml), IL-10 (10 ng/ml) or IFN-ß (10 ng/ml) for 8 h. The expression of CXCL-8 and G3PDH mRNA was analyzed by Northern blotting. Approximately 10 μg per lane total cellular RNA was used. (B) Autoradiographic densities of each mRNA band were quantitated using a Bio-Image Analyzer (Fuji film Co., Tokyo, Japan). The results were standardized against the levels of G3PDH, and are presented as relative density. The level of expression detected in untreated cells equaled 1.

Statistical analysis. The significance of differences in numerical data was evaluated using the χ^2-test, or Student's t-test. The probability level of $p<0.05$ was considered as the limit of significant difference.

Results

Expression of CXCL-8 mRNA in human pancreatic carcinoma cells. We firstly investigated whether pancreatic carcinoma cells can express CXCL-8 mRNA constitutively in RPMI-1640 medium without FBS. As shown in Fig. 1, CXCL-8 mRNA was detected in most pancreatic carcinoma cells by Northern

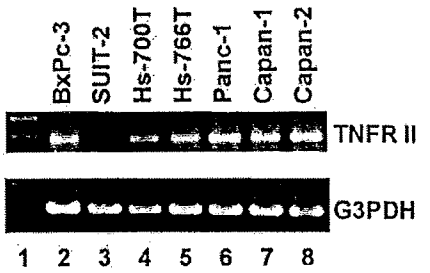

Figure 3. Pancreatic carcinoma cells expressed TNF receptor (TNFRII) mRNA. Carcinoma cells were cultured at 70-80% confluent in a 25-cm² flask. They were then incubated with serum-starved medium for 24 h, and mRNA was isolated. Approximately 5 μg per lane total cellular RNA was used for RT-PCR analysis.

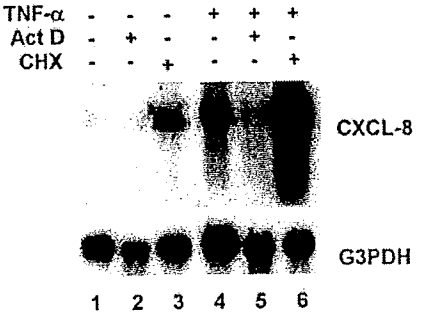

Figure 4. Effect of actinomycin D or cycloheximide on the expression of CXCL-8 mRNA by TNF-α in Hs-700T cells. Hs-700T cells were serum-starved and then treated with TNF-α (10 ng/ml) in combination with actinomycin D (4 μM), or cycloheximide (50 μM). Total cellular RNA (10 μg) was extracted and analyzed by Northern blotting.

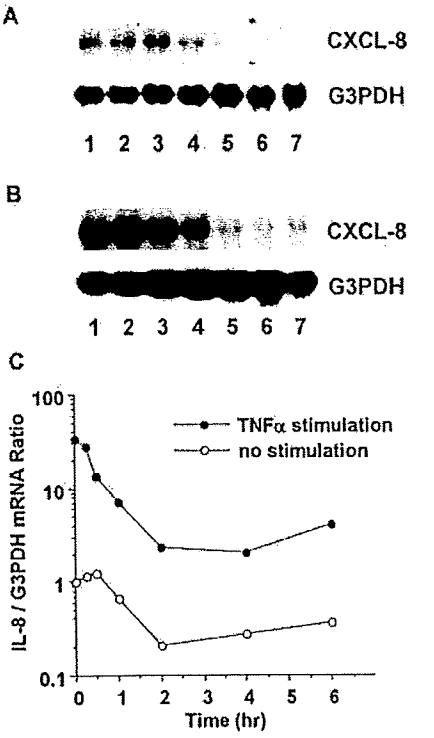

Figure 5. Stability of CXCL-8 mRNA transcript by TNF-α in Hs-700T cells. Carcinoma cells were serum-starved and then treated with CXCL-8 (10 ng/ml) for 4 h. Additionally, cells were incubated with actinomycin D (4 μM) for various lengths of time. Total cellular RNA (10 μg) was extracted and analyzed by Northern blotting. (A) No treatment. (B) TNF-α treatment. (C) Autoradiographic densities of each mRNA band were quantitated using a Bio-Image Analyzer (Fuji film Co., Tokyo, Japan). The results were standardized against the levels of G3PDH, and are presented as relative density. The level of expression detected in untreated cells after the stimulation of actinomycin D for 15 min equaled 1.

blotting. Especially, Hs-700T expressed a large amount of CXCL-8 mRNA transcript (lane 3).

Regulation of CXCL-8 mRNA expression by various cytokines in Hs-700T cells. We examined the effect of TNF-α, IL-1ß, LIF, IL-6, CXCL-8, IL-10 or IFN-ß on CXCL-8 mRNA expression in Hs-700T cells. In comparison with cells incubated in medium (Fig. 2A and B, lane 1), TNF-α, IL-1ß, LIF, IL-6, CXCL-8 or IFN-ß further augmented the expression levels of CXCL-8 mRNA in Hs-700T cells (Fig. 2A and B, lanes 2-6 and 8). TNF-α was markedly upregulated to maximum effect (6.2-fold), whereas IL-10 was not significantly upregulated (0.9-fold) in Hs-700T cells (Fig. 2A and B, lane 7). Similarly, we observed upregulation of CXCL-8 mRNA expression by these cytokines, especially TNF-α and IL-1ß, in other pancreas carcinoma cells, such as BxPc-3, SUIT-2, Hs-766T and Panc-1 cells (data not shown).

Expression of TNF receptor mRNA in pancreas carcinoma cells. TNF-α binds two kinds of receptors, the 55-kDa, type I (TNFR I) and the 75-kDa, type II (TNFR II), which mediate gene expression through TNFR associated factor 2 (TRAF2) cooperatively. TNFRI induces apoptosis through TNFR-associated death domain (TRADD) protein (10). We analyzed the expression of TNFR II mRNA after serum-starvation in pancreatic carcinoma cells by RT-PCR analysis. As shown in Fig. 3, the expression of TNFR II mRNA was detectable in most pancreatic carcinoma cells constitutively. These results indicated that TNF-α could induce CXCL-8 expression through TNFR II in pancreatic carcinoma cells.

Effect of actinomycin D or cycloheximide on the expression of CXCL-8 mRNA by TNF-α in Hs-700T cells. To investigate whether TNF-α induces *de novo* protein synthesis, we analyzed the effect of transcript synthesis inhibitor, actinomycin D (Act D), and protein synthesis inhibitor, cycloheximide (CHX), on the expression of CXCL-8 mRNA by Northern blotting. Cells were stimulated with Act D or CHX for 3 h, and followed by the stimulation of TNF-α or not. As shown in Fig. 4, TNF-α enhanced CXCL-8 mRNA expression in comparison with untreated cells (lanes 1 and 4) and Act D markedly suppressed CXCL-8 mRNA expression (lanes 2 and 5). CHX augmented CXCL-8 mRNA which was induced by TNF-α or not (lanes 3 and 6). The increase of CXCL-8 mRNA transcripts was dependent on *de novo* mRNA transcription and they did not require other protein synthesis. These results indicate that TNF-α promotes *de novo* synthesis of CXCL-8 in Hs-700T cells.

Effect of TNF-α on the stability of CXCL-8 transcripts in Hs-700T cells. We next evaluated whether TNF-α affects the

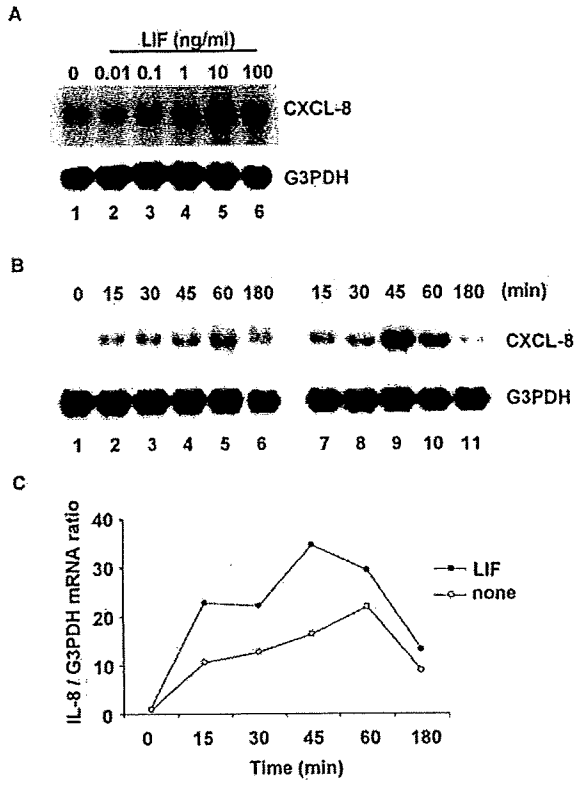

Figure 6. Regulation of CXCL-8 mRNA expression by LIF in Hs-700T cells. Carcinoma cells were serum-starved and then treated with indicated concentrations of human recombinant LIF for 8 h. Total cellular RNA was extracted and the expression of CXCL-8 mRNA was analyzed by Northern blotting. (A) CXCL-8 mRNA induction by dosage of LIF, 0-100 ng/ml. (B) Kinetics of CXCL-8 mRNA induction by 10 ng/ml concentration of LIF. (C) Autoradiographic densities of each mRNA band were quantitated using a Bio-Image Analyzer (Fuji film Co., Tokyo, Japan) in kinetics study. The results were standardized against the levels of G3PDH, and are presented as relative density. The level of expression detected in untreated cells equaled 1.

