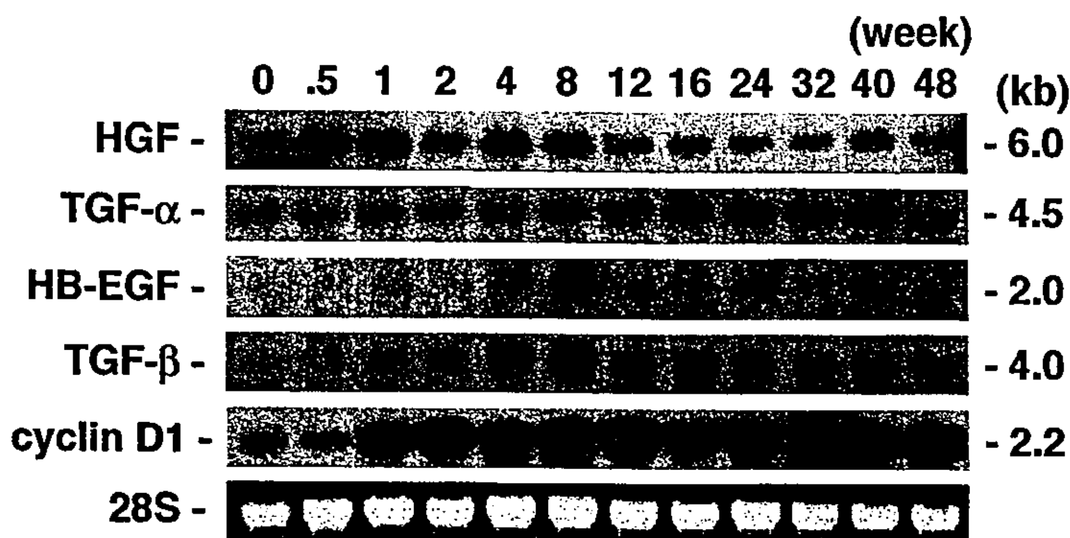

Fig. 4. Enhanced expression of growth factors and cyclin D1 in the liver tissues of rats fed a CDAA diet. Northern blot analysis demonstrated the expression of growth factors and cyclin D1 in the liver of rats fed a CDAA diet at the indicated time-points. Total RNA (10 μg) was analyzed as described in Section 2 for HGF, TGF-α, HB-EGF, TGF-β1, and cyclin D1. HGF expression was rapidly stimulated after CDAA diet feeding (from day 3 to 8 weeks), whereas stimulation of TGF-α expression was observed mainly in the late phase. Expression of HB-EGF increased slightly from 4 to 8 weeks. TGF-β1 expression was stimulated highly from 4 to 8 weeks, followed by a gradual decrease until 48 weeks. Enhanced expression of cyclin D1 was observed from 1 to 48 weeks. The expression pattern of each transcript was confirmed by several experiments using different individual animals at selected time points.

tistical significance 4 weeks after the start of the CDAA diet ($P < 0.001$) (Fig. 5B). Furthermore, the PCNA-positivity was much higher in GST-P-positive nodules than in the surrounding GST-P-negative areas at 8 weeks ($P < 0.001$), and an increase in PCNA-positive hepatocytes in both areas continued throughout the experimental period with a peak at 24 weeks (Fig. 5B).

Since a choline-deficient diet has been demonstrated to induce p53-independent programmed cell death [26], we immunohistochemically examined apoptosis in hepatocytes using an anti-single-stranded DNA antibody (Fig. 6). Although the number of apoptotic hepatocytes was much lower than the number of PCNA-positive cells (Fig. 5B), the number of apoptotic hepatocytes increased slightly 3 days after the start of the CDAA diet. This was followed by a marked increase at 4 weeks. After a peak at 8 weeks, the number of apoptotic cells rapidly decreased until 16 weeks ($P < 0.001$), and only a few were detected at 48 weeks.

4. Discussion

Early changes in rats fed a CDAA diet are due to oxidative injury to DNA and other subcellular components and result in the elevation of serum ALT and liver triglyceride [27,28]. The most abundant oxidative DNA damage, caused by 8-hydroxydeoxyguanosine (8-OHdG), has previously been detected after 1 day of feeding the CDAA diet. In this study, the level of serum ALT was markedly elevated beginning 1–4 weeks after CDAA diet administration, meaning that CDAA diet-induced hepatic injury mainly occurred in the early phase of this treatment. The number of platelets was significantly increased by day 3, while an increase in serum ALT was not observed at this point. We are not able to fully explain this phenomenon. Cytokines, such as interleukin (IL)-6, which is also known to play an important role in liver regeneration, may cause the transient increase in platelet levels [29]. Although platelets are known to release reactive oxygen species (ROS) [30], Hensley et al. have reported that administration of a CDAA diet caused complex 1 dysfunction and increased H_2O_2 generation in liver mitochondria [31].

