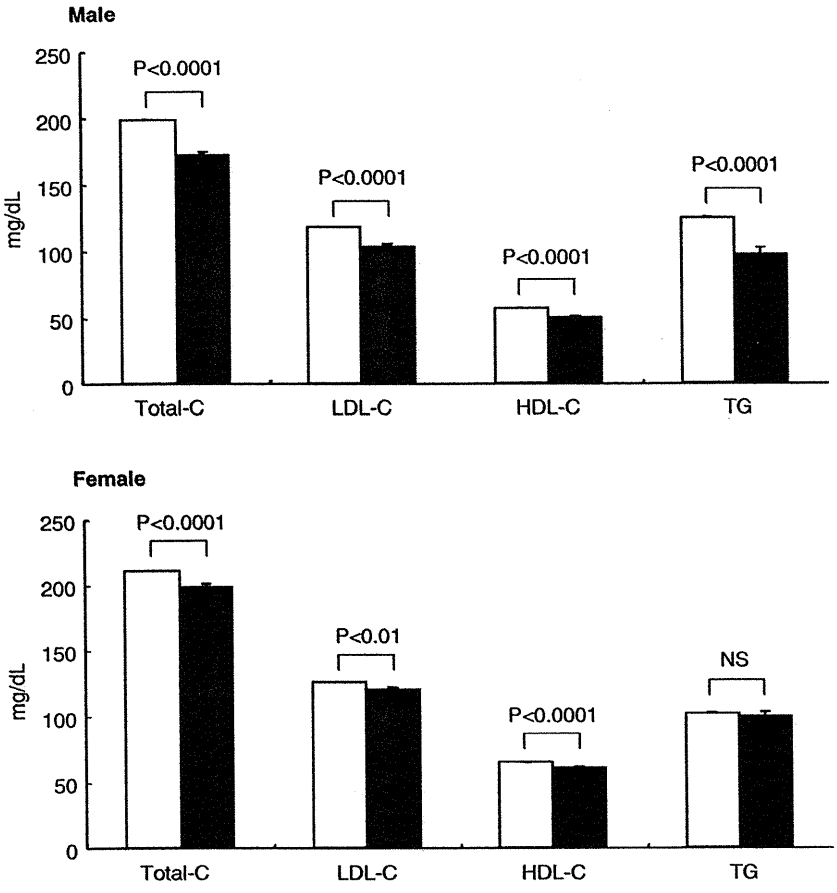


**Figure 1** Comparison of serum total cholesterol (Total-C), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and triglyceride (TG) concentrations between HCV-negative and -positive subjects with normal serum aminotransferase levels (ALT ≤30 and AST ≤30) without obesity (BMI <25). Data are expressed as the mean ± SD. Original data were reported previously by Miyazaki *et al.*[58] (Male: □ HCV-negative ($N$ = 25 323), ■ HCV-positive ($N$ = 118); Female: □ HCV-negative ($N$ = 64 706), ■ HCV-positive ($N$ = 210))

closely associated with SVR.[77–82] In contrast, amino acid substitutions at positions 70 and 91 of the core region have been reported to be associated with non-virological response to therapy.[70,83,84] The overall rate of SVR was only 44% in patients with a low number of substitutions in ISDR (0-1) but was increased 83% in selected subgroups of younger patients (<60 years) with the wild-type sequence at Core amino acid 70, and higher concentrations of LDL cholesterol (≥120 mg/dL).[75]

Recent studies have shown that single nucleotide polymorphisms located in the gene region encoding interleukin 28B (*IL28B*, also called *IFNλ3*) are strongly associated with the response to PEG-IFN plus ribavirin therapy.[85–87] The latest decision tree model, starting with one of the *IL28B* polymorphisms (rs8099917), includes platelet counts, ISDR and serum HCV-RNA levels, but does not include serum LDL cholesterol concentrations.[76] However, the total cholesterol, LDL cholesterol and ApoB concentrations are significantly higher in chronic hepatitis C patients carrying another *IL28B* major (responder) allele (CC in rs12979860) compared with those with minor (non-responder) alleles (CT or TT).[88] Therefore, the association between serum LDL cholesterol concentration and SVR may be reflected in this decision tree model by the underlying link between *IL28B* genotypes and LDL cholesterol concentrations.

It is not clear why a higher probability of SVR is predicted in patients with high serum cholesterol concentrations. As mentioned above, we cannot exclude the possibility that the high cholesterol levels in patients with HCV only reflect having the *IL28B* major (responder) allele. Otherwise, it may just reflect the wild-type sequence at Core amino acid 70 because substitution in the core protein correlated significantly with low concentration of LDL cholesterol.[89] However, in chronic hepatitis C patients, serum LDL cholesterol concentrations correlate negatively with hepatic LDLR mRNA expression[67] and monocyte LDLR protein expression.[90] These results indicate that high serum cholesterol

concentrations are associated with downregulated LDLR, one of the putative receptors mediating HCV cell entry. In addition, adequate amounts of natural LDLR ligands, LDL and VLDL, might inhibit lipoprotein-mediated HCV cell entry via LDLR.[91]

## ABNORMAL CHOLESTEROL METABOLISM IN HEPATOCYTES

STEATOSIS IS FREQUENTLY observed in HCV infection.[92] A comprehensive analysis of HCV core gene transgenic mice has shown that steatosis is mediated in large part by the HCV core protein.[93,94] At least four mechanisms have been suggested regarding the development of steatosis via the HCV core protein: (i) Inhibited tyrosine phosphorylation of insulin receptor substrate (IRS)-1 causes insulin resistance, which increases the peripheral release and hepatic uptake of fatty acids;[95] (ii) The suppression of MTP activity inhibits the secretion of VLDL from the liver;[96] (iii) Upregulated sterol regulatory element-binding protein (SREBP)-1c via the liver X receptor (LXR)-α pathway stimulates fatty acid synthesis in the liver.[97] In addition, the interaction between nuclear proteasome activator PA28γ and HCV core protein plays a critical role in the activation of this LXRα pathway; (iv) Downregulated peroxisome proliferator-activated receptor (PPAR)-α inhibits β-oxidation of fatty acids.[98,99] On the other hand, Huh-7 cells transfected with NS2[100] or 4B (NS4B)[101] from HCV have shown that these nonstructural proteins also upregulate SREBP-1c and appear to contribute to HCV-associated steatosis.

Steatosis is defined as an accumulation of lipid droplets, consisting mainly of triglycerides. The above mechanisms of steatosis may explain triglyceride accumulation in livers infected with HCV. However, the effects of HCV infection on hepatic cholesterol metabolism are poorly understood. Significant amounts of cholesterol are likely to be included within the lipid droplets, but data regarding the quantity of cholesterol in livers infected with HCV are limited. Woodhouse *et al.* reported for the first time that cholesterol concentrations in HCV-infected human hepatoma cell line Huh-7.5 were increased 56% compared with those in non-infected Huh-7.5 cells.[102] To understand the changes in cholesterol metabolism as a consequence of HCV infection, key enzymes in the cholesterol biosynthetic pathway (Fig. 2) including rate-limiting HMG-CoA reductase (HMGCR) have been studied. In HCV core gene transgenic mice, hepatic transcription levels of HMGCR and HMG-CoA synthase (HMGCS) tended to be higher compared with controls, but the difference was not statistically significant.[97] Similar results were reported in JFH1-infected Huh-7 cells, in which transcription levels of HMGCR and squalene synthase appeared to be somewhat elevated; although statistical analysis was not performed because measurements were only made in duplicate.[103]