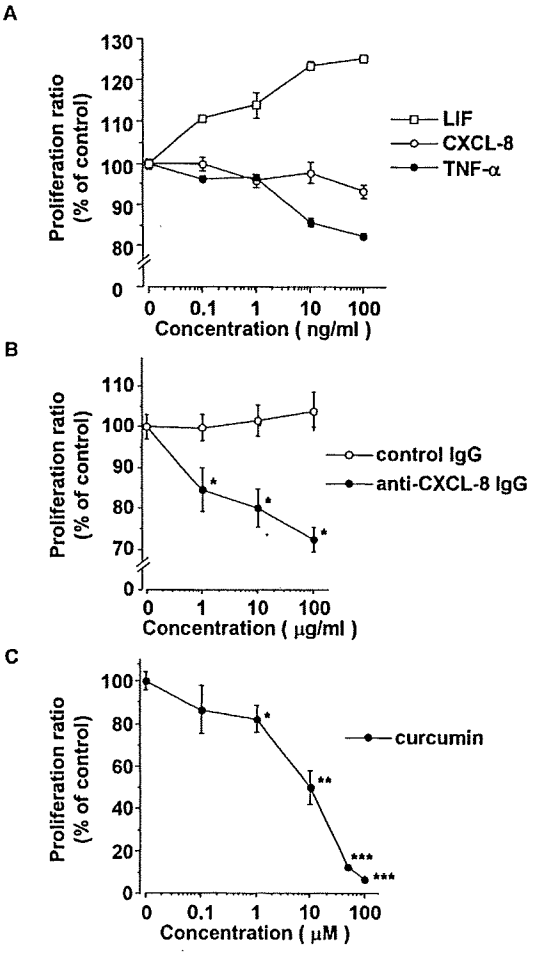

Figure 7. Effect of CXCL-8, TNF-α, LIF, anti-CXCL-8 IgG, or curcumin on cell proliferation of Hs-700T cells. Carcinoma cells ($1x10^5$ cells/ml) were exposed to various concentrations of CXCL-8, TNF-α, LIF or anti-CXCL-8 IgG (0-100 ng/ml) in triplicate using the 96-well microtitre plates for 48 h. Then, MTT assay was performed as described in Materials and methods. Data were shown as mean ± SD of three-four wells. Statistical significance was evaluated by the Mann-Whitney t-test. (A) Effect of CXCL-8, TNF-α or LIF. (B) Effect of anti-CXCL-8 IgG or control IgG. *P<0.001. (C) Effect of curcumin. *P=0.0162, **P=0.0007, ***P<0.0001.

stability of CXCL-8 mRNA post-transcriptionally in Hs-700T cells. Cells were incubated in medium with or without TNF-α for 3 h, and followed by addition of Act D for the indicated time. The half-life of CXCL-8 mRNA was 36.5 min by TNF-α and 35.2 min by no stimulation (Fig. 5). TNF-α did not affect CXCL-8 mRNA stabilization in comparison with control, indicating that TNF-α did not contribute to protection from CXCL-8 mRNA degradation after transcription.

Regulation of CXCL-8 mRNA expression by LIF in Hs-700T cells. Previously we reported that Hs-700T cells promoted cell growth by the stimulation of LIF. We clarified the underlying mechanism of induction of LIF, *c-fos*, *junB*, and cyclinE mRNA (8). CHX suppressed endogenous LIF mRNA expression after the stimulation of TNFα or LIF (data not shown). This led us to assume that induction of LIF requires *de novo* other protein synthesis. We evaluated whether LIF induces the expression of CXCL-8 mRNA by Northern blotting in Hs-700T cells. Cells were serum-starved and then stimulated with various LIF concentrations. As shown in Fig. 6A, addition of LIF induced endogenous CXCL-8 mRNA expression in a dose-dependent fashion, especially >0.1 ng/ml concentration of LIF (lanes 3-6). Additive LIF also induced the expression of CXCL-8 mRNA in the early phase of kinetics and maximally after stimulation for 45 min (Fig. 6B and C).

Suppression of cell growth by neutralizing anti-CXCL-8 IgG or curcumin in Hs-700T cells. The promotion of cell growth might depend on CXCL-8 expression in Hs-700T cells. To clarify this hypothesis, we examined whether the stimulation of CXCL-8 promotes cell growth in Hs-700T cells. We firstly investigated the effect of recombinant CXCL-8, TNF-α and LIF on cell proliferation by MTT assay. As shown in Fig. 7A, LIF promoted cell growth in a dose-dependent manner, however CXCL-8 did not have a significant effect after incubation for 48 h. TNF-α suppressed cell growth at a dose of 10-100 ng/ml and cellular morphology of treated cells partially exhibited loss of volume, rounding shape and chromatin condensation, all being morphological features of cells in apoptosis. As Hs-700T expressed both CXCL-8

(Fig. 1) and its receptor mRNA (data not shown) constitutively, CXCL-8 might function as an autocrine growth factor. Anti-CXCL-8 IgG suppressed cell growth with increasing dose and its inhibitory effects were significant with 1-100 μg/ml of anti-CXCL-8 IgG (Fig. 7B). The promoter of CXCL-8 consists of consensus sequence motif of NF-κB, which is strongly activated by TNF-α. Curcumin is a strong inhibitor of NF-κB activation. We examined the effect of curcumin on growth of Hs-700T cells by MTT assay. Carcinoma cells were incubated in RPMI-1640 medium with curcumin or not for 48 h. As shown in Fig. 7C, curcumin inhibited cell growth in a dose-dependent manner and a high dose of curcumin, >50 μM, blocked it completely. These results suggested that constitutive expression of a high concentration of CXCL-8 played a major biological role and an optimal dose of CXCL-8 could affect cell growth effectively in Hs-700T cells.

Discussion

In our previous study, CXCL-8 was produced constitutively and commonly in a variety of human carcinoma cells derived from lung, stomach, pancreas, esophagus, colon, gall bladder, breast, and melanoma (5). High amounts of CXCL-8 expression have been reported in various human malignancies, including leukocytes, melanocytes, mesothelium, brain, ovary, prostate, kidney, neck, breast, colon, and stomach (11). Thus, CXCL-8 was produced at high incidence and amount in most carcinoma cells. All examined pancreatic carcinoma cell lines produced CXCL-8 (12). This is consistent with our results.

Clinically, pancreatic cancer is a disease with the worst prognosis, which can metastasize to the liver and invade surrounding tissues easily. Serum concentration of IL-6, CXCL-8, IL-10, and IL-1RA were elevated in patients who had pancreatic cancer in comparison with healthy individuals. Serum concentration of CXCL-8 correlated with weight loss, but not with survival (13). Thus, CXCL-8 could be an important molecule in pancreatic cancer bearing patients. Metabolic imbalance between vascularization and tumor formation in aggressive pancreatic cancer could lead to low blood flow and low extracellular pH in tumor microenvironment. Hypoxia and acidosis enhanced expression of CXCL-8 by activation of NF-κB and AP-1 in pancreatic cancer cells (14).

In the present study, we demonstrated that inflammatory cytokines, especially TNF-α and LIF, induced CXCL-8 expression in pancreatic carcinoma cells. Act D did not affect post-transcriptionally the half life of CXCL-8 mRNA after the stimulation of TNF-α. TNF-α upregulated CXCL-8 drastically in *de novo* pathway. In contrast, IL-1ß induces stabilization of CXCL-8 mRNA in malignant breast cancer cells via the 3' untranslated region: involvement of divergent RNA-binding factors HuR, KSRP and TIAR (15). Nitric oxide also upregulated the expression of CXCL-8 by an increase in CXCL-8 gene transcription and mRNA stability in pancreatic cancer (16). LIF induced CXCL-8 mRNA in a dose- and time-dependent manner in Hs-700T cells. LIF expression correlated with CXCL-8 expression in psoriasis, but not skin cancers (17,18).

Previous studies have revealed that CXCL-8 contributes to cancer progression, such as proliferation, metastasis, and angiogenesis (19) in a variety of tumor microenvironments. We also demonstrated that neutralizing anti-CXCL-8 IgG suppressed cell growth in Hs-700T which produced a high amount of CXCL-8 in the culture supernatants. This growth promoting activity is consistent with other pancreatic carcinoma cells, including SUIT-2 and Capan-1 cells. CXCL-8, produced by carcinoma cells, acts as an autocrine growth factor in pancreatic carcinoma cells. However, addition of CXCL-8 failed to promote cell growth. An optimal dose of CXCL-8 has a growth activity function, but excess doses do not. Receptors of CXCL-8, CXCR-1 and CXCR-2, might be insufficient to express and supply after receptor internalization in Hs-700T cells. Although TNF-α induced CXCL-8 mRNA expression strongly in *de novo* synthesis pathway, TNF-α suppressed cell proliferation in any tested dose, 0.1-100 ng/ml, in Hs-700T cells. Apoptotic activity of TNF-α is dominant via receptor containing death domain, particularly 55-kDa TNF receptor (TNFRI), despite the strong inducer of CXCL-8. Our previous reports demonstrated that exogenous LIF promoted cell growth by the mechanisms upregulating endogenous LIF and LIFR expression in Hs-700T cells which produced a small amount of LIF (8,20). In this study, LIF induced expression of CXCL-8 transcript at levels that were limited in comparison with TNF-α. Thus, our data suggest that CXCL-8 functions as an autocrine growth factor which facilitates pancreatic cancer progression, and its expression is influenced by inflammatory cytokines, such as TNF-α and LIF. CXCL-8 also could act as a paracrine and endocrine factor, and affect the interaction with stromal tissues, including fibroblasts, endothelial cells, and infiltrative leukocytes in pancreatic tumor microenvironments.