The relative liver weight increased from 2 to 4 weeks of CDAA diet feeding, then decreased until 12 weeks, and consequently became similar sized to livers of untreated rats. These findings may be explained by the histopathological changes observed in this study as well as the data reported in previous investigations [24,25]. In the livers of rats fed a CDAA diet, fat deposition quickly expands, leading to a diffuse fatty liver within a week. In addition, an increase in hepatocyte death was detected from 4 weeks. In contrast, the relative weight of the spleen gradually increased 8 weeks after the start of CDAA diet administration in parallel with a decrease in platelet levels, and enlargement of the spleen continued throughout the experimental period. These symptoms progress in conjunction with cirrhosis development.

Following CDAA diet-induced hepatocyte injury, the expression of growth and growth-inhibitory factors was stimulated (Fig. 3). HGF expression was upregulated in the early phase of CDAA diet feeding, followed by enhanced expression of TGF-α in the late phase. These results indicate that hepatocytes are persistently exposed to growth stim-

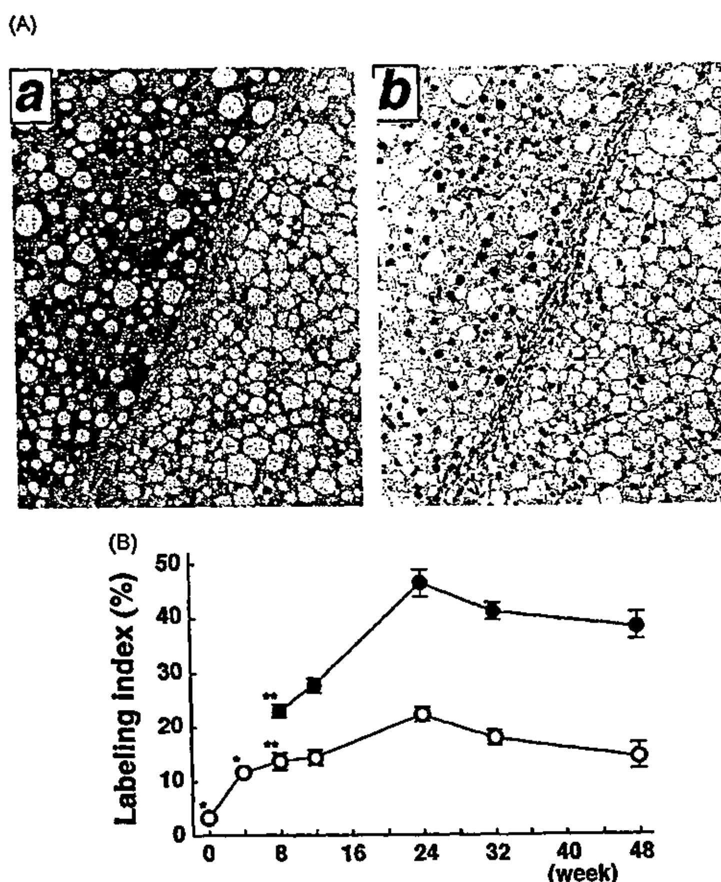

Fig. 5. Administration of a CDAA diet induced accelerated proliferation of hepatocytes throughout the experimental period. Proliferation of hepatocytes in the GST-P-positive nodules and the surrounding GST-P-negative areas was evaluated immunohistochemically using anti-PCNA antibodies as described in Section 2. (A) The same sections of liver tissue were stained with anti-GST-P or anti-PCNA antibody (a or b, respectively) [original magnification 100×]. (B) The number of PCNA-positive cells per 3×10^3 hepatocytes in both GST-P-negative areas (open circle) and GST-P-positive nodules (closed circle) was indicated as a PCNA-labeling index ($n = 3$). The PCNA-positivity reached a peak at 24 weeks, and was much higher in GST-P-positive nodules than in the surrounding GST-P-negative areas ($^*P < 0.001$, $^{**}P < 0.001$).

uli during exposure to a CDAA diet. Conversely, expression of TGF-β1, a growth-inhibitory factor for hepatocytes [15–17], was also stimulated during this treatment. TGF-β1 has been demonstrated to inhibit growth factor-induced cyclin D1 expression and DNA synthesis in primary cultured rat hepatocytes [10]. TGF-β1 also induces hepatocyte apoptosis both in vitro and in vivo [32,33]. Recent investigations have reported that the sensitivity of hepatocytes to TGF-β-mediated growth inhibition is modulated by the expression levels of TGF-β receptors during liver regeneration [34,35]. Furthermore, hepatocytes that adapted to survive in low choline were resistant to TGF-β1 [6,26]. Additionally, other cytokines, such as IL-1α and IL-1β also play a role in terminating DNA synthesis in hepatocytes induced by partial hepatectomy [36]. In this study, despite stimulation of TGF-β1 expression, enhanced expression of cyclin D1 and an increase in PCNA-positive hepatocytes were observed during CDAA diet administration, and the number of apoptotic hepatocytes markedly decreased following a peak at 8 weeks. Although we did not examine expression of TGF-β receptors and other cytokines in the rat livers, continuous stimulation of growth and growth inhibitory factors induced by prolonged exposure to a CDAA diet may affect TGF-β receptor expression and its signaling pathway directly or indirectly, leading to modulation of susceptibility of hepatocytes to TGF-β1.