The above data were obtained from core gene transgenic mice livers and human hepatoma cell lines. However, cholesterol metabolism is subject to marked interspecies variation[104] and is very different between normal livers and hepatomas.[105] Therefore, the mRNA expression data in HCV-infected human liver reported by Nakamuta *et al.* are extremely valuable.[66,67] In their reports, transcription levels of HMGCR, HMGCS, farnesyl-diphosphate synthase and squalene synthase were all upregulated significantly in livers from chronic hepatitis C patients compared with controls. *De novo* cholesterol synthesis in the liver is mainly regulated by SREBP-2, which is synthesized in the endoplasmic reticulum and released as mature a transcription factor to the nucleus by sterol-sensitive proteolysis.[106,107] In Huh-7 cells transfected with NS4B, the protein expression levels of both precursor and mature forms of SREBP-2 were increased.[101] In contrast, hepatic transcription levels of SREBP-2 were not upregulated in core gene transgenic mice.[97] Therefore, NS4B rather than core protein may stimulate cholesterol biosynthesis in these models. In the livers from chronic hepatitis C patients, however, the upregulation of HMGCR is not associated with the transcription level of SREBP-2.[66] It is emphasized again that cholesterol metabolism is sometimes regulated differently among human and rodent livers, and the cultured human hepatoma cell line.

When hepatic transcription levels in chronic hepatitis C patients is compared with controls,[66,67] a striking abnormality in the regulation of cholesterol metabolism is pointed out, namely, upregulation of enzymes involved in the cholesterol biosynthetic pathway and marked downregulation of LDLR. Under physiological conditions, transcription of HMGCR, HMGCS, squalene synthase and LDLR are coordinately regulated by SREBP-2 because the 5′ flanking promoter regions of these four genes contain closely-related sequences designated as sterol regulatory elements.[108,109] In fact, significant positive correlations between HMGCR and LDLR mRNA levels have been observed in livers from chronic hepatitis C patients,[67] as well as in normal human livers.[110,111] The reason for the different transcriptional balance of hepatic HMGCR and LDLR between chronic hepatitis C patients and control subjects

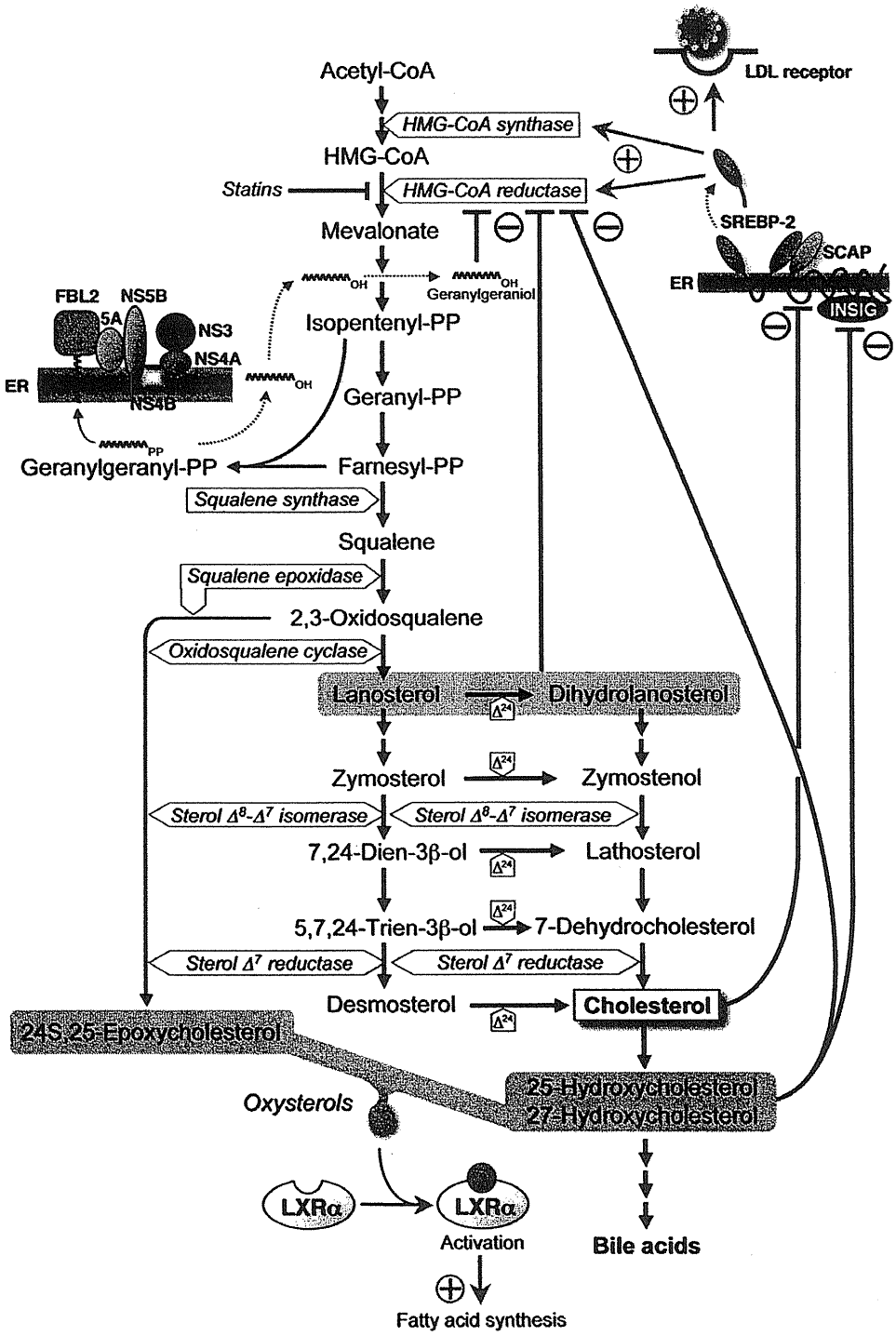

Figure 2 Cholesterol biosynthetic pathway and its feedback regulation. HMG-CoA reductase (HMGCR), HMG-CoA synthase (HMGCS) and the LDL receptor (LDLR) are transcriptionally and coordinately upregulated by a transcription factor, SREBP-2 (as a cleaved mature form). Statins competitively inhibit HMGCR activity, whereas intermediates and derivatives of cholesterol downregulate HMGCR protein by two different mechanisms: (1) Cholesterol and oxysterols (yellow background) interact with SREBP cleavage-activating protein (SCAP) and insulin-induced gene (INSIG), respectively, and suppress the maturation (activation) of SREBP-2; (2) Oxysterols, some intermediate sterols (gray background) and a nonsterol intermediate, geranylgeraniol (geranylgeranyl-OH), accelerate ubiquitination followed by degradation of HMGCR protein. Geranylgeranyl pyrophosphate (geranylgeranyl-PP) is required for geranylgeranylation of host protein FBL2 that binds to NS5A in a reaction that is crucial for HCV RNA replication. Oxysterols are natural ligands of LXRα, which upregulates key enzymes involved in fatty acid biosynthesis as well as SREBP-1c. Abbreviations: ER, endoplasmic reticulum; $\Delta^{24}$, sterol $\Delta^{24}$ reductase.

remains unresolved. However, if hepatic cholesterol concentrations are increased in patients, as reported by the study of HCV-infected Huh-7.5 cells,[102] the abnormality in patients is considered to reside in the regulation of HMGCR rather than LDLR.