Curcumin is a food element which has inhibitory activity of NF-κB. We examined previously that curcumin-down-regulated NF-κB activation correlated with CXCL-8 production and suppressed cell growth significantly in pancreatic carcinoma cell lines, SUIT-2 (7). NF-κB and IκB kinase are constitutively active in human pancreatic cells, and their downregulation by curcumin is associated with the suppression of proliferation and the induction of apoptosis (21). In the present study, curcumin also suppressed cell proliferation in Hs-700T cells. It could be beneficial to use an NF-κB inhibitor, such as curcumin, to treat pancreatic cancer with high CXCL-8 production.

These results suggest that CXCL-8 could be a molecular target to develop new strategies for clinical anti-cancer therapy and diagnosis in pancreatic cancer.

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ORIGINAL ARTICLE

Simplified Staging System for Predicting the Prognosis of Patients With Resectable Liver Metastasis

Development and Validation

Masami Minagawa, MD, PhD; Junji Yamamoto, MD, PhD; Tomoo Kosuge, MD, PhD; Yutaka Matsuyama, PhD; Shin-ichi Miyagawa, MD, PhD; Masatoshi Makuuchi, MD, PhD

Hypothesis: Although several staging systems for colorectal liver metastasis have been proposed, simple and generally accepted staging systems are not available for this disease. We hypothesized that more detailed analysis of primary colorectal cancer may make it possible to develop a simple staging system and that its stratification ability may be demonstrated by validation against data from unrelated patients.

Design: Retrospective analysis of prospectively documented data, development of a stage, and validation against an unrelated cohort.

Setting: Four tertiary referral centers.

Patients: Twenty-two clinicopathologic factors were examined in 369 consecutive patients who underwent curative resection for liver metastasis from colorectal cancer (original cohort). Using the independent prognostic factors, a simplified staging system was developed and was validated by data from 229 unrelated patients (validation cohort).

Main Outcome Measures: Kaplan-Meier survival curve analyses between different prognostic groups in the cohorts.

Results: Multivariate analysis revealed several independent prognostic variables, including hepatic lymph node metastasis (relative risk 4.39), 4 or more colorectal lymph node metastases (RR 1.50), carcinoembryonic antigen level of 50 ng/mL or higher (RR 1.29), and multiple hepatic metastases (RR 1.27). Patients with hepatic lymph node metastasis were assigned to stage 4, and the remaining patients were divided according to number of factors: none, stage 1; 1, stage 2; 2 or 3, stage 3. In the original cohort, median survival in stages 1, 2, 3, and 4 was 7.2,.3.5, 2.0, and 1.3 years, respectively. In the validation cohort, these values were 9.6, 4.1, 2.8, and 1.6 years, respectively.

Conclusions: The proposed simplified staging system was easy to use, was highly predictive of patient outcome, and permitted categorization of patients into treatment groups. Although we validated this staging system, further validation and improvements are needed.

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LIVER METASTASES FROM COlorectal cancer are classified by Union Internationale Contre le Cancer (UICC) staging criteria as stage IV, although the prognosis of patients with this disease varies widely.[1] Hepatic resection for colorectal liver metastasis remains the only treatment that has curative potential.[2] Many controversies exist about the treatment of liver metastasis, such as the effectiveness of adjuvant chemotherapy, the timing of resection for synchronous metastasis, and the operative indications for multiple metastasis or extrahepatic metastasis. As a result, there is an increasing need for a simple staging system that can reflect the prognosis and permit the stratification of patients for clinical trials.

Several staging systems for colorectal liver metastasis have been proposed. Gennari,[3-5] Fortner,[6] and Gayowski[7] and their colleagues proposed staging systems based on the size, number, and intrahepatic and extrahepatic extent of metastatic nodules. Cady and Stone[8] developed a prognostic scoring system that weighs individual factors. Nordlinger,[9] Fong,[10] Iwatsuki,[11] and Schindl[12] and their colleagues developed staging systems by analyzing prognostic factors, but 5 to 7 factors had to be explored to determine the stage.

See Invited Critique at end of the article

What are the requirements of a good staging system? First, it should be simple and easy to use. Second, it must provide reliable information on the prognosis of the disease. Third, it should permit the categorization of patients into various treat-

ment groups. Based on these criteria, well-defined and generally accepted staging systems are not available for this disease. The primary goals of this study were to develop a staging system that will fulfill these requirements and to validate its prognostic reliability in an unrelated group of patients.

METHODS

Between January 1, 1980, and December 31, 2002, 388 patients with hepatic metastasis from colorectal cancer underwent liver resection at the Department of Surgery, National Cancer Center (1980-1990), the First Department of Surgery, Shinshu University (1990-1994), and the Department of Hepato-Biliary-Pancreatic Surgery, University of Tokyo (1994-2002). The last author (M.M.) participated in all of the operations. Nineteen of these resections were not radical because of gross residual disease within or outside the liver, and the remaining 369 patients were included in the original cohort.

Selection criteria for surgery were the possibility of complete removal of all hepatic and extrahepatic lesions and the possibility of preserving at least 40% of the normal hepatic parenchyma. The total number of hepatic metastases, their unilateral or bilateral presentation, and the existence of extrahepatic metastasis were not considered exclusion criteria. No ablative strategies were used along with resection in any of these patients. The treatment policy for synchronous metastasis was simultaneous resection regardless of the number and extent of liver metastasis and the location of the primary cancer.

In all cases, the preoperative diagnostic workup included ultrasonography and plain and contrast-enhanced computed tomography to stage liver involvement and chest radiography, chest computed tomography, barium enema, and colonoscopy to assess the presence or absence of extrahepatic disease. Patients with advanced disease underwent bone scintigraphy or positron emission tomography. Intraoperative bimanual liver palpation and intraoperative ultrasonography (IOUS) were also performed in all patients, and all of the resections were IOUS-guided procedures. The mean duration of follow-up in the original cohort was 4.11 years (range, 1.1 months to 18.8 years).

The validation cohort consisted of 229 patients with colorectal liver metastases who underwent curative hepatic resections by colleagues of the last author (M.M.): 77 at the National Cancer Center between January 1, 1991, and December 31, 1997 (M.M. moved to Shinshu University in 1990), and 152 at Cancer Institute Hospital between January 1, 1997, and December 31, 2003. The selection criteria for hepatectomy and the preoperative and intraoperative diagnostic workup in these groups were comparable with those of the original cohort. The mean duration of follow-up in the validation cohort was 3.95 years (range, 2.5 months to 13.5 years). This retrospective study was approved by the institutional review boards in the respective institutions.

Survival time was calculated from the date of hepatic resection to death or censored date. Patients who died of colorectal cancer were treated as event observations, and patients who died of unrelated causes and were alive at the last follow-up were treated as censored observations. Survival curves were constructed using the Kaplan-Meier product-limit method and compared using the log-rank test. Significant prognostic factors in a univariate analysis were entered into a Cox proportional hazards model using stepwise selection to identify independent predictors of death. Statistical significance was defined as $P<.05$. A software program (SAS version 8; SAS Institute Inc, Cary, NC) was used for the statistical analyses.

RESULTS

The 3-, 5-, and 10-year survival of the original cohort were 52%, 38%, and 26%, respectively. There was no in-hospital death. We analyzed the effects of 15 clinicopathologic factors at hepatic resection (**Table 1**) and 7 at primary colorectal resection (**Table 2**) on survival after curative hepatic resection. Multiple liver metastases ($P<.001$), diameter of 5 cm or greater ($P=.02$), interval between primary cancer and liver resection less than 6 months ($P=.04$), carcinoembryonic antigen (CEA) level of 50 ng/mL or greater ($P<.001$), a resection margin less than 5 mm ($P=.006$), hepatic lymph node metastasis ($P<.001$), extrahepatic metastasis ($P=.03$), and extrahepatic invasion ($P=.03$) showed significant prognostic value for survival in a univariate analysis. Unilateral distribution of metastases was a favorable factor ($P<.001$), and 148 of 222 patients with unilateral metastasis had a solitary metastasis. Excluding patients with a single metastasis, distribution was not significant in patients with multiple metastases ($P=.64$) (Table 1). Survival curves stratified by the number of liver metastases are shown in **Figure 1**A. The prognosis according to the serum CEA level at hepatic resection is shown in Figure 1B. In this article, patients were divided into 2 groups according to the serum CEA level at hepatic resection ($\geq$50 and $<$50 ng/mL) because the χ^2 statistic by the log-rank test reached a maximum ($\chi^2=21.8$) when the boundary was set at 50 ng/mL.

Invasion to the serosa or another organ of primary colorectal cancer (pT4 by UICC classification) ($P=.02$), number of colorectal lymph node metastases of 4 or more (pN2 by UICC classification) ($P<.001$), and lymphatic duct involvement by the primary cancer ($P=.03$) also predicted an adverse outcome (Table 2). Nodal status of the primary cancer and long-term survival are shown in Figure 1C.

MULTIVARIATE ANALYSIS OF OUTCOME

The univariate prognostic factors were entered into a multivariate model to identify independent predictors of long-term survival. Hepatic lymph node metastases had the greatest impact on survival (relative risk, 4.39), followed by 4 or more colorectal lymph node metastases (pN2) (relative risk, 1.50), CEA level of 50 ng/mL or greater (relative risk, 1.29), and multiple metastases (relative risk, 1.27) (**Table 3**).