Recent reports have demonstrated that the cells existing in CDAA diet-induced preneoplastic nodules escaped from TGF-β-mediated growth inhibition and have a selective growth advantage. This indicates the possibility that the TGF-β1 signaling pathway is disturbed in preneoplastic nodules induced by a CDAA diet [37,38]. TGF-β1 is produced as a small, biologically inactive, latent complex, which consists of mature TGF-β1 and latency-associated peptide (LAP). The latent TGF-β binding protein (LTBP) facilitates fixation of latent TGF-β1 in the extracellular matrix, resulting in a reservoir of latent TGF-β1 [39]. In contrast to diet-induced hepatocarcinogenesis, expression of TGF-β1 mRNA and latent TGF-β1 protein was observed in non-parenchymal cells surrounding or within the preneoplastic nodules or HCCs in a rat model of chemical hepatocarcinogenesis, however, mature TGF-β1 protein was detected only

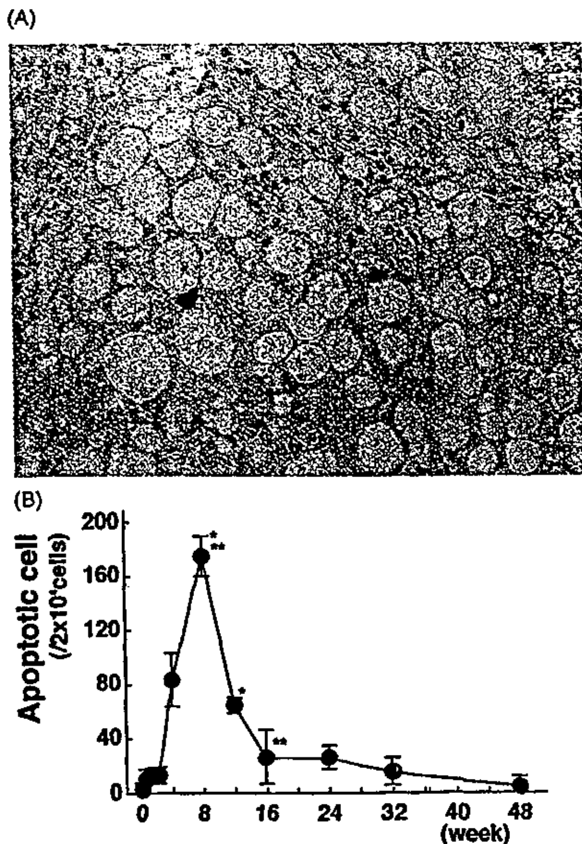

Fig. 6. Hepatocyte apoptosis was decreased following a peak in the early weeks of CDAA diet feeding. (A) Apoptosis of hepatocytes was examined by immunohistochemistry using an anti-single-stranded DNA antibody [23] as described in Section 2 [original magnification 400×]. (B) The number of apoptotic cells per 2×10^4 hepatocytes was counted ($n = 3$). The number of apoptotic hepatocytes rapidly decreased after a peak at 8 weeks of CDAA diet administration, and only a few apoptotic cells were detected at 48 weeks ($^*P = 0.004$, $^{**}P < 0.001$).

in nonparenchymal cells and connective tissues associated with HCCs, but was not observed in preneoplastic nodules [40]. In this study, the number of PCNA-positive hepatocytes continued to increase throughout the experimental period, and the PCNA-positivity in GST-P-positive nodules was much higher than that in GST-P-negative areas. Although the difference between GST-P-positive and GST-P-negative cells, both of which are positive for PCNA, is not fully understood and, also, details of the activation process of latent TGF-β in liver have not been elucidated, the regulatory effect of TGF-β1 on growth of GST-P-positive cells may contribute to the development of HCCs.

Following CDAA diet-induced hepatocyte injury in the early phase, both growth and growth-inhibitory stimuli were induced, and accelerated proliferation and decelerated apoptosis of hepatocytes was also observed. These results allow us to hypothesize that altered hepatic microenvironments in the early phase of a CDAA diet administration may contribute to development of preneoplastic nodules. In this context, we screened genes differentially expressed in rat livers treated with a CDAA diet for 8 weeks, and isolated osteoactivin (OA) cDNA [41]. Expression of OA was stimulated in rat livers during CDAA diet-induced cirrhosis development and in human HCCs. Further experimentation is underway to clarify the role of OA in the lengthy process of cirrhosis-associated hepatocarcinogenesis.

In this study, we have demonstrated that expression of growth and growth-inhibitory factors was stimulated following CDAA diet-induced liver injury, and that hepatocyte proliferation was accelerated during CDAA diet feeding, while hepatocyte apoptosis was decelerated. Although further investigations are necessary to understand the impaired mechanisms of liver regeneration following repeated hepatocyte injury induced by a CDAA diet, the results presented here indicate the possibility that imbalance between proliferation and apoptosis of hepatocytes, which are exposed to growth and growth-inhibitory stimuli over lengthy periods, is involved in CDAA diet-induced hepatocarcinogenesis.