Regarding the transcriptional regulation of HMGCR in patients with HCV infection, upregulation was not consistent with the mRNA expression of SREBP-2.[66] The expression of SREBP-2 implies the amount of the precursor (inactive) form of SREBP-2 but not the mature (active) form. The conversion from the precursor to mature form is catalyzed by sterol-sensitive proteolysis and is inhibited by the increased cell cholesterol or oxysterols.[112] In livers infected with HCV, sterol-sensitive proteolysis may be suppressed because of increased tissue sterols. Therefore, HMGCR appears to be upregulated independent of SREBP-2. Actually, regulation of HMGCR by the cAMP/protein kinase A/cAMP-responsive element binding protein (cAMP/PKA/CREB) signaling pathway has been reported,[113] but further investigation is needed to clarify the mechanism.

Moreover, it is not yet clear whether or not HMGCR activity is coordinately upregulated with mRNA expression of HMGCR in patients with HCV infection. Indeed, our preliminary data of serum biomarker sterol concentrations suggest that endogenous cholesterol biosynthesis is not upregulated in chronic hepatitis C patients.[114] There are at least three possible mechanisms that may downregulate HMGCR activity post-transcriptionally in these patients. First, HCV NS4A and NS4B proteins can inhibit protein synthesis in their host cells by translational shutoff.[115,116] Second, under the condition of increased cell sterols, degradation of HMGCR protein is stimulated by sterol-accelerated ubiquitination.[117] Third, previous reports suggest that HCV infection directly induces insulin resistance of the liver.[118–120] Insulin increases HMGCR phosphatase activity and stimulates dephosphorylation of HMGCR protein, resulting in increased HMGCR activity.[121] By contrast, as in the case of insulin resistance, HMGCR activity may decrease due to phosphorylation of the enzyme.

## ANTI-HCV THERAPY BY CHOLESTEROL MODULATORS

HCV MODULATES CHOLESTEROL metabolism in host hepatocytes and ultimately affects cholesterol homeostasis in the whole body. Although it is not clear whether or not the alteration of cholesterol metabolism in chronic hepatitis C patients is essential for HCV replication, the inhibition of cholesterol biosynthesis in host cells may suppress the replication of the virus because cholesterol is an important molecule for the structure and life cycle of HCV. In fact, it has been reported that statins, competitive inhibitors of HMGCR, suppress HCV replication *in vitro*.[122,123] However, the anti-HCV activity of statins has been considered to occur due to their anti-geranylgeranylation effects of cellular proteins rather than their cholesterol lowering activity.[124,125] The inhibition of HMGCR by statins results in reduced intracellular mevalonate concentrations and consequently leads to a reduction of geranylgeranyl pyrophosphate. This compound is required for geranylgeranylation of a host protein called FBL2, which binds to NS5A in a reaction crucial for HCV RNA replication (Fig. 2).[126] The anti-HCV effects of statins are reduced by the addition of mevalonate or geranylgeraniol, but not farnesol, suggesting that HCV RNA replication requires one or more geranylgeranylated proteins.[122,124,125] In addition, GGTI-286, an inhibitor of geranylgeranyltransferase I, exhibited a negative effect on HCV replication *in vitro*.[127] Curiously, however, while pravastatin alone does not inhibit HCV replication, pravastatin inhibits HMGCR activity as effectively as other statins (i.e. atorvastatin, fluvastatin, simvastatin, lovastatin and mevastatin).[122,123] The reason for this has not been elucidated, but the fact does not preclude the possibility that anti-HCV activity would occur as a result

of their pleiotropic effects, but not as a result of the inhibition of HMGCR. In addition, the hydrophobicity of the statin may be related to the anti-HCV efficacy, i.e. pravastatin is water-soluble whereas other statins are lipophilic.

The anti-HCV effects of fluvastatin monotherapy have been reported in patients with chronic hepatitis C, but were modest, variable and often short-lived.[128] However, another report has shown that fluvastatin monotherapy did not exhibit anti-HCV activity in HIV/HCV co-infected subjects.[129] The effects of other statin monotherapies using atorvastatin,[130] simvastatin[131] or rosuvastatin[132] have also been reported, but *in vivo* anti-HCV activity was not proven at conventional doses. The combination of statin with PEG-IFN plus ribavirin therapy has also been studied. Sezaki *et al.* reported that fluvastatin could be used to increase the response to PEG-IFN plus ribavirin, especially in aged women who respond poorly to PEG-IFN plus ribavirin therapy.[133] Another report by Milazzo *et al.* showed that fluvastatin in addition to PEG-IFN plus ribavirin therapy did not increase the SVR but did significantly improve the RVR rate in HIV/HCV co-infected patients.[134] Thus, statin monotherapy is not sufficiently effective for treatment of HCV infection, but the combination with PEG-IFN plus ribavirin may accelerate the elimination of HCV in some cases.

Although it may depend on the dose and nature of the statin, the administration of statins sometimes stimulates fatty acid biosynthesis in host cells,[135–137] which is not preferable for the treatment of HCV infection. Statins competitively inhibit HMGCR activity and induce compensatory expression of HMGCR and SREBP-2. This overexpression of SREBP-2 appears to stimulate enzymes involved in fatty acid biosynthesis, which are basically regulated by SREBP-1c.[137] Moreover, the reduction of intracellular geranylgeranyl pyrophosphate concentrations by statins also appears to be a factor that induces fatty acid biosynthesis because geranylgeranyl pyrophosphate is an antagonist of LXRα.[138,139] On the other hand, statins are known to upregulate LDLR on the cell membrane, so that cell entry of HCV virion may be enhanced. These varied effects of statins may counterbalance the direct antiviral activity of these compounds.

The anti-HCV effects of other inhibitors different from statins have also been studied. Bezafibrate is a synthetic ligand of PPARα and is known to decrease serum VLDL and LDL.[140] Bezafibrate monotherapy of chronic hepatitis C patients significantly reduced serum HCV-RNA titers and alanine aminotransferase levels.[141] This compound did not inhibit HMGCR activity in human liver but was suggested to downregulate other enzymes involved in the synthesis of cholesterol because serum concentrations of lathosterol, a biomarker for cholesterol biosynthesis, were decreased during bezafibrate therapy.[142]

The recent explosion of research into HMGCR and related proteins has provided new insights into the feedback regulation of cholesterol biosynthesis (Fig. 2).[143] Cholesterol and oxysterols simultaneously downregulate HMGCR, HMGCS and LDLR by blocking the activation of SREBP-2 in the endoplasmic reticulum.[144] In contrast, oxysterols, some intermediate sterols and a nonsterol intermediate, geranylgeraniol, can accelerate ubiquitination and the subsequent degradation of HMGCR protein.[145,146] The addition of 25-hydroxycholesterol, one of the oxysterols, to Huh-7 cells bearing HCV replicons can lead to an antiviral state within the host cell.[147–149] 25-Hydroxycholesterol appears to downregulate not only HMGCR and LDLR but also fatty acid biosynthesis.[147,149] Similar effects on *de novo* cholesterol and fatty acid biosynthesis have been observed by an antibiotic cerulenin. This compound mainly inhibits HMGCS and fatty acid synthase,[150] and suppresses HCV replication *in vitro*.[148] U18666A is a unique compound that has multiple actions on cholesterol metabolism including the inhibition of intracellular cholesterol trafficking and the activities of 2,3-oxidosqualene cyclase, sterol $\Delta^8$-$\Delta^7$ isomerase and sterol $\Delta^{24}$ reductase.[151] This compound also has anti-HCV activity on cultured cells,[152] which appears to be due to the blockade of cholesterol availability and the accumulation of intermediate sterols followed by downregulation of HMGCR.