METHOD FOR DETERMINING THE STAGE

Regional lymph node metastasis of the liver was clearly the most influential factor and was associated with a 4.39-fold increase in the likelihood of death if it was positive. Thus, these patients were assigned to stage 4. The other 3 independent prognostic factors (number of lymph node metastases around the primary cancer $\geq$4, CEA level $\geq$50 ng/mL, and multiple liver metastases) cannot be considered complete contraindications to resection because each alone was still associated with a sufficiently favorable outcome to justify an aggressive surgical procedure, and the

Table 1. Factors at Hepatic Resection

Variable	Patients, No.	Survival, Median (95% CI), y	5-y Survival, %	*P* Value*
Sex				.73
F	138	3.3 (2.7-4.8)	41	
M	231	3.1 (2.5-3.7)	37	
Age, y				.80
<60	174	3.0 (2.4-4.0)	38	
≥60	195	3.3 (2.8-4.0)	39	
No. of liver metastases				<.001
Single	156	4.8 (3.3-6.9)	49	
Multiple	213	2.4 (2.1-2.9)	31	
Diameter, cm				.02
<5	245	3.5 (2.8-4.6)	42	
≥5	122	2.7 (2.0-3.2)	32	
Distribution				<.001
Unilateral	222	3.6 (3.1-5.2)	44	
Bilateral	141	2.3 (2.1-2.8)	27	
Distribution of multiple metastases				.64
Unilateral	74	2.7 (1.9-3.5)	34	
Bilateral	137	2.3 (2.1-2.8)	28	
Presentation of liver metastasis				.19
Synchronous†	187	2.8 (2.3-3.4)	35	
Metachronous	182	3.5 (2.8-4.8)	41	
Interval between primary cancer and liver resection, mo				.04
≥6	183	3.5 (3.0-4.8)	42	
<6	186	2.5 (2.3-3.3)	35	
Carcinoembryonic antigen at hepatectomy, ng/mL				<.001
<50	234	4.0 (3.3-5.3)	44	
≥50	124	2.1 (1.7-2.8)	26	
Resection margin, mm				.006
<5	230	2.7 (2.3-3.5)	34	
≥5	95	4.3 (3.2-5.8)	47	
Vascular invasion				.70
Negative	332	3.1 (2.7-3.6)	38	
Positive	30	3.3 (1.9-5.4)	41	
Biliary invasion				.496
Negative	350	3.1 (2.8-3.5)	38	
Positive	19	4.2 (1.9-NC‡)	39	
Hepatic lymph node metastasis				<.001
Negative	365	3.2 (2.8-3.6)	39	
Positive	4	1.3 (0.4-NC‡)	NC	
Extrahepatic metastasis				.03
Negative	333	3.2 (2.8-3.8)	39	
Positive	29	2.6 (1.4-3.1)	23	
Extrahepatic invasion				.03
Negative	350	3.1 (2.8-3.8)	39	
Positive	12	2.7 (1.2-3.3)	17	

Abbreviations: CI, confidence interval; NC, not calculated.
*By the log-rank test.
†Metastasis that had been diagnosed before the primary colorectal surgery or found at primary surgery.
‡Indicates that the survival curve remains above a survival rate of 50%.

increase in the likelihood of death ranged from 1.27 to 1.50. Therefore, these criteria were used to determine whether some combination could be used to dictate the choice of clinical options. Patients who had none of these 3 factors were assigned to stage 1, those with 1 factor to stage 2, and those with 2 or 3 factors to stage 3 (**Figure 2**). Survival curves for the original cohort, classified according to this simplified staging system, are shown in **Figure 3**. This simple staging was found to be highly predictive of the long-term outcome ($P<.001$) (Figure 3), and the differences in survival between the stages were significant (**Table 4**). Next, the original cohort was divided into 2 groups—synchronous vs metachronous metastasis—and the prognostic value of this simplified staging system was evaluated in each group. In the 187 patients with synchronous metastasis, 5-year survival for stages 1, 2, 3, and 4 were 65%, 38%, 18%, and 0%, respectively ($P<.001$). In the 182 patients with metachronous metastasis, 5-year survival for stages 1, 2, 3, and 4 were 54%, 48%, 30%, and 0%, respectively ($P<.001$).

VALIDATION OF THE SIMPLIFIED STAGING SYSTEM

The 3-, 5-, and 10-year survival of the validation cohort were 61%, 44%, and 35%, respectively. Of the 229 pa-

Table 2. Factors at Resection of Colorectal Cancer

Variable	Patients, No.	Survival, Median (95% CI), y	5-y Survival, %	*P* Value*
Diameter of primary lesion, cm				.33
<5	120	3.5 (2.8-5.2)	41	
≥5	148	3.0 (2.4-4.2)	37	
Location of colorectal cancer				.41
Colon	214	3.1 (2.8-4.2)	40	
Rectum	152	3.1 (2.4-4.0)	35	
Depth of wall invasion†				.02
pT1-pT3	181	3.7 (3.1-5.0)	42	
pT4	159	2.8 (2.2-3.3)	35	
No. of LN metastases around primary cancer†				<.001
0-3 (pN0-pN1)	230	4.2 (3.3-5.8)	47	
≥4 (pN2)	87	1.8 (1.3-2.1)	19	
Lymphatic duct invasion				.03
Negative	101	3.9 (3.1-7.0)	44	
Positive	211	2.8 (2.3-3.3)	34	
Vascular invasion of primary cancer				.53
Negative	132	3.2 (2.6-3.9)	38	
Positive	179	3.1 (2.5-4.0)	37	
Differentiation of primary adenocarcinoma				.88
Well-differentiated	156	3.3 (2.7-4.0)	37	
Moderately/poorly differentiated	129	2.7 (2.3-3.9)	37	
Others	7	3.0 (0.6-NC)	19	

Abbreviations: CI, confidence interval; LN, lymph node; NC, not calculated (indicates that the survival curve remains above a survival rate of 50%).
*By the log-rank test.
†By TNM stage of the Union Internationale Contre le Cancer.

tients, 64 were assigned to stage 1, 93 to stage 2, 67 to stage 3, and 5 to stage 4. Median survival time and 5- and 10-year survival rates for each stage are summarized in Table 4. The assigned stage was highly predictive of patient outcome ($P<.001$) (**Figure 4**).

COMMENT

In the 1980s, 2 staging systems were developed by Gennari et al[3-5] and Fortner et al.[6] These systems were based on the degree and extent of metastatic tumors and not on factors regarding primary colorectal cancer.[3-6] Cady and Stone[8] proposed a scoring index based on 4 risk factors: surgical margin, CEA level, disease-free interval, and number of liver nodules. This staging also did not include factors regarding colorectal cancer, although the researchers pointed out that patients with poor differentiation and greater than 5 lymph node metastases in the primary cancer should have a poor prognosis, which would be governed by biological factors.[13] Gayowski et al[7] proposed a modified TNM staging system based on several factors: unilateral or bilateral, single or multiple, 2 cm or smaller or larger than 2 cm, and vascular or ductal invasion to a major branch. In this system, all metastases with bilateral distribution are considered modified T4. Generally, most patients with a single tumor have a unilateral distribution, and those with multiple nodules have a bilateral distribution. The worse outcome associated with multiple nodules affects the outcome with a bilateral distribution. As we have shown previously,[14] the prognosis of patients with multiple tumors did not differ according to the distribution in the liver. In a multicenter study by Nordlinger et al,[9] 1568 patients who had metastases confined to the liver and who received curative resection were analyzed, and 7 factors were found to be significant in a multivariate analysis: age 60 years or older, size 5 cm or larger, pT4 by UICC classification, pN1 or greater by UICC classification, disease-free interval of less than 2 years, 4 or more nodules, and margins less than 1 cm. Three stages were established based on the number of factors present: 0 to 2, 3 to 4, and 5 to 7. A similar method was used by Fong et al[10] and Iwatsuki et al[11] in 1999, but cases with a positive margin, extrahepatic disease, or hepatic lymph node metastasis were either excluded or assigned to the highest stage. Patients without these factors were divided according to the number of the following factors: node-positive primary cancer, disease-free interval of less than 12 months, more than 1 hepatic tumor, largest hepatic tumor greater than 5 cm, and CEA level greater than 200 ng/mL by Fong et al[10] and 3 or more tumors, tumor size greater than 8 cm, disease-free interval of 30 months or less, and bilateral tumor by Iwatsuki et al.[11] These staging systems were based on a multivariate survival analysis and reflected the prognosis but used 7 factors. Thus, all of the factors must be explored to determine the stage, which may make it difficult to use these staging systems.