Acknowledgements

We thank Ms. Akemi Chuman and Ms. Himiko Fujiwara for technical assistance. This work was supported in part by grants-in-aid from Ministry of Science, Education, Sports and Culture (14570484) and the Ministry of Health, Labor and Welfare of Japan.

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［原著］

学生定期健康診断におけるエコー健診導入の意義
－肥満学生での検討－

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CAMPUS HEALTH, 41 (2), 111－116, 2004

要旨：今回，我々は宮崎大学の定期健康診断を受診した3471名（男性2263名，女性1208名）の内BMI27以上の学生（164名）に対し，腹部エコー検査（UST）が有用であるか検討した。同意が得られた130名（男性92名，女性38名）を対象に，上腕並びに背部皮下脂肪厚，血液生化学検査（AST, ALT, r-GTP, UA, T-Cho, TG, HDL-C），USTを施行した。130名の内，アルコール常習者は認めず，脂肪肝は98名75%にみられた。BMI30以上では約90%に脂肪肝を認め，BMI 27-30でも約55%に脂肪肝を認めた。特に女性（60.5%）に対し男性（81.5%）は有意に脂肪肝が高率に認められた。BMI 27-30の内，生活習慣病（高脂血症，高尿酸血症，肝障害）合併例は85名中52名（61%），BMI30以上では45名中40名（89%）であった。BMI 27-30の内UST正常群での生活習慣病合併例は38名中16名（42%）（M15／24，F1／14）に対し，脂肪肝群では47名中36名（76%）（M32／38，F4／9）でいずれの群でも男性が有意に高かった。BMI30以上で高度脂肪肝群の生活習慣病合併例は34名中34名100%であった。若年時においても肥満は生活習慣病発症に関与していた。軽度脂肪肝群で肝障害（NAFLD）を有していたのは31名中9名30%であったのに対し，高度脂肪肝群では56名中39名69.6%であった。ロジステイック解析による高度脂肪肝に寄与する因子はBMIが最も強く続いて性別であった。今回のUSTにより，BMI27-30では，脂肪肝例，正常例があることを認めた。その中で，特に高度脂肪肝群では高率に生活習慣病，NAFLDを認めた。以上の結果より，学生定期健康診断にUSTを導入し，脂肪肝の有無を判定することに意義があると考えられた。

キーワード：学校健診，肥満，生活習慣病，脂肪肝，腹部エコー検査

はじめに

食生活の変遷とともに大学生の肥満は年々増加している。肥満は，糖尿病，高脂血症，高血圧など，40代から増加する生活習慣病の誘因であり，肥満を予防し改善することは，生活習慣病を予防する上で極めて重要なことである。特に，大学生などの若年からの肥満は，将来の生活習慣病を惹起する可能性が極めて高く，改善すべき重要な課題と考えられる。また，最近肥満に起因する脂肪肝のみならず，高度の肝機能障害をきたすnon alcoholic fatty liver disease（NAFLD）ならびに肝硬変および肝細胞癌に進展する可能性の示唆されているnon alcoholic steatohepatitis（NASH）が注目されている[1]。今回，

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我々は宮崎大学学生定期健康診断にてBMI27以上の学生に対し，エコー検診と血液生化学検査を施行し，BMIならびに脂肪肝と生活習慣病ならびにNAFLDの因果関係について検討すると同時に，学生健康診断に腹部エコー検査を導入する意義について検討した。