## PERSPECTIVES

A UNIQUE FEATURE of HCV is that the viral life cycle depends on host cholesterol metabolism. Therefore, monitoring and controlling host cholesterol metabolism in chronic hepatitis C patients contributes to the treatment of this viral infection. Serum cholesterol concentration can be a marker of resistance to therapy. Recent technical innovations have made it possible to obtain metabolite profiling of human serum,[153–155] which may lead to the discovery of more sensitive and reliable biomarkers to evaluate host and/or viral conditions. As for drugs, many clinical trials targeting lipid metabolic pathways are being conducted using statins, eicosapentaenoic acid (EPA)[156] or bisphosphonate.[66] EPA suppresses transcription of SREBP-1c[157]

and bisphosphonate inhibits geranylgeranyl pyrophosphate synthase.[158] In addition to U18666A, a number of preclinical compounds that inhibit enzymes in cholesterol biosynthesis have been reported e.g. squalene synthase inhibitors (ER-27856,[159] RPR-107393[159]), squalene epoxidase inhibitor (NB-598[159]), sterol $\Delta^7$ reductase inhibitors (AY-9944,[160] BM15.766,[160] YM-9429[161]) etc. Moreover, downregulation of SR-BI may inhibit cell entry of the HCV virion and may constitute an additional adjuvant therapy. SR-BI expression is directly upregulated by the farnesoid X receptor (FXR; bile acid receptor)[162,163] and an antagonist of FXR has already been discovered.[164]

Finally, cholesterol metabolism regulates fatty acid synthesis through activation of LXRα (Fig. 2). The natural ligands of LXRα are believed to be oxysterols. The activation of LXRα by the interaction with oxysterols causes upregulation of key enzymes involved in fatty acid biosynthesis as well as SREBP-1c. Although serum oxysterol concentrations in chronic hepatitis C patients were not significantly elevated in our preliminary investigations,[114] oxysterol levels between serum and liver may be quite different, as suggested by cholesterol and triglyceride levels in the patients. If the hepatic oxysterol concentrations are high in the patients, lowering the levels by the inhibition of synthesis or by the promotion of metabolism into more polar and inactive compounds (bile acids etc.) may be another way to improve metabolic abnormalities in these patients.

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II. C型肝炎

C型慢性肝炎に対する治療学の進歩
C型肝炎に対するインターフェロン以外の治療法

# 肝庇護療法：ウルソデオキシコール酸，強力ミノファーゲンC

Alternative therapies for hepatitis C: UDCA and SNMC

松﨑靖司　池上　正　齋藤吉史

Key words：肝庇護療法，ウルソデオキシコール酸，強力ミノファーゲンC，発がん抑制，病診連携

## はじめに

C型慢性肝炎の治療目標は肝がんの発現阻止にほかならない．この目標を達成するために幾つかの治療法がある．その中で最初に考慮されるのは，抗ウイルス療法である．しかしその有効率は完全なものとはいえないのが現状である．更に，副作用の問題など，様々な理由で抗ウイルス療法，特にインターフェロン(IFN)療法の恩恵に与れない患者群がいまだ存在する．現段階で最も問題になるのは，線維化が進行しつつあり，近い将来肝がんの超ハイリスク群に移行していくと思われる集団である．したがってこの肝硬変への伸展を抑制し，更には発がんを抑制することは臨床医としては重要な課題である．このように抗ウイルス療法を行えない患者には，肝庇護療法にて肝炎を鎮静化し，肝発がんを抑制する必要がある．

近年，幾つかの肝炎を巡る訴訟が起こっている．肝炎診療をきちんと行わないことへのなかなか厳しい判定がなされる時代となってきた．抗ウイルス療法，特にIFN治療を行い，無効であった場合の予後につき，更に治療方針を明確にICせずに肝がんができた場合など，問題となることもありうる．

最新の情報を把握し，病診連携をきちんととり，日常診療を行うことが医療現場では肝要である．

本稿では，慢性肝炎に対して現在行われている肝庇護療法につき概説する．

## 1. 肝庇護療法の位置づけ

肝庇護療法はHCVを排除しないものの，肝炎を鎮静化し肝細胞の再生を促すことにより，肝線維化進展を抑える治療法である．C型慢性肝炎で肝庇護療法の適応になるのは，肝臓の炎症マーカーであるALTが異常値を示す患者で，抗ウイルス療法にてウイルス排除ができなかった患者，IFN療法の副作用により抗ウイルス療法を実施できない患者，実施できても規定の投与期間を完遂できない患者，また抗ウイルス療法を望まない患者が主な対象者となる．肝庇護療法の歴史は古く，これまで多くの治療法が試みられている．その中でもウルソデオキシコール酸(UDCA)とグリチルリチン製剤の注射薬の先発品である強力ミノファーゲンC(SNMC)は，有用性において科学的な根拠を有して使用されている治療法である．

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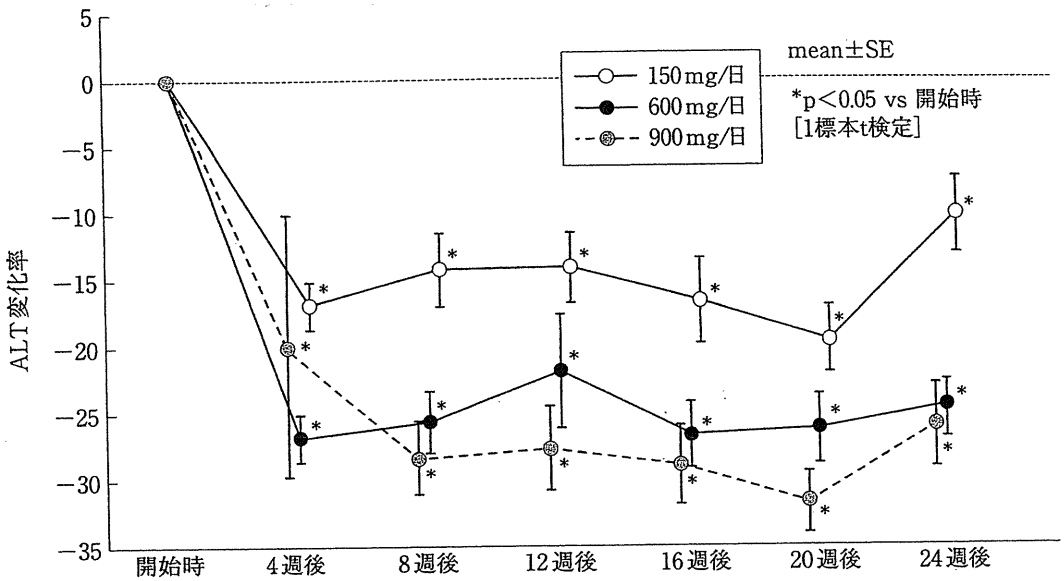


| | 開始時 | 4週後 | 8週後 | 12週後 | 16週後 | 20週後 | 24週後 |
|---|---|---|---|---|---|---|---|
| 150mg/日 (n) | (188) | (186) | (185) | (183) | (179) | (175) | (170) |
| 600mg/日 (n) | (193) | (186) | (190) | (190) | (189) | (182) | (181) |
| 900mg/日 (n) | (185) | (181) | (182) | (179) | (179) | (177) | (168) |