It is essential for a good staging system to provide reliable information on the prognosis of the disease. To show that a staging system actually reflects the prognosis, it must be verified by validation against data from unrelated patients. The staging system proposed by Fong et al[10] was validated by Mann et al[15] in Australia. Schindl et al[12] developed a prognostic scoring system using Dukes stage, number of metastases, CEA level, alkaline phosphatase level, and albumin level and validated its prog-

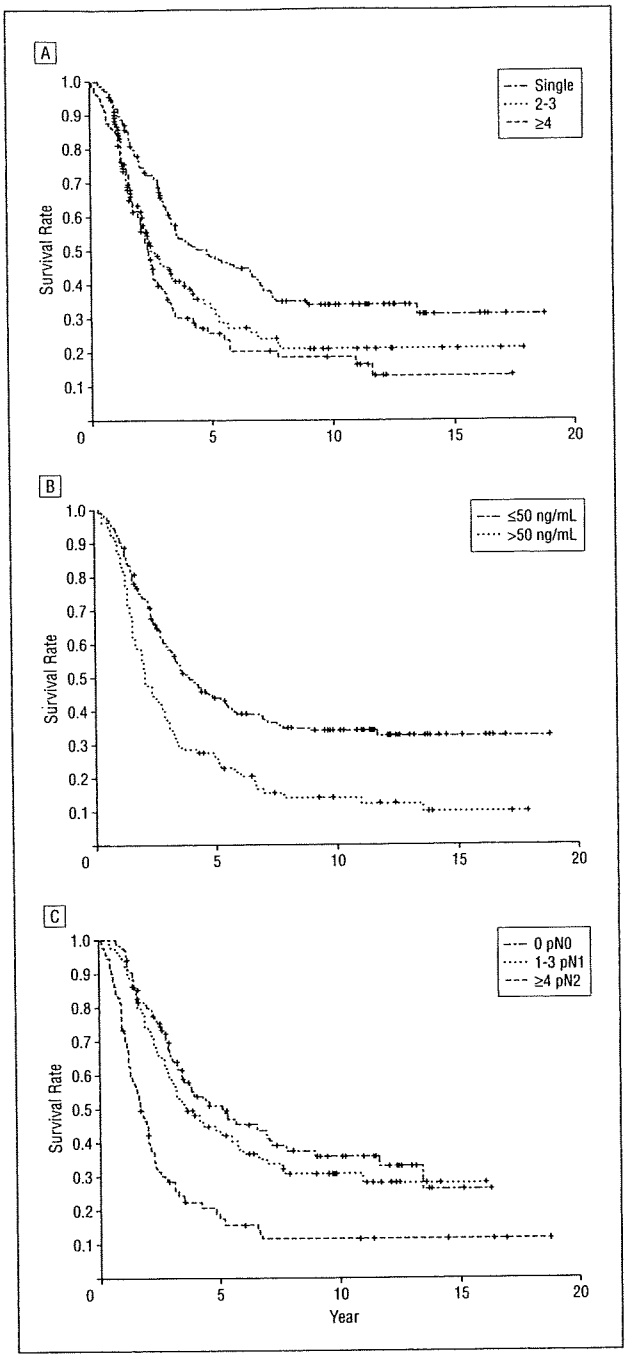

Figure 1. Kaplan-Meier survival analyses for patients in the original cohort. A, Stratified by the number of liver metastases. Median survival in 156 patients with a single metastasis was 4.8 years (95% confidence interval [CI], 3.3-6.9 years), in 116 patients with 2 to 3 nodules was 2.5 years (95% CI, 2.1-3.8 years), and in 97 patients with 4 or more deposits was 2.3 years (95% CI, 1.9-2.8 years) (1 vs 2-3, $P=.003$; 1 vs ≥4, $P<.001$; and 2-3 vs ≥4, $P=.33$). B, Stratified by the serum level of carcinoembryonic antigen at hepatectomy. Median survival in 234 patients with a level of less than 50 ng/mL was 4.0 years (95% CI, 3.3-5.3 years) and in 124 patients with a level of 50 ng/mL or more was 2.1 years (95% CI, 1.7-2.8 years) ($P<.001$). C, Stratified by the number of colorectal lymph node metastases. Median survival in 114 patients without lymph node involvement (pN0 by Union Internationale Contre le Cancer classification) was 5.2 years (95% CI, 3.5-7.2 years), in 116 patients with 1 to 3 lymph node metastases (pN1) was 3.7 years (95% CI, 2.8-5.8 years), and in 87 patients with 4 or more lymph node metastases (pN2) was 1.8 years (95% CI, 1.3-2.1 years) (0 vs 1-3, $P=.29$; and 1-3 vs ≥4, $P<.001$).

Table 3. Multivariate Analysis Using the Cox Proportional Hazards Model

Variable	Relative Risk (95% CI)	*P* Value
Hepatic LN metastasis		.005
Negative	1 (Reference)	
Positive	4.4 (1.8-8.1)	
No. of LN metastases around primary cancer		<.001
0-3	1 (Reference)	
≥4	1.5 (1.3-1.8)	
Carcinoembryonic antigen at hepatectomy, ng/mL		.002
<50	1 (Reference)	
≥50	1.3 (1.1-1.5)	
No. of liver metastases		.005
1	1 (Reference)	
≥2	1.3 (1.1-1.5)	

Abbreviations: CI, confidence interval; LN, lymph node.

nostic reliability in an unrelated group of patients. The robustness of the present staging system was tested by validation against data from patients who were not included in the original cohort. The survival rates of each stage in the validation cohort approximate those in the original cohort, and the *P* values for stage 1 vs 2 and stage 3 vs 4 are significant. Regarding stage 2 vs 3, it seems reasonable to predict that it will be significant with increasing numbers of patients because the median survival time of each stage is monotonically decreasing with advancing the stage. Consequently, the present staging system may provide reliable information on the prognosis of patients with colorectal liver metastasis.

Extrahepatic extension, such as extrahepatic metastasis, extrahepatic invasion, local recurrence at the primary cancer, and hepatic node metastases, has been analyzed as a whole in most previous studies. Patients with these factors have long been considered to be contraindicated for hepatectomy because of their dismal outcome. However, lung metastases, intraperitoneal dissemination, and local recurrence have gradually gained acceptance for resection in some institutions because a favorable prognosis can be anticipated if the tumors are removed completely.[16-19]

The incidence of macroscopic involvement of hepatic lymph nodes in patients who underwent hepatic resection reported in the literature is 3% to 6%, and 4 of 7 studies[2,7,9,11,18,20,21] reported 5-year survival of 0%. In contrast, Elias et al[18] showed 5-year survival of 27% in such patients after hepatectomy and lymph node dissection. The rate of microscopic involvement of hepatic lymph nodes has been reported to be 11% to 28%.[22-28] Although hepatectomy and lymph node dissection were performed in these patients, 5-year survival was reportedly 0% to 5%.[24,25,28] Rodgers and McCall[29] reviewed 15 studies that gave survival data on node-positive patients: 145 patients received hepatic resection, and only 5 (3.4%) survived 5 years. Based on these findings together with the present results, patients with hepatic lymph node metastasis were assigned to stage 4 in the simplified staging system. We should not operate on patients with hepatic lymph node metastasis.

Although many researchers[2,9,14,30-34] have noted that primary colorectal cancer affects the prognosis of patients who received hepatectomy for liver metastases, some[7,13,20,24,35-40] have reported contrary results. This discrepancy may be due to rates of synchronous and metachronous metastasis in each study. As our group[41] previously noted, the significant prognostic factors in patients with synchronous metastasis are different from those in patients with metachronous metastasis. In patients with synchronous metastasis, independent prognostic factors were 4 or more lymph node metastases around the colorectal cancer ($P<.001$) and multiple liver metastases ($P=.003$), whereas in patients with metachronous metastasis, CEA level ($P=.002$), 4 or more lymph node metastases around the colorectal cancer ($P=.03$), and hepatic lymph node metastasis ($P=.03$) were independently significant.[41] Factors associated with colorectal cancer play a more important role in synchronous metastasis. In a study in which most patients have metachronous metastasis, the stage of the primary cancer may not play an important role in the prognosis.

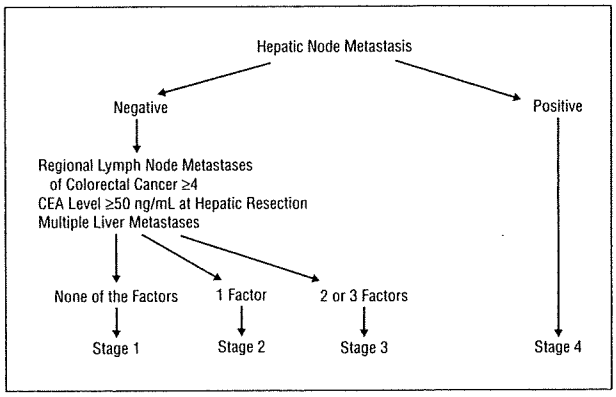

Figure 2. Algorithm used to determine the stage in this simplified staging system. CEA indicates carcinoembryonic antigen.

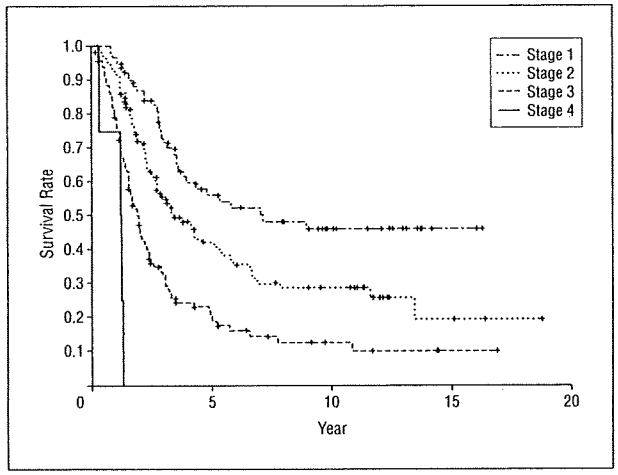

Figure 3. Kaplan-Meier survival analysis for patients in the original cohort stratified according to the simplified staging system.