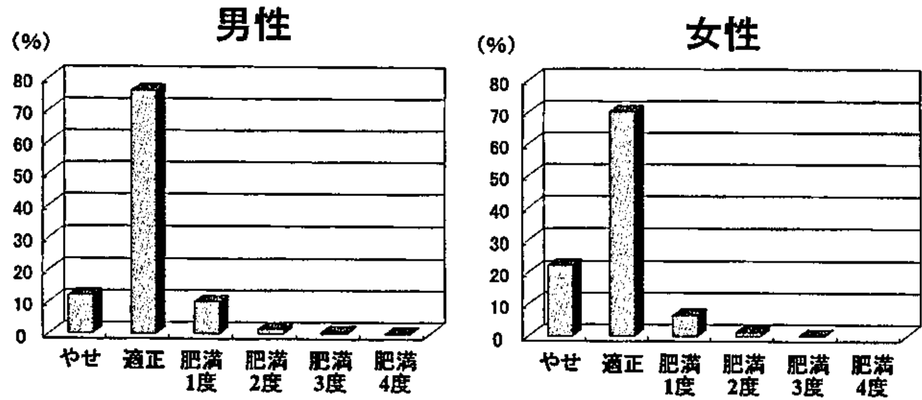

図１．肥満度分布

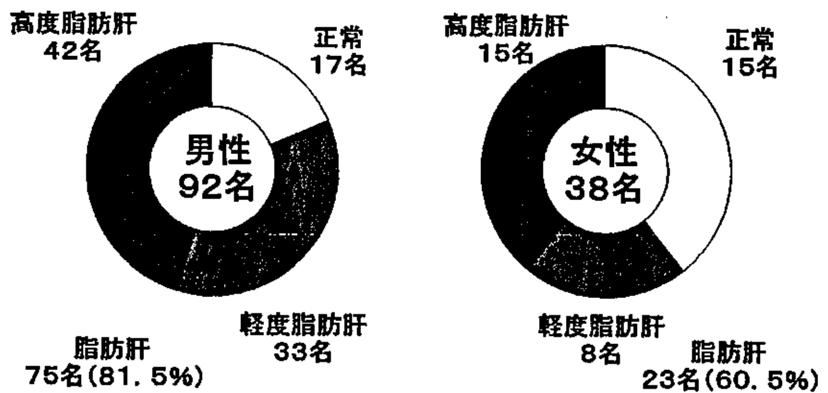

図２．BMI27以上の学生の腹部エコーの結果（男女別）

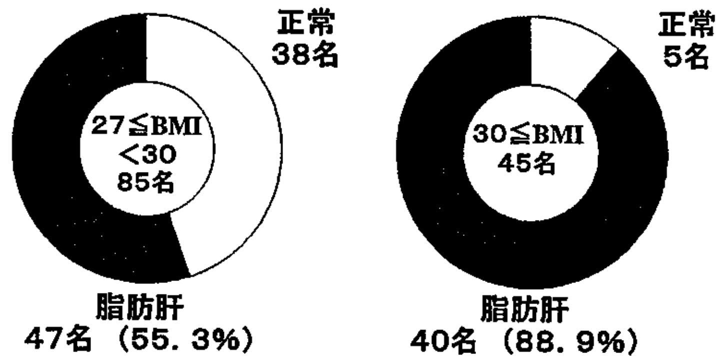

図３．BMI27以上の学生の腹部エコーの結果
(BMI27以上30未満学生とBMI30以上学生の比較)

対象と方法

平成14年度，宮崎大学学生定期健康診断を受診した3471名（男性2263名，女性1208名）のうち，BMI27以上の学生164名（平均年齢20歳）を対象とした。同意が得られた130名に対し，腹部エコー検査，上腕部皮下脂肪厚，背部皮下脂肪厚，血液生化学検査（AST，ALT，r-GTP，UA，T-Cho，TG，HDL-Cho）を施行した。腹部エコー検査は，SonoSite180plus（オリンパス）を用い，脂肪肝の判定は，エコー減衰率，エコー輝度，肝脈管像ならびに肝腎コントラスト比を用いて行い，明らかな肝腎コントラストを認めるものを軽度脂肪肝，明らかな肝腎コントラストに加え，肝深部エコー減衰が高度で肝静脈描出不良のものを高度脂肪肝，いずれも認めないものを正常と判定し３群に分類した[2]。BMIは体重（Kg）／身長（m）^{2}にて計算した。統計処理はStatView version 5.0で，Mann-Whitney U検定にて有意差検定とロジステイック解析を行った。

結　果

日本肥満学会の肥満度分類による当大学の肥満度分布は，やせ（BMI<18.5；男性12%，女性22.1%）（以下同順），適正（18.5≦BMI<25；76%，70%）肥満１度（25≦BMI<30；10%，6.4%）肥満２度（30≦BMI<35；1.4%，1.2%）肥満3度（35≦BMI<40；0.5%，0.1%）肥満４度（40≦BMI；0.1%，0）であった（図１）。このうち，BMI27以上の学生は164名存在し，検査同意が得られた130名（92名，38名）に対し，各種２次検査を施行した。また，アルコール常飲者ならびにB型肝炎およびC型肝炎患者は認められなかった。