**図1　UDCA(ウルソ®)検証的試験の結果：ALT(GPT)変化率の推移**

## 2. ウルソデオキシコール酸

経口肝庇護療法の第一選択薬としては，UDCA(ウルソ®)が挙げられる．UDCAは胆汁酸製剤であり，古来より動物性生薬として珍重された'熊胆'の成分である．我が国においてウルソ®錠投与が肝疾患に対して'慢性肝疾患における肝機能の改善として，UDCA(ウルソ®)を，通常，成人1回50mgを1日3回経口投与する.'として保険適用となり50余年が既に経過した．慢性肝疾患(慢性肝炎，肝硬変)患者に対してのコントロール試験は，UDCA(ウルソ®)150mg/日を4週間投与したところ，UDCA(ウルソ®)投与群はプラセボ投与群に比しAST(GOT)，ALT(GPT)値が有意に低下したと，1976年に報告されたのが最初である[1]．慢性肝炎に対して著者らはUDCA 300mg/日を投与し，投与前に比べAST，ALTが有意に改善することも見いだした[2]．慢性肝炎に対するUDCAの有効性の成績は，二重盲検法により報告された[3-5]．

以前からUDCA(ウルソ®)は150mg/日の使用が可能であったが，全国規模での二重盲検コントロール試験が国内63施設において実施された．その結果，UDCA(ウルソ®)150mg/日投与群に比べ600mg/日および900mg/日投与群での投与開始4-24週後におけるAST，ALTおよびγ-GTP値の改善が有意の差をもって認められた(**図1**)．有効性と併せて安全性に問題ないことが確認され[6]，2007年3月にUDCA(ウルソ®)はC型慢性肝疾患に対する効能追加の承認に至った．現在，C型慢性肝疾患に対する効果的なUDCA(ウルソ®)投与量は600-900mg/日である．副作用については，胃不快感，下痢，便秘などの消化器症状が時にみられるが，その程度は軽微なものである．

C型慢性肝炎における長期投与の成績は，C型慢性肝炎患者にUDCA(ウルソ®)600mg/日(必要に応じ900mg/日へ増量)を1年以上投与したところ，ALT(GPT)の変化率(中央値)は−43.4％(257例)と有意な低下を認め，効果の持続が確認されている[6]．

作用機序については，著者らはUDCAを投与したときの血清胆汁酸分画の検討より，体内胆汁酸プールの変換の重要性を考えている[7]．UDCAの肝細胞保護作用に関しては，様々な角度より検討されている．しかし，いまだUDCA

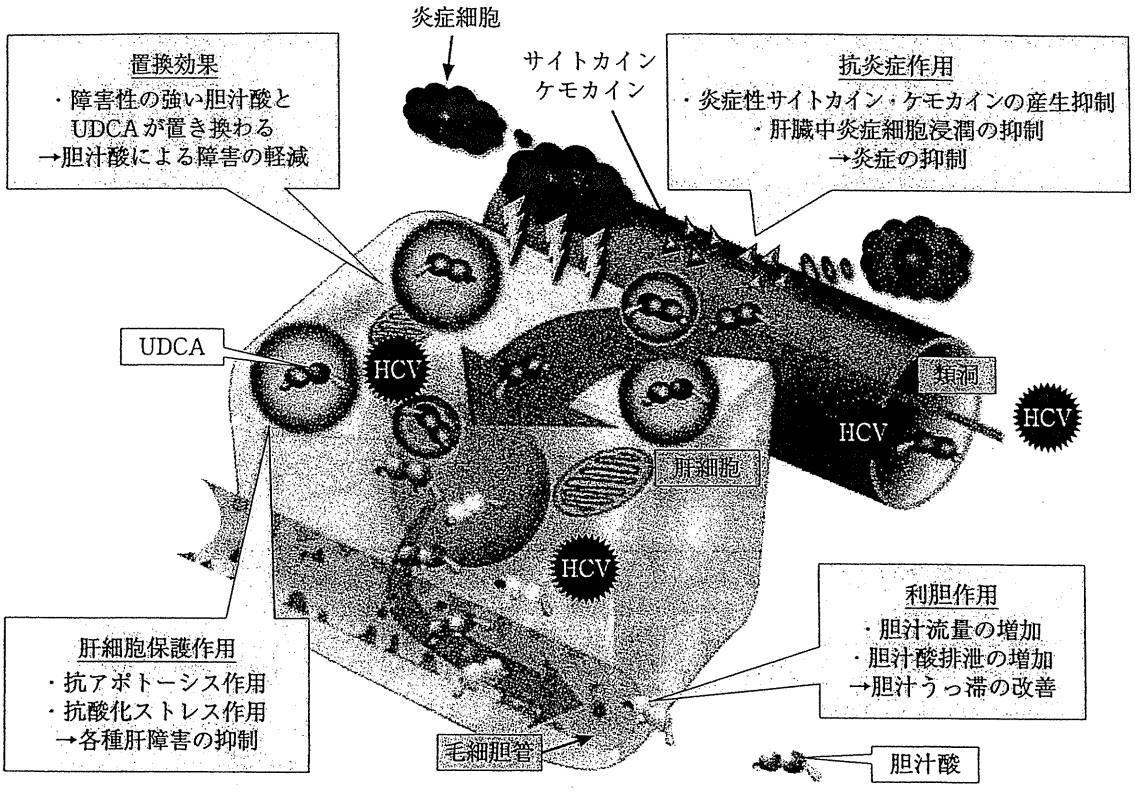


**図2 UDCAの肝機能改善作用**

作用発現機序にはナゾの部分が多く存在しているのも実状である．図2に示すごとく，現在考えられている作用機序を以下にまとめてみる．UDCAの投与により，上記のごとく細胞障害性の胆汁酸がUDCAに置き換わり肝細胞膜が保護されると考えられている．またUDCAには抗酸化ストレス作用，免疫調整作用，抗アポトーシス作用もあり，肝細胞の保護に働いているとも報告されている．これら複合的な機序により，PBCばかりでなく，C型慢性肝炎に対してもUDCAは肝機能の改善効果を発揮するものとされる．

同剤の慢性C型肝炎患者に対する発がん予防効果については，前向き研究はないものの，TaraoらはUDCA投与を受けた群では，受けない群に比べて5年以上の観察期間中での肝細胞がんの発生が有意に低かったとの後ろ向き研究の結果を発表している[8]．UDCA投与群と非投与群でALTの値自体には有意な差がみられなかったとしている点で，ALT改善とは別の発がん予防効果を推測している．つまり，著者らも報告しているように，UDCAの発がん防止作用に抗炎症作用以外の存在を示唆するものである[9]．

## 3. 強力ミノファーゲンC(SNMC)

甘草中の成分であるグリチルリチンが主成分であり，我が国では古くから肝障害や蕁麻疹の治療のため用いられてきた．作用機序の本体はグリチルリチンのもつ弱ステロイド作用とされ，抗炎症効果によりALTの改善をみると考えられている．

SNMCは，国内36施設における慢性肝炎133症例を対象に，1日40mL，1カ月間連日投与の二重盲検比較試験が行われた．その結果，SNMC投与群の有効率は，プラセボ群に比し，明らかな有意の差をもって有効であることが認められた．この結果により，1979年‘慢性肝疾患における肝機能異常の改善’が承認された[10]．