In most studies, the factors of colorectal cancer were represented in terms of Dukes stage. We analyzed it more precisely: patients without mesenteric lymph node metastasis and those with 1 to 3 lymph node metastases had a similar prognosis, and those with 4 or more metastases showed a significantly worse outcome (Figure 1C). Therefore, it is more reasonable to separate patients according to the number of lymph node metastases (≥4 vs 0-3) than Dukes stage (A-B vs C). Moreover, the depth of the wall invasion by colorectal cancer is known to affect the prognosis. A tumor without regional lymph node invasion is classified as Dukes stage A if it invades the muscularis propria or less and as Dukes stage B if it infiltrates the subserosa or more. According to the present analysis on the depth of invasion and prognosis, tumors that perforated the visceral peritoneum or directly invaded other organs or structures (T4 by UICC classification) had a significantly poor outcome after hepatic resection, and no difference in survival was observed between tumors that invaded the submucosa (T1) or muscularis propria (T2) and tumors that invaded through the muscularis propria into the subserosa or into nonperitonealized pericolic or perirectal tissues (T3). A similar result was reported by Kato et al.[42] Therefore, it may be more reasonable to separate patients with liver metastasis into T1 to T3 and T4 than Dukes stages A and B-C.

Many studies have demonstrated that the preoperative CEA level has prognostic value. However, little is known about the biological function of CEA, which might act as an adhesion molecule when expressed on the cell surface or as a secreted immune modulator.[43-47] It has also been noted that the tumor burden may not correlate with CEA levels,[48,49] that the prognostic value of a high serum CEA level was comparable with that of the presence of intraperitoneal tumor cells,[50] that CEA en-

Table 4. Kaplan-Meier Analysis in the Original and Validation Cohorts

	Original Cohort				Validation Cohort			
Stage	Patients, No.	Survival, Median (95% CI), y	5-y Survival, %	10-y Survival, %	Patients, No.	Survival, Median (95% CI), y	5-y Survival, %	10-y Survival, %
1	78	7.2 (3.9-NC)	56	46	64	9.6 (4.4-NC)	61	50
2	129	3.5 (2.7-5.3)	42	29	93	4.1 (2.8-6.3)	43	33
3	111	2.0 (1.6-2.3)	22	13	67	2.8 (2.0-3.8)	33	24
4	4	1.3 (0.4-NC)	0	0	5	1.6 (0.2-NC)	0	0
1 vs 2		$P=.004$				$P=.03$		
2 vs 3		$P<.001$				$P=.14$		
3 vs 4		$P=.01$				$P=.003$		

Abbreviations: CI, confidence interval; NC, not calculated (indicates that the survival curve remains above a survival rate of 50%).

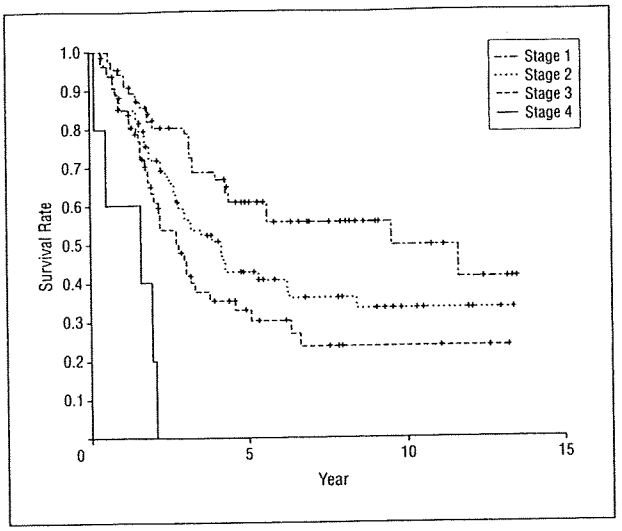

Figure 4. Kaplan-Meier survival analysis for patients in the validation cohort stratified according to the simplified staging system.

hances liver metastasis by functioning as an attachment factor,[51] and that an increased posthepatectomy CEA level was independently associated with extrahepatic recurrence.[52] Based on these results, the precise function of CEA is not clear: a high serum CEA level may reflect a highly malignant nature of cancer cells, which induces peritoneal dissemination, liver metastasis, and extrahepatic recurrence. In the present series, a CEA level of 50 ng/mL or more was an independent prognostic factor that contributed to the construction of the staging in association with the number of mesenteric lymph node metastases and multiple liver metastases.

Solitary metastasis was a favorable prognostic factor in a multivariate analysis. The prognosis of 97 patients with 4 or more nodules was similar to that of 116 patients with 2 to 3 deposits (Figure 1A). This result may be a consequence of the complete removal of hepatic and extrahepatic metastases and treatment of postresectional recurrence. In the present series, all of the patients underwent careful examination by means of IOUS and IOUS-guided hepatectomy. Makuuchi et al[53] first introduced IOUS in 1979. Twenty-five years later, modern diagnostic instruments still cannot replace IOUS regarding its sensitivity in depicting liver nodules.[54] Choti et al[40] demonstrated that the patient's prognosis after hepatic resection was significantly improved with the use of IOUS. In our experience, approximately 1.5-fold as many nodules are visualized by means of IOUS in patients with 4 or more metastases, and, thus, one third of the nodules cannot be detected even with extracorporeal diagnostic modalities. If these nodules are left in place, the prognosis of patients with 4 or more metastases will be dramatically worsened. These occult nodules in 4 or more metastases may have played an important role in the poor prognosis. Characteristically, liver metastasis, especially 4 or more metastases, can easily lead to recurrent nodules in the remnant liver. The treatment of such recurrences can strongly affect the prognosis. Our choice of treatment for recurrent metastasis is repeated resection, performed immediately and without neoadjuvant chemotherapy. With this treatment, the prognosis of patients with multiple metastases has been remarkably improved.[55,56]

This simplified staging system is easy to use, is highly predictive of patient outcome and survival, and permits the categorization of patients into various treatment groups. Patients with hepatic lymph node metastasis, who are categorized to stage 4 using the simplified staging system, should be excluded from hepatic resection. Patients in stage 1, 2, or 3 should receive hepatic resection, but it may be appropriate to apply adjuvant chemotherapy to patients with stage 3 disease. Our simplified staging system was validated by data from unrelated patients. However, further verification and refinement by other medical centers are necessary.

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Special Report

Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan†

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In the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan, 18 213 individuals were newly registered as patients with primary liver cancer at 645 medical institutions over a period of 2 years (from 1 January 2002 to 31 December 2003). Of these patients, 94.2% had hepatocellular carcinoma (HCC) and 4.1% had intrahepatic cholangiocarcinoma (ICC). In addition, 24 705 follow-up patients were registered in the survey. Epidemiological and clinicopathological factors, diagnosis and treatment were investigated in the newly registered patients, and the cumulative survival rates of newly registered patients in the 12th to 17th follow-up surveys conducted between 1992 and 2003 were calculated for each histological type (HCC, ICC, and combined HCC and ICC) and stratified by background factors and treatment. The data obtained in this follow-up survey should contribute to future research and medical practice for primary liver cancer.

Key words: combined hepatic carcinoma, cumulative survival rate, follow-up survey, hepatocellular carcinoma, intrahepatic cholangiocarcinoma

INTRODUCTION

SINCE 1969, THE Liver Cancer Study Group of Japan (LCSGJ) has conducted 16 nationwide follow-up surveys of primary liver cancer in patients in member hospitals and cooperative institutions in Japan, with the goal of promoting research and clinical treatment of liver cancer.[1–11] The 17th Nationwide Follow-up Survey of Primary Liver Cancer was conducted over a 2-year period from 1 January 2002 to 31 December 2003, and 18 213 patients with primary liver cancer were newly registered at 645 institutions. In addition, 24 705 registered patients were followed up with a valid response rate of 70.0%. Items related to epidemiological and clinicopathological factors, diagnosis and treatment were investigated in the newly registered patients. Cumulative survival rates of newly registered patients in the 12th to 17th follow-up surveys conducted between 1992 and 2003 were calculated for each histological type and based on background factors and treatment.

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METHODS

Basic statistics

THE SUBJECTS WERE 18 213 patients with primary liver cancer who underwent treatment or autopsy during a 2-year period from 1 January 2002 to 31 December 2003 at 645 institutions in Japan. Doctors in each institution completed a form developed by the Follow-up Survey Committee of the Liver Cancer Study Group of Japan (chairperson: Masatoshi Kudo). In cases with an inconsistency between the clinical, pathological and autopsy diagnoses, the autopsy and pathological diagnoses were given first and second priority, respectively. Of the 18 213 patients, 94.2% had hepatocellular carcinoma and 4.1% had intrahepatic cholangiocarcinoma (Table 1). The results in the tables are categorized into hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined HCC and ICC,

Table 1 Classification of primary liver cancer

Diagnosis	Male $n = 13\,017$	Female $n = 5196$	Total $n = 18\,213$
HCC	12 341	4818	17 159 (94.2%)
ICC	470	279	749 (4.1%)
Combined	93	30	123 (0.7%)
Cystadenocarcinoma	15	6	21 (0.1%)
Hepatoblastoma	8	4	12 (0.1%)
Sarcoma	11	8	19 (0.1%)
Others	79	51	130 (0.7%)

Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma. After Ikai *et al.* (2007), with permission from the Japan Society of Hepatology.

for which more than 100 newly registered cases appeared in the current follow-up survey. The abbreviations in the tables conform to the *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, Second English Edition.*[12]

Cumulative survival rate

The cumulative survival rates of newly registered patients in the 12th to 17th follow-up surveys whose final prognosis was determined to be survival or death (excluding patients with unknown outcomes) were calculated for each histological type (HCC, ICC, and combined HCC and ICC) and based on different background factors and treatment, including hepatectomy, local ablation therapy, and transcatheter arterial embolization. In the report of the 16th Nationwide Follow-up Survey of Primary Liver Cancer and in prior reports, patients who died due to liver-unrelated causes ('other causes' in Table 2) were considered as censored cases and patients who died due to liver-related events were considered to be uncensored cases. In the present report, however, patients who had died from either liver-related or liver-unrelated causes were considered to be uncensored cases in estimating cumulative survival rates.