腹部エコー検査にて，脂肪肝は130名中98名75.3%にみられた（図２）。男性では軽度脂肪肝

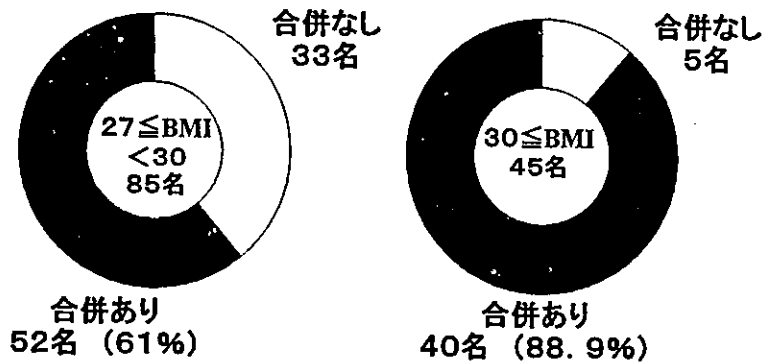

図4．BMI27以上の学生の生活習慣病の合併率

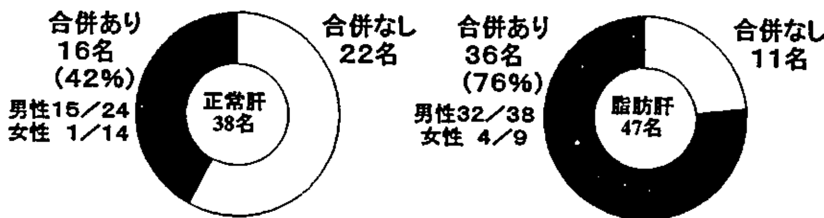

図5．脂肪肝と生活習慣病合併の関連
（BMI27以上30未満学生での検討）

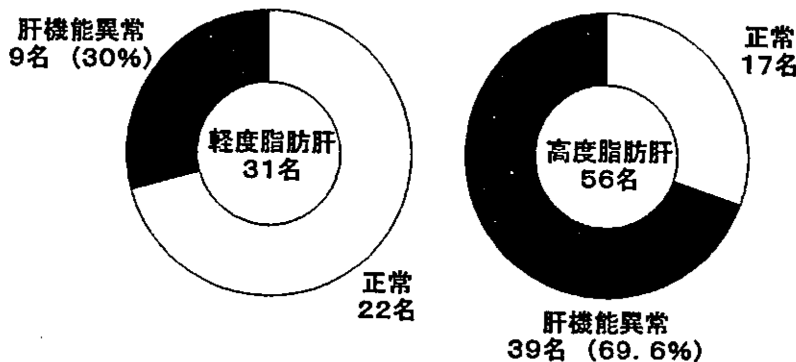

図6．脂肪肝群における肝障害合併率

が33名，高度脂肪肝が42名存在し，92名中75名（81.5%）と高率に脂肪肝が認められた。女性は軽度脂肪肝が8名，高度脂肪肝が15名に見られ，全体では，38名中23名（60.5%）と男性が有意に脂肪肝の併発を伴っていることが分かった。

ここで，BMI27以上30未満とBMI30以上の2群に分けて検討を加えた。BMI27以上30未満の学生の内，脂肪肝がみられたのは85名中47名55.3%であったのに対し，BMI30以上では45名中40名88.9%と有意に高率であった（図3）。また，BMI27以上30未満の学生の内，なんらかの生活習慣病〔高脂血症（T-CHO>220，TG>150），高尿酸血症（UA>7.0），肝機能障害（ALT>35，AST>40）〕を併発していた学生は85名中52名61%であったのに対しBMI30以上の学生は45名中40名89%であった（図4）。

BMI30以上で高度脂肪肝の生活習慣病併発例は34名中34名100%であったため，BMI27以上30未満の学生に対象を絞り，脂肪肝の有無で生活習慣病の合併率に有意差があるか否か検討した。BMI27以上30未満の学生でエコー正常群の生活習慣病併発例は38名中16名42%に対し，脂肪肝群では47名中36名76%と有意に脂肪肝群が高率に生活習慣病を併発していた。また，エコー正常群の内男性は24名中15名62.5%の生活習慣病併発率に対し，女性は14名中1名7.1%の併発率であった。脂肪肝群でも男性は38名中32名84.2%の生活習慣病併発率に対し，女性は9名中4名44.4%の併発率でいずれも男性が有意に高かった（図5）。

脂肪肝を基礎に持つNAFLDについて検討した。軽度脂肪肝の学生の内NAFLDを併発していたのは31名中9名30%（ALT平均54）であったのに対し高度脂肪肝の学生は56名中39名69.6%（ALT平均75）で有意に高度脂肪肝群が高率にNAFLDを発症していた（図6）。

また，腹部エコー検査にて，正常群，軽度脂肪肝群，高度脂肪肝群の3群に分類し各種検査項目での有意差を検討した（表1）。BMI値と血清ALT値は正常群と軽度脂肪肝群には有意差は見られなかったが，正常群と高度脂肪肝群，軽度脂肪肝群と高度脂肪肝群間に有意差が認められ，高度脂肪肝群が有意にBMI値と血清ALT値が高い結果であった。血清AST値と中性脂肪およびHDLコレステロール値では，正常群と高度脂肪肝群に有意差が認められ，高度脂肪肝群がAST値と中性脂肪が高くHDLコレステロール値が低い結果であった。総コレステロール値は軽度脂肪肝群と高度脂肪肝群間に有意差が認められ，高度脂肪肝群が有意に高い結果であった。尿酸値は正