更に1日投与量40mLでは効果の不十分な症例もあることから，国内11施設における慢性肝炎，肝硬変178症例を対象に，1日40mL，3

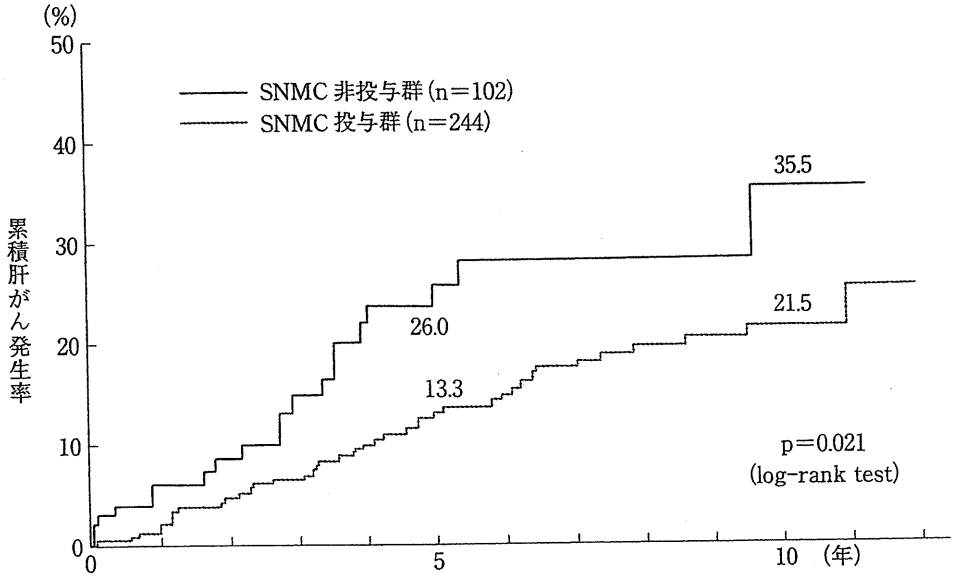


**図3 ALT値高値例におけるSNMC投与有無別にみた累積肝がん発生率**
(文献[13]より引用)

週間連日静注投与を行い，2週目のALT値が正常値上限値の1.5倍以下までに改善しなかった症例93例を対象に，40mL継続投与群と100mL増量投与群との用量別比較試験が行われた．その結果，100mL増量投与群が40mL継続投与群に比し，有意にALT値を改善することが認められた．この結果により1994年，慢性肝疾患の用法・用量に関する承認事項の一部変更が承認され，1日100mLを限度とした大量投与法が可能となった[11]．

C型慢性肝炎に対しての肝炎鎮静効果を無作為コントロール試験にて，SNMC 100mL/日×3/週と，SNMC 100mL/日×3/週にUDCA 600mg/日連日服用群の2群で比較した報告がある．その結果，併用群がALT改善率は有意にSNMC 100mL/日に比し良好であることが報告されている[12]．SNMCとUDCAの併用が炎症の鎮静化に有効であると考えられる．このような肝庇護薬の使用方法もあり，UDCAの投与タイミングを工夫することで，より炎症の鎮静化を図る努力をする必要があろう．

SNMCの肝発がん予防については，やはり我が国における後ろ向き研究であるが，投与群が非投与群に比べ有意に肝発がん率が低下したとする報告がある[13]（**図3**，**表1**）．SNMC投与継続群，非投与群をレトロスペクティブに15年間追跡した検討である．その結果，SNMC継続投与によりほぼ半数に発がん率の低下がみられ，ALT値が正常値の2倍以下に下降した症例では明らかな発がん抑制がみられた．この報告からC型慢性肝炎後の発がん予防には，ウイルスの排除が第一であるが，炎症を抑制してトランスアミナーゼ値を落ち着かせておくことも重要で

**表1 強力ミノファーゲン注は肝発がんのリスクを低下させる**(文献[13]より引用)

| factors | category | risk ratio (95%CI) | p |
|---|---|---|---|
| 線維化レベル | | | |
| | F1 | 1 | |
| | F2-3 | 2.94(1.20-7.21) | 0.018 |
| | F4 | 9.21(3.73-22.8) | <0.001 |
| 性別 | | | |
| | 1: female | 1 | |
| | 2: male | 2.80(1.35-5.81) | 0.006 |
| SNMC注の有無 | | | |
| | 1: no | 1 | |
| | 2: yes | 0.49(0.27-0.86) | 0.014 |

342名の慢性C型肝炎患者のうち，SNMC投与を行った群での肝発がん率は5年で13.3%，10年で21.5%，非投与群では5年で26%，10年で35.5%であった．比例ハザードモデルを用いた解析では，SNMC注射の有無が有意に発がん率を低下させる要因として抽出された．

あるという認識が得られた.

## 4. UDCA, SNMC投与のタイミング：ALT値から

C型慢性肝炎におけるUDCAの使用現況は，肝炎等克服緊急対策研究事業の平成13年度～15年度報告書によると[14]，IFN無効・非適応例に対するUDCA単独治療成績は，ALT値正常化が37％，正常値の1.5倍以下まで改善が30％で，目標値までの改善は計67％とされている．2010年度版のC型肝炎治療ガイドラインでは，進展予防(発がん予防)の治療の項目として，2番目に'IFN非適応例およびIFNでALT値，AFP値の改善が得られない症例は肝庇護剤(SNMC，UDCA)，瀉血療法を単独あるいは組み合わせて治療する.'と明記している．更に3番目に'肝炎進展予防(発がん予防)を目指した治療のALT目標値はstage 1(F1)では，持続的に基準値の1.5倍以下にcontrolする．stage 2-3(F2-F3)では，極力正常値ALT≦30 IU/Lにcontrolする.'と記されている．C型慢性肝炎の患者のALT値を基準値(30 IU/L)以下にすることが推奨されているのである．これは，C型慢性肝炎患者については，ALT値が基準値以下に低下している症例では，そうでない患者に比べ明らかに肝がん発がんのリスクが低いという，幾つかの後ろ向き調査の結果を根拠としている．

よって，基準値30 IU/Lを超え，更にALT値が80 IU/L以下くらいの比較的低値の患者が，UDCA投与でALT値正常化が得られやすいと考えられている．つまりALT値が30 IU/Lを超えたらUDCAは開始した方がよいということになるであろう．SNMCはUDCAにてトランスアミナーゼの改善が図れない場合や，AST，ALT値が100を超えるような場合は使用した方がよいであろう．これらに関しては経験的なものであり科学的根拠はない．

## おわりに

C型慢性肝炎に対する肝庇護療法について，UDCA，SNMC療法の位置づけと具体的な治療法を述べた．'抗ウイルス療法'と'肝庇護療法'は治療の両輪である．2つの治療法のターゲットは，それぞれウイルスの合成阻害と肝の炎症抑制であり，その役割は異なる．

ALT値が低ければ低いほど，肝発がん率は低率であるという臨床データが蓄積されてきた．ALT値を極力低値に改善し，その状態を維持することが必要であろう．つまり，日常診療上，軽度の肝機能数値の異常がみられても，積極的に正常値以下にUDCAやSNMCなどの肝庇護療法を利用し改善させることが，肝がん撲滅の道へつながる．また，健康診断などで肝機能異常(ALT，AST，γ-GTP)を指摘された場合，判断が難しい場合などにおいては，肝臓専門医に一度相談されることを推奨する．これは重要な病診連携である．肝疾患診療におけるネットワークを構築することが重要な課題と考える．

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HEPATOLOGY

# Characteristics and prognosis of patients with hepatocellular carcinoma after the year 2000 in Japan

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**Key words**
after the year 2000, early detection, hepatocellular carcinoma, liver function, prognosis, surveillance.