RESULTS

Basic statistics

Causes of death during the study period

FOR HCC, THE mortality of newly registered patients during the study period was 15.8%: the death rate due to cancer was 55.1% and death rates due to hepatic failure, gastrointestinal bleeding and rupture of esophagogastric varices were 21.5%, 2.0% and 3.1%, respectively. Of the patients who did not survive, 44 died within 30 days after surgery; these patients represented 0.8% of the 5327 patients who underwent surgery. For

Table 2 Causes of death of patients with primary liver cancer

	HCC	ICC	Combined
Alive	13 946	454	75
Total deaths of between 2002 and 2003	2 700	270	44
Cancer death	1 487 (55.1%)	216 (80.0%)	30 (68.2%)
Hepatic failure	581 (21.5%)	28 (10.4%)	5 (11.4%)
Gastrointestinal bleeding	55 (2.0%)	2 (0.7%)	2 (4.5%)
Rupture of esophageal varices	85 (3.1%)	0 (0.0%)	3 (6.8%)
Rupture of tumor	172 (6.4%)	0 (0.0%)	1 (2.3%)
Operative death	44 (1.6%)	5 (1.9%)	1 (2.3%)
Other causes	276 (10.2%)	19 (7.0%)	2 (4.5%)
Unknown	402	20	3

Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma. After Ikai *et al.* (2007), with permission from the Japan Society of Hepatology.

Table 3 Clinical profile of patients with primary liver cancer

	HCC	ICC	Combined
Diagnosis	*n* = 33 731	*n* = 1505	*n* = 216
Computed tomography	13 160 (39.0%)	581 (38.6%)	89 (41.2%)
Magnetic resonance imaging	2 767 (8.2%)	181 (12.0%)	14 (6.5%)
Ultrasonography	9 257 (27.4%)	366 (24.3%)	59 (27.3%)
Selective angiography	6 495 (19.3%)	200 (13.3%)	34 (15.7%)
Histopathological finding	1 746 (5.2%)	115 (7.6%)	17 (7.9%)
Others	306 (0.9%)	62 (4.1%)	3 (1.4%)
Encephalopathy	*n* = 16 004	*n* = 699	*n* = 115
None	15 439 (96.5%)	696 (99.6%)	113 (98.3%)
Mild	425 (2.7%)	1 (0.1%)	0 (0.0%)
Coma occasionally	140 (0.9%)	2 (0.3%)	2 (1.7%)
Ascites	*n* = 16 321	*n* = 709	*n* = 116
Absent	14 230 (87.2%)	662 (93.4%)	105 (90.5%)
Slight	1 259 (7.7%)	18 (2.5%)	5 (4.3%)
Moderate	832 (5.1%)	29 (4.1%)	6 (5.2%)
Serum bilirubin (mg/mL)	*n* = 16 506	*n* = 685	*n* = 113
0.0–0.9	9 353 (56.7%)	427 (62.3%)	78 (69.0%)
1.0–1.9	5 535 (33.5%)	135 (19.7%)	26 (23.0%)
2.0–3.0	974 (5.9%)	23 (3.4%)	6 (5.3%)
≥3.1	644 (3.9%)	100 (14.6%)	3 (2.7%)
Serum albumin (g/dL)	*n* = 16 326	*n* = 668	*n* = 108
<2.8	1 252 (7.7%)	42 (6.3%)	3 (2.8%)
2.8–2.9	884 (5.4%)	35 (5.2%)	4 (3.7%)
3.0–3.5	4 886 (29.9%)	130 (19.5%)	24 (22.2%)
>3.5	9 304 (57.0%)	461 (69.0%)	77 (71.3%)
ICG R_{15} (%)	*n* = 11 003	*n* = 438	*n* = 89
≤14	3 736 (34.0%)	295 (67.4%)	51 (57.3%)
15–24	3 372 (30.6%)	100 (22.8%)	17 (19.1%)
25–40	2 558 (23.2%)	38 (8.7%)	17 (19.1%)
>40	1 337 (12.2%)	5 (1.1%)	4 (4.5%)
Prothrombin activity (%)	*n* = 15 256	*n* = 630	*n* = 107
<40	217 (1.4%)	8 (1.3%)	1 (0.9%)
40–49	348 (2.3%)	7 (1.1%)	3 (2.8%)
50–70	3 375 (22.1%)	62 (9.8%)	15 (14.0%)
71–80	3 546 (23.2%)	74 (11.7%)	21 (19.6%)
>80	7 770 (50.9%)	479 (76.0%)	67 (62.6%)
Platelet count ($\times 10^4/mm^3$)	*n* = 16 476	*n* = 673	*n* = 112
<3.0	130 (0.8%)	2 (0.3%)	0 (0.0%)
3.0–4.9	880 (5.3%)	3 (0.4%)	1 (0.9%)
5.0–9.9	5 437 (33.0%)	45 (6.7%)	20 (17.9%)
10.0–14.9	4 907 (29.8%)	75 (11.1%)	27 (24.1%)
15.0–19.9	2 839 (17.2%)	141 (21.0%)	31 (27.7%)
20.0–99.9	2 226 (13.5%)	398 (59.1%)	33 (29.5%)
>100	57 (0.3%)	9 (1.3%)	0 (0.0%)
Liver damage classification by LCSGJ	*n* = 14 295	*n* = 594	*n* = 105
A	8 478 (59.3%)	483 (81.3%)	75 (71.4%)
B	4 700 (32.9%)	81 (13.6%)	27 (25.7%)
C	1 117 (7.8%)	30 (5.1%)	3 (2.9%)

Table 3 *Continued*

	HCC	ICC	Combined
Child–Pugh classification	*n* = 15 651	*n* = 654	*n* = 112
A	11 119 (71.0%)	541 (82.7%)	87 (77.7%)
B	3 603 (23.0%)	94 (14.4%)	23 (20.5%)
C	929 (5.9%)	19 (2.9%)	2 (1.8%)
AFP (ng/mL)	*n* = 15 831	*n* = 496	*n* = 110
<15	5 756 (36.4%)	415 (83.7%)	37 (33.6%)
≤199	5 786 (36.5%)	58 (11.7%)	32 (29.1%)
≤399	902 (5.7%)	8 (1.6%)	8 (7.3%)
≤999	907 (5.7%)	7 (1.4%)	8 (7.3%)
≤9999	1 450 (9.2%)	7 (1.4%)	15 (13.6%)
≤99 999	704 (4.4%)	1 (0.2%)	8 (7.3%)
≥100 000	326 (2.1%)	0 (0.0%)	2 (1.8%)
AFP-L3 (%)	*n* = 6321	*n* = 76	*n* = 44
ND	2 234 (35.3%)	53 (69.7%)	10 (22.7%)
<5.0	1 349 (21.3%)	7 (9.2%)	1 (2.3%)
≤9.9	491 (7.8%)	3 (3.9%)	2 (4.5%)
≤14.9	309 (4.9%)	0 (0.0%)	2 (4.5%)
≤19.9	189 (3.0%)	1 (1.3%)	2 (4.5%)
≥20.0	1 749 (27.7%)	12 (15.8%)	27 (61.4%)
PIVKA-II (mAU/mL)	*n* = 14 209	*n* = 341	*n* = 96
<40	5 833 (41.1%)	289 (84.8%)	46 (47.9%)
≤99	2 004 (14.1%)	19 (5.6%)	8 (8.3%)
≤299	1 795 (12.6%)	12 (3.5%)	13 (13.5%)
≤499	641 (4.5%)	0 (0.0%)	3 (3.1%)
≤999	778 (5.5%)	7 (2.1%)	6 (6.3%)
≤2999	985 (6.9%)	7 (2.1%)	4 (4.2%)
≤9999	892 (6.3%)	3 (0.9%)	7 (7.3%)
≥10 000	1 281 (9.0%)	4 (1.2%)	9 (9.4%)
CEA (ng/mL)	*n* = 5716	*n* = 637	*n* = 79
<2.5	2 280 (39.9%)	219 (34.4%)	31 (39.2%)
≤4.9	2 078 (36.4%)	163 (25.6%)	19 (24.1%)
≤9.9	1 067 (18.7%)	100 (15.7%)	18 (22.8%)
≤19.9	211 (3.7%)	50 (7.8%)	6 (7.6%)
≤49.9	40 (0.7%)	48 (7.5%)	2 (2.5%)
≤99.9	14 (0.2%)	22 (3.5%)	1 (1.3%)
≥100	26 (0.5%)	35 (5.5%)	2 (2.5%)
CA 19-9 (U/mL)	*n* = 4533	*n* = 635	*n* = 67
<37	2 896 (63.9%)	206 (32.4%)	27 (40.3%)
≤99	1 134 (25.0%)	76 (12.0%)	16 (23.9%)
≤299	384 (8.5%)	84 (13.2%)	13 (19.4%)
≤999	70 (1.5%)	79 (12.4%)	6 (9.0%)
≤2999	26 (0.6%)	71 (11.2%)	3 (4.5%)
≤9999	13 (0.3%)	61 (9.6%)	1 (1.5%)
≥10 000	10 (0.2%)	58 (9.1%)	1 (1.5%)

AFP, alpha-fetoprotein; AFP-L3, lectin-reactive alpha-fetoprotein; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembrionic antigen; Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; ICG R_{15}, indocyanine green retention rate at 15 min; LCSGJ, Liver Cancer Study Group of Japan; ND, not determined; PIVKA-II, protein induced by Vitamin K absence-II.