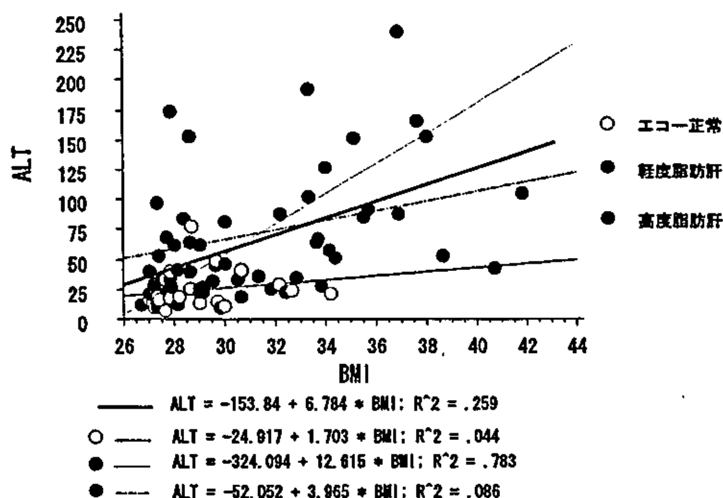

図7．腹部エコー結果とALTおよびBMIの関連

表1．脂肪肝と各種検査（平均値）の関連

		正常肝	軽度脂肪肝	高度脂肪肝
BMI	(Kg/m²)	28.4	29.0	31.9
体脂肪率	(%)	30.9	29.9	34.9
AST	(IU/L)	18.5	33.4	38.9
ALT	(IU/L)	21.7	54.0	75.1
γ-GTP	(IU/L)	24.0	45.9	47.9
UA	(mg/dl)	5.9	6.7	7.1
T-CHO	(mg/dl)	181.4	179.4	191.7
HDL	(mg/dl)	50.4	47.1	44.2
TG	(mg/dl)	99.1	119.5	133.9
上腕部皮下脂肪厚	(mm)	23.3	22.6	24.6
背部皮下脂肪厚	(mm)	24.5	26.4	28.2
最高血圧	(mmHg)	141	140	144
最低血圧	(mmHg)	83	81	87

*：P<0.05

常群に比し脂肪肝群が有意に高値であった。体脂肪率ならびに最低血圧は高度脂肪肝群が有意に軽度脂肪肝群に比し高い結果であった。上腕ならびに背部皮下脂肪厚は3群間に有意差は見られなかった。また，NAFLDとBMIの関係について検討した結果，BMIが高くなるほど，ALT値が高くなる傾向が見られ，両者には正の相関関係が見られた。腹部エコー検査別に解析すると，高度脂肪肝群はBMI値に関係なく一様にALT値が高値であったが，軽度脂肪肝群はBMIとALT間に有意に正の相関が認められた（図7）。ロジステイック解析による高度脂肪肝に寄与する因子はBMIが最も強く続いて性別であった（表2）。

考　察

2001年日本病院会での全国人間ドックの調査によると，1984年では肝機能異常者は10%以下であったが，2001年では25%を超え，異常値増加率の第1位であった[3)]。この肝機能異常の著しい増加は，肥満に基づく脂肪肝あるいは，脂肪性肝炎に基づく血清ALT値の上昇と考えられている。肥満に基づく脂肪性肝炎は一般に脂肪食の多い西欧諸国で近年著しい増加が見られている。米国では，人口の20%に脂肪肝が，また，2～3%に脂肪性肝炎が見られると報告されている[4)]。従来，脂肪性肝炎は40歳以降に頻度が高くなると考えられてきたが，今回我々の結果によると，BMI27以上の学生の内75%に脂肪肝が認められ，特に高度脂肪肝群が42%見られた（図2）。このことは，NAFLDが従来長期にわたる肥満の結果と考えられてきたこれまでの見解とは異なり，若年時で比較的短期間の肥満によってもNAFLDが発症しうることを示唆している。特にBMI30

表2．高度脂肪肝群と正常肝群のロジスティック解析

	係数	標準誤差	係数/標準誤差	カイ2乗	p値	Exp（係数）
エコー正常群vs高度脂肪肝群	-14.766	4.154	-3.555	12.638	.0004	3.864E-7
性：M	-1.523	.720	-2.115	4.471	.0345	.218
BMI	.310	.135	2.297	5.275	.0216	1.364
UA	.486	.249	1.950	3.804	.0511	1.626
TG	.012	.005	2.164	4.682	.0305	1.012
GPT	.077	.022	3.538	12.515	.0004	1.080

以上の高度肥満群では45名中40名88.9%に脂肪肝がみられ，この内高度脂肪肝例はすべて肝機能異常，高脂血症ならびに高尿酸血症を合併していた。中尾らの報告にも見られるように年齢に関わらず若年でもBMIに相関して，肝機能異常，高脂血症ならびに高尿酸血症が認められた[5]。また，現在注目されている疾患にnon alcoholic steato-hepatitis（NASH）がある。肥満に基づく脂肪肝が1st hitでそれに引き続くインシュリン抵抗性などの代謝異常[6]，酸化ストレス[7]，エンドトキシン[8]等が2nd hitの2 hit theoryにより発症すると考えられている。今回の調査にて生活習慣病（高脂血症，高尿酸血症，肝機能障害）を併発していた学生はBMI27以上30未満の学生では85名中52名61%，BMI30以上の学生では45名中40名89%（図4）に昇り，若年肥満で比較的短期間の肥満歴であっても既に何らかの代謝異常を併発していた。特に BMI27以上30未満の学生でエコー正常群の生活習慣病併発例は38名中16名（男性15／24，女性1/14）42%であるのに対し，脂肪肝群では47名中36名（男性32／38，女性４／９）76%でいずれも男性が有意に高かった。また，BMI30以上で高度脂肪肝の生活習慣病併発例は34名中34名100%であった。すなわち，NAFLD からNASHへの移行に必要条件が既に20歳代にて存在していると言うことである。また，軽度脂肪肝の学生の内，肝機能障害を併発していたのは31名中９名30%（ALT平均54）であったのに対し高度脂肪肝の学生は56名中39名69.6%（ALT平均75）であり（図６），ロジステイック解析による高度脂肪肝に寄与する因子はBMIが最も強く続いて性別であった（表２）。すなわち，男性の肥満を如何に解消していくかが重要なことと考えられる。将来重篤な合併症を引き起こす素地が若年より存在していることを我々保健管理医が自覚することは，予防医学の観点からも極めて重要なことである。