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## Abstract

**Background and Aim:** The survival rate of patients with hepatocellular carcinoma (HCC) improved through the 1990s in Japan, primarily due to advances in the detection of small HCC under the establishment of surveillance systems. We investigated how the characteristics of patients with HCC changed and whether this trend is continuing after the year 2000.

**Methods:** The characteristics and survival rates of patients with initial HCC (not a recurrence) who were diagnosed after the year 2000 until 2008 were analyzed and compared with those of patients in whom HCC was diagnosed in the 1990s or before.

**Results:** In comparison to 8 years before the year 2000, the percentage of patients with better liver function at diagnosis of HCC increased after the year 2000, whereas the size of maximal HCC tumors did not change in comparison to patients before the year 2000. The survival rate of patients continued increasing after the year 2000.

**Conclusions:** The prognosis of patients with HCC continues to improve after the year 2000. This is not due to further improvements in the detection of small-sized HCC; the detection of small HCC had reached a plateau in the 1990s. Rather, this improvement appears to be due in part from the continued increase in the distribution of patients with better liver function at diagnosis.

## Introduction

Hepatocellular carcinoma (HCC) is among the most common cancers worldwide. It is the sixth most common cancer in the world, and the third most common cause of cancer-related death.[1,2] In Japan, HCC is the third most common cause of death from cancer in men, and the fifth in women.[3] The prognosis of patients with HCC has improved due to improvements in the management of such patients, including the development of novel treatment options or techniques and increased early detection of HCC.

We previously observed the improvement of the survival rate of patients with HCC during the years 1976–2000, particularly in the 1990s.[4] In that observation, we found that the increase in the early detection of HCC associated with the establishment of surveillance systems for HCC was the strongest contributing factor in the improvement of patient survival.[4,5] However, it has not been revealed whether this trend persists after the year 2000 into the 21st century.

In the present study, we investigated how the characteristics of patients with HCC changed and whether the improvement of patient survival continues after the year 2000.

## Methods

### Patients and analyses

The entire protocol was approved by the hospital ethics committee and carried out in compliance with the Declaration of Helsinki. Between 1981 and 2008, a total of 2013 patients were diagnosed as having initial HCC (not a recurrence) at Ogaki Municipal Hospital (Ogaki, Japan). Diagnosis was confirmed by histological findings on the basis of resected hepatic tumors or ultrasonography-guided needle biopsy specimens. In cases in which resection was not indicated and it was necessary to avoid biopsy of the tumor because of the possibility of needle tract seeding of the cancer cells in association with biopsy, especially in patients with advanced tumors, the diagnosis of HCC was based on the imaging findings of selective hepatic angiography and computed tomography (CT). These included hypervascularity on angiographic images and a high-density mass on arterial-phase dynamic CT images, and a low-density mass on portal-phase dynamic CT images. When findings indicative of HCC were not obtained by means of dynamic CT or angiography, CT during hepatic arteriography and CT during arterial portography or T1- and T2-weighted imaging

associated with superparamagnetic iron oxide-enhanced magnetic resonance imaging (MRI) were performed after the 1990s.

Individual decisions regarding treatment were made primarily on the basis of the treatment guidelines for HCC in Japan. Patients were initially assessed for eligibility for hepatic resection. In hepatic resection, the tumor was resected with an ample margin as hepatectomy, and enucleation of the HCC tumor without margin was not performed as surgical treatment. Only patients who had class A liver function by Child–Pugh classification[6] (with some exceptions) and 15-min retention of indocyanin green test of ≤30%, and had no more than three HCC tumors, were considered for surgical treatment. When patients declined or were deemed ineligible for surgical treatment, they underwent non-surgical treatment. Patients were first considered to be offered locoregional ablative therapies (LAT). Patients who had no more than three HCC tumors with a maximal tumor size ≤3 cm were considered for LAT. Before the year 1995, percutaneous ethanol injection (PEIT) was performed for all patients as LAT, because other modalities for LAT were not available. Some patients underwent percutaneous microwave thermocoagulation (PMCT) during 1996–2000. After the year 2000 when radiofrequency ablation (RFA) became available for LAT, all patients underwent RFA with some exceptions. Patients who were ineligible for both surgery and LAT were offered transcatheter arterial chemoembolization (TACE). No patient underwent liver transplantation as a treatment, because it is extremely difficult to find a cadaveric donor for transplantation in Japan due to religious reasons. In addition, living-donor liver transplantation was not performed at our institution during the study period. No patients received molecular-targeted drugs during the study period.

The etiology of underlying liver disease, characteristics and the progression of HCC, liver function at the time of HCC diagnosis, and patient survival rates were analyzed on the basis of clinical records. The Child–Pugh classification was determined as an indicator of liver function. Tumor staging was performed according to the American Joint Committee on Cancer (AJCC) classification system.[7] In cases in which pathological evaluation was not available, vascular invasion was assessed by means of dynamic CT and angiography. The initial treatment for HCC was also investigated. Patients were stratified into seven periods by year of HCC diagnosis: 1981–1984, 1985–1988, 1989–1992, 1993–1996, 1997–2000, 2001–2004, and 2005–2008.

All patients were followed up from 0.1 months to 241.1 months (median follow-up period: 19.1 months) at our institution after diagnosis and treatment. Patients were followed up with ultrasonography, and CT or MRI was performed every 3–6 months. In addition, regular monitoring of serum tumor markers (α-fetoprotein [AFP] and des-gamma-carboxy prothrombin [DCP]) was performed every 3 months. When the elevation of tumor markers was observed, additional imaging examinations (usually by CT or MRI) were performed to check the presence of HCC. When the recurrence of HCC was confirmed, patients underwent treatment for recurrent HCC, as well as the treatment for initial HCC.

### Statistical analysis

Values were expressed as mean ± standard deviation, unless otherwise indicated. Differences in percentages between groups were analyzed by the $\chi^2$-test. Differences in mean quantitative values were analyzed by Mann–Whitney *U*-test. The date of HCC diagnosis was defined as time zero in the calculation of patient survival rates. Surviving patients and patients who died from a cause other than liver disease were censored. Patients who died from an HCC-related cause or liver failure were not censored. The Kaplan–Meier method[8] was used to calculate survival rates, and the log–rank test[9] was used to analyze differences in survival.

The Cox proportional hazards model[10] was used for the multivariate analysis of factors related to survival. The variables analyzed were the period of the diagnosis of HCC (1981–2008), patient age and sex, Child–Pugh class, tumor stage by AJCC, and initial treatment. Data analyses were performed with the JMP statistical software package (version 6.0, Macintosh version; SAS Institute, Cary, NC, USA). All *P*-values were derived from two-tailed tests, and $P < 0.05$ was accepted as statistically significant.

## Results

### Patient characteristics and HCC

The demographic characteristics of the 2013 patients included in this study are summarized in Table 1. The study patients included 1495 men and 518 women, with a mean age of 65.0 ± 9.6 (range: 21–93) years. Liver function at diagnosis of HCC was Child–Pugh class A in 1137 (56.5%) patients. HCC was stage I in 797 (39.6%) patients and stage II in 574 (28.5%) patients, according to the TNM stage classification of the AJCC.