Table 4 Hepatitis B and C virus-associated antigen and antibody

	HCC	ICC	Combined
HBsAg	*n* = 16 340	*n* = 696	*n* = 115
Negative	13 803 (84.5%)	653 (93.8%)	93 (80.9%)
Positive	2 531 (15.5%)	43 (6.2%)	22 (19.1%)
Undetermined	6 (0.0%)	0 (0.0%)	0 (0.0%)
HBsAb	*n* = 5281	*n* = 179	*n* = 54
Negative	4 248 (80.4%)	147 (82.1%)	40 (74.1%)
Positive	1 004 (19.0%)	30 (16.8%)	14 (25.9%)
Undetermined	29 (0.5%)	2 (1.1%)	0 (0.0%)
HBcAb	*n* = 4149	*n* = 134	*n* = 40
Negative	1 983 (47.8%)	78 (58.2%)	13 (32.5%)
Positive	2 138 (51.5%)	56 (41.8%)	27 (67.5%)
Undetermined	28 (0.7%)	0 (0.0%)	0 (0.0%)
HBeAg	*n* = 3320	*n* = 93	*n* = 28
Negative	2 801 (84.4%)	89 (95.7%)	25 (89.3%)
Positive	506 (15.2%)	4 (4.3%)	3 (10.7%)
Undetermined	13 (0.4%)	0 (0.0%)	0 (0.0%)
HBeAb	*n* = 3195	*n* = 91	*n* = 27
Negative	1 689 (52.9%)	51 (56.0%)	17 (63.0%)
Positive	1 455 (45.5%)	40 (44.0%)	10 (37.0%)
Undetermined	51 (1.6%)	0 (0.0%)	0 (0.0%)
HCVAb	*n* = 16 504	*n* = 700	*n* = 115
Negative	5 004 (30.3%)	564 (80.6%)	64 (55.7%)
Positive	11 488 (69.6%)	134 (19.1%)	51 (44.3%)
Undetermined	12 (0.1%)	2 (0.3%)	0 (0.0%)

Combined, combined hepatocellular and cholangiocarcinoma; HBcAb, antibody to hepatitis B core antigen; HBeAb, antibody to hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAb, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; ICC, intrahepatic cholangiocarcinoma.
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ICC, the mortality of newly registered patients during the study period was 36.3% and death rates due to cancer and hepatic failure were 80.0% and 10.4%, respectively (Table 2).

Past history

Of patients with HCC, 78.2% and 59.9% had a past history of chronic hepatitis and liver cirrhosis, respectively, whereas only 18.2% and 6.4% of ICC patients had this history, respectively. Interferon therapy had been given to 16.1% of HCC patients due to concomitant chronic hepatitis, and 28.8% and 22.3% of HCC patients and 9.1% and 12.1% of ICC patients had a past history of blood transfusion and habitual alcohol intake, respectively.

Clinical diagnosis

Clinical diagnosis of primary liver cancer in patients with HCC was made at a mean age of 65.5 years in males and 69.4 years in females. For patients with ICC, the corresponding mean ages were 66.5 years in males and 68.3 years in females. The mean ages were higher than those in the 16th survey. The male to female ratios for HCC and ICC patients were 2.55 and 1.64, respectively.

In patients with HCC, the level of liver injury at the time of diagnosis, based on the liver damage classification of the LCSGJ, was class A, B and C in 59.3%, 32.9% and 7.8% of patients, respectively, whereas 71.0%, 23.0% and 5.9% of HCC patients were in the Child–Pugh Class A, B and C categories, respectively (Table 3). Of the HCC patients, 36.4%, 36.5% and 27.1% had serum alpha-fetoprotein (AFP) levels of <15 ng/mL, 15–199 ng/mL and ≥200 ng/mL, respectively, and 64.4%, 4.9% and 30.7% of patients with HCC had serum levels of lectin-reactive AFP (AFP-L3) of <10%, 10.0–14.9% and ≥15%, respectively. Of the HCC patients, 41.1%, 14.1% and 44.8% had a protein induced by vitamin K absence or antagonist-II (PIVKA-II) level of <40 mAU/mL, 40–99 mAU/mL and

Table 5 Tumor characteristics by imaging studies

	HCC	ICC	Combined
Tumor size by imaging studies (cm)	*n* = 15 788	*n* = 604	*n* = 106
≤1	687 (4.4%)	2 (0.3%)	3 (2.8%)
≤2	4 436 (28.1%)	58 (9.6%)	11 (10.4%)
≤3	3 939 (24.9%)	106 (17.5%)	17 (16.0%)
≤5	3 495 (22.1%)	181 (30.0%)	30 (28.3%)
≤10	2 336 (14.8%)	200 (33.1%)	35 (33.0%)
≤15	598 (3.8%)	48 (7.9%)	10 (9.4%)
≤20	175 (1.1%)	8 (1.3%)	0 (0.0%)
≤25	50 (0.3%)	0 (0.0%)	0 (0.0%)
>25	72 (0.5%)	1 (0.2%)	0 (0.0%)
No. tumors by imaging studies	*n* = 16 187	*n* = 655	*n* = 110
1	9 365 (57.9%)	509 (77.7%)	65 (59.1%)
2	2 850 (17.6%)	42 (6.4%)	16 (14.5%)
3	1 265 (7.8%)	21 (3.2%)	7 (6.4%)
4	505 (3.1%)	9 (1.4%)	2 (1.8%)
5	254 (1.6%)	4 (0.6%)	4 (3.6%)
≥6	1 948 (12.0%)	70 (10.7%)	16 (14.5%)
Portal vein invasion by imaging studies	*n* = 15 169	*n* = 562	*n* = 110
Image-Vp0	13 184 (86.9%)	366 (65.1%)	76 (69.1%)
Image-Vp1	463 (3.1%)	39 (6.9%)	9 (8.2%)
Image-Vp2	449 (3.0%)	57 (10.1%)	6 (5.5%)
Image-Vp3	616 (4.1%)	85 (15.1%)	12 (10.9%)
Image-Vp4	457 (3.0%)	15 (2.7%)	7 (6.4%)
Hepatic vein invasion by imaging studies	*n* = 14 387	*n* = 544	*n* = 104
Image-Vv0	13 775 (95.7%)	469 (86.2%)	93 (89.4%)
Image-Vv1	215 (1.5%)	19 (3.5%)	5 (4.8%)
Image-Vv2	180 (1.3%)	32 (5.9%)	2 (1.9%)
Image-Vv3	217 (1.5%)	24 (4.4%)	4 (3.8%)
Bile duct invasion by imaging studies	*n* = 14 219	*n* = 527	*n* = 104
Image-B0	13 859 (97.5%)	291 (55.2%)	95 (91.3%)
Image-B1	141 (1.0%)	46 (8.7%)	4 (3.8%)
Image-B2	100 (0.7%)	69 (13.1%)	4 (3.8%)
Image-B3	82 (0.6%)	81 (15.4%)	0 (0.0%)
Image-B4	37 (0.3%)	40 (7.6%)	1 (1.0%)
Distant metastases by imaging studies			
Lung	259	25	3
Bone	207	17	1
Adrenal gland	57	4	2
Lymph node	199	113	9
Brain	12	1	0
Peritoneum	43	15	2
Others	30	10	0
Esophageal or gastric varices	*n* = 4894	*n* = 34	*n* = 18
F1, RC (−)	2 604 (53.2%)	24 (70.6%)	9 (50.0%)
F2 or RC (+)	1 990 (40.7%)	8 (23.5%)	7 (38.9%)
Rupture	300 (6.1%)	2 (5.9%)	2 (11.1%)

b0, absence of invasion of the bile ducts; B1, invasion of (or tumor thrombus in) the third order or more peripheral branches of the bile duct, but not of second order branches; B2, invasion of (or tumor thrombus in) the second order branches of the bile duct; B3, invasion of (or tumor thrombus in) the first order branches of the bile duct; B4, invasion of (or tumor thrombus in) the common hepatic duct; Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; Vp0, absence of invasion of (or tumor thrombus in) the portal vein; Vp1, invasion (or tumor thrombus in) distal to the second order branches of the portal vein, but not of the second order branches; Vp2, invasion of (or tumor thrombus in) second order branches of the portal vein; Vp3, invasion of (or tumor thrombus in) first order branches of the portal vein; Vp4, invasion of (or tumor thrombus in) the main trunk of the portal vein and/or contra-lateral portal vein branch to the primarily involved lobe; Vv0, absence of invasion of (or tumor thrombus in) the hepatic vein; Vv1,invasion of (or tumor thrombus in) peripheral branches of the hepatic vein; Vv2, invasion of (or tumor thrombus in) the right, middle, or left hepatic vein, the inferior right hepatic vein, or the short hepatic vein; Vv3, invasion of (or tumor thrombus in) the inferior vena cava. After Ikai *et al.* (2007), with permission from the Japan Society of Hepatology.