結　語

今回のUSTにより，BMI27-30では脂肪肝例，正常例があることを認めた。特に高度脂肪肝群では高率に生活習慣病，NAFLDを認めた。学生定期健康診断にUSTを導入し，脂肪肝の有無を判定することに意義があると考えられた。USTの導入は，人的及び経費的にも労力を伴うが，肥満学生に視覚的に脂肪肝の病態を認識させることが，今後の生活習慣を改善させると考えられた。将来この集団からNASHへの移行を如何に阻止するかが今後の課題である。

謝　辞

本研究に貴重な御助言頂きました高知大学医学部第１内科西原利治氏，本研究に御協力を頂きました当センター看護師の高山文子，俵迫つや子氏，宮崎大学医学部第２内科研究室外山恵子氏に深甚なる謝意を表します。

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Abstract

The introduction of abdominal ultra-sonography test into student annual health examination.Examination for overweight students

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CAMPUS HEALTH, 41 (2), 111−116, 2004

Key words : health examination, obesity, lifestyle-related disease, fatty liver, UST

We examined the usefulness of abdominal ultra-sonography test (UST) by annual health examination (2263 men and 1208 women) for the students with BMI more than 27. We performed 130 (92 men and 38 women) from 164 students whose BMI was more than 27. Lipopexia of arm and back, biochemical blood tests (AST, ALT, r-GTP, UA, T-Cho, TG, HDL-C) and UST were performed. Fatty liver was found 98 out of 130 students (75%). The fatty liver existed in the student of about 90% of 30 or more BMI, and the fatty liver existed even in the student of BMI 27-30 to about 55%. Fifty-two out of 85 students of BMI 27-30 and forty out of 45 students had from 30 or more BMI had the complication of lifestyle-related disease. Lifestyle-related disease was appeared sixteen (M15/24, F1/14) out of 38 students with normal UST and thirty-six (M32/38, F4/9) out of 47 students with fatty liver in the students of BMI 27-30. All 34 students with excessive fatty liver had the complication of lifestyle-related disease from 30 or more BMI. By the logistic analysis, BMI was significantly associated with severe fatty liver. It turns out that subjects of a fatty liver and a normal subjects in the students of BMI 27-30 by UST. Especially the student of an advanced fatty liver group had merged a lifestyle-related disease and NAFLD to high rate. It is important to introduce UST into annual health examination, and to judge the existence of a fatty liver.

Int. J. Cancer: **110**, 621–625 (2004)
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Publication of the International Union Against Cancer

LETTER TO THE EDITOR

Role of HTLV-1 Proviral DNA Load and Clonality in the Development of Adult T-Cell Leukemia/Lymphoma in Asymptomatic Carriers

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Dear Sir,

Human T-lymphotropic virus type 1 (HTLV-1) is the causative agent for adult T-cell leukemia/lymphoma (ATL).[1–3] Only a small proportion of HTLV-1 carriers eventually develop ATL after a long latency.[4] However, the critical events in the leukemogenic process remain unclear. In general, viral load is an important factor affecting the outcome of virus-associated disease. The HTLV-1 proviral DNA load in the peripheral blood mononuclear cells (PBMCs) of carriers exhibits a wide range of values.[5] Enhanced expression of HTLV-1 Tax, which transactivates transcription of viral mRNA and of host genes that control cell proliferation, induces amplification of infected cells (*i.e.*, the number of proviral copies).[6] One of the host genes encodes the IL-2 receptor (IL-2R), which is overexpressed on the surface of ATL cells.[7] We have observed that subjects with detectable *tax/rex* mRNA have a higher number of IL-2Rα-positive T cells.[8] We also have found a positive association between HTLV-1 proviral load and the level of soluble IL-2R in asymptomatic carriers,[5] as well as the number of morphologically abnormal lymphocytes on a peripheral blood smear among carriers.[9,10] In addition, the HTLV-1 proviral load within a carrier is stable over many years.[5] It has been postulated that clonal proliferation of HTLV-1-infected cells likely is responsible for maintaining the proviral load level in a carrier.[11,12] HTLV-1 Tax may contribute to the clonal proliferation of HTLV-1-infected cells by promoting their abnormal growth.[6] Tax also exerts dysregulation of the cell cycle by binding to its inhibitors and