With the exception of 356 (17.7%) patients who had not received treatment, all patients underwent treatment for HCC within 2 weeks after the diagnosis of HCC. Treatment included hepatectomy in 459 (22.8%) patients and LAT in 392 (19.5%) patients. Among patients receiving LAT, 190 patients were treated by PEIT and 189 patients were treated by RFA. HCC was treated by TACE in 618 (30.7%) patients. The diagnosis of HCC in 459 patients who underwent hepatectomy was based on a histological examination of tumor tissue taken from resected specimens. In patients treated by LAT, the diagnosis of HCC was made based on fine-needle biopsy of specimens from 162 of the 392 patients (41.3%). In the remaining 230 patients treated by LAT, the diagnosis was made based on the imaging findings. HCC was diagnosed by the imaging findings in all 618 patients who underwent TACE. A histological diagnosis was made in 21 of the 188 patients (11.2%) who underwent treatment other than surgery, LAT, or TACE, and 20 of the 356 patients (5.6%) who did not undergo treatment. In total, HCC was diagnosed histologically in 662 (32.9%) patients.

### Characteristics and treatment for HCC by period

We analyzed the trends in the characteristics of patients with HCC by period. The numbers of patients who were diagnosed as having initial HCC (not a recurrence) were 141 patients during the period 1981–1984, 220 during 1985–1988, 292 during 1989–1992, 305 during 1993–1996, 334 during 1997–2000, 366 during 2001–2004, and 355 during 2005–2008. This number increased during the 1980s and 1990s and peaked during 2001–2004. Patient age at the diagnosis was increasing throughout the study period. The

**Table 1** Clinical characteristics of study patients ($n = 2013$)

| | |
|---|---|
| Age (mean ± SD, years) (range) | 65.0 ± 9.6 (21–93) |
| Sex ratio (female/male) | 518 (25.7%)/1495 (74.3%) |
| Etiology of underlying liver disease (HBV/HCV/HBV, HCV/non-HBV, non-HCV/non-HBV) | 368 (18.3%)/1175 (58.4%)/23 (1.1%)/223 (11.1%)/224 (11.1%) |
| Child–Pugh class (A/B/C)† | 1137 (56.5%)/650 (32.3%)/226 (11.2%) |
| Albumin (mean ± SD, g/dL) | 3.50 ± 0.56 |
| Total bilirubin (mean ± SD, mg/dL) | 1.19 ± 1.28 |
| Diagnostic modality (histology/other) | 662 (32.9%)/1351 (67.1%) |
| AJCC tumor stage (I/II/III/IV) | 797 (39.6%)/574 (28.5%)/554 (27.5%)/88 (4.4%) |
| Tumor size (mean ± SD, cm) (range) | 5.70 ± 3.37 (0.5–29.4) |
| Tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm) | 572 (28.4%)/677 (33.6%)/764 (38.0%) |
| Tumor number (single/multiple) | 870 (43.2%)/1143 (56.8%) |
| Vascular invasion (absent/present) | 1398 (69.4%)/615 (30.6%) |
| Initial treatment | |
| No treatment | 356 (17.7%) |
| Hepatectomy | 459 (22.8%) |
| LAT | 392 (19.5%) |
| TACE | 618 (30.7%) |
| Other | 188 (9.3%) |

†Category of Child–Pugh class A includes patients without cirrhosis. Other treatment included repeated arterial infusion chemotherapy ($n = 93$), one-shot arterial infusion of anticancer drug ($n = 61$), systemic chemotherapy ($n = 26$), and radiation ($n = 8$). AJCC, American Joint Committee on Cancer; HBV, hepatitis B virus; HCV, hepatitis C virus; LAT, locoregional ablative therapy, including percutaneous ethanol injection, percutaneous microwave thermocoagulation, and radiofrequency ablation; non-HBV, hepatitis B virus was negative (hepatitis C virus was not tested before 1990); non-HBV, non-HCV, both hepatitis B virus and hepatitis C virus were negative; SD, standard deviation; TACE, transcatheter arterial chemoembolization.

**Table 2** Clinical characteristics of study patients between periods 1992–2000 and 2001–2008

| | Periods 1992–1996 and 1997–2000 ($n = 639$) | Periods 2001–2004 and 2005–2008 ($n = 721$) |
|---|---|---|
| Age (mean ± SD, years) (range)[1] | 64.7 ± 8.8 (36–93) | 68.2 ± 9.3 (21–91) |
| Sex ratio (female/male) | 172 (26.9%)/467 (73.1%) | 203 (28.2%)/518 (71.8%) |
| Etiology of underlying liver disease (HBV/HCV/HBV, HCV/non-HBV, non-HCV) | 94 (14.7%)/463 (72.5%)/12 (1.9%)/70 (10.9%) | 114 (15.8%)/503 (69.8%)/9 (1.2%)/95 (13.2%) |
| Child–Pugh class (A/B/C)[2] | 380 (59.5%)/197 (30.8%)/62 (9.7%) | 497 (68.9%)/169 (23.5%)/55 (7.6%) |
| Albumin (mean ± SD, g/dL)[3] | 3.31 ± 0.62 | 3.59 ± 1.09 |
| Total bilirubin (mean ± SD, mg/dL) | 1.33 ± 1.76 | 1.20 ± 1.37 |
| AJCC tumor stage (I/II/III/IV)[4] | 266 (41.6%)/190 (29.7%)/157 (24.6%)/26 (4.1%) | 369 (51.2%)/199 (27.6%)/124 (17.2%)/29 (4.0%) |
| Tumor size (mean ± SD, cm) (range) | 4.28 ± 3.39 (0.5–19.0) | 4.07 ± 3.25 (0.5–19.2) |
| Tumor size (≤2 cm/>2 cm and ≤5 cm/>5 cm) | 221 (34.6%)/221 (34.6%)/197 (30.8%) | 237 (32.9%)/300 (41.6%)/184 (25.5%) |
| Tumor number (single/multiple)[5] | 282 (44.1%)/357 (55.9%) | 392 (54.4%)/329 (45.6%) |
| Vascular invasion (absent/present)[6] | 487 (76.2%)/152 (23.8%) | 599 (83.1%)/122 (16.9%) |
| AFP (median, ng/mL) (range)[7] | 38.0 (0.0–595 000) | 24.7 (0.8–2 402 000) |
| DPC (median, mAU/mL) (range)[8] | 62.0 (10.0–8 000) | 38.2 (10.0–75 000) |
| Antiviral therapy for HBV infection[9] | 8 (7.5%) | 72 (58.5%) |
| Antiviral therapy for HCV infection[10] | 36 (7.6%) | 73 (14.3%) |
| Eradication of HCV by antiviral therapy | 8 (1.7%) | 17 (3.3%) |

[1]$P < 0.0001$; [2]$P = 0.0013$; [3]$P < 0.0001$; [4]$P = 0.0011$; [5]$P = 0.0002$; [6]$P = 0.0020$; [7]$P = 0.0003$; [8]$P = 0.0027$; [9]$P < 0.0001$; [10]$P = 0.0012$. †Category of Child-Pugh class A includes patients without cirrhosis. AFP, α-fetoprotein; AJCC, American Joint Committee on Cancer; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; non-HBV, non-HCV, both hepatitis B virus and hepatitis C virus were negative; SD, standard deviation.

mean age was 60.6 ± 9.1 during the period 1981–1984, 61.4 ± 10.0 during the period 1985–1988, 62.3 ± 9.2 during the period 1989–1992, 63.8 ± 8.5 during the period 1993–1996, 65.5 ± 9.0 during the period 1997–2000, 68.0 ± 9.1 during the period 2001–2004, and 68.5 ± 9.5 during the period 2005–2008. The prevalence of patients with Child–Pugh class A liver function at diagnosis and the prevalence of patients with AJCC tumor stage I continued increasing after the period 1985–1988. In contrast, the prevalence of patients with maximal tumor size < 2 cm markedly increased between the period 1985–1988 and the period 1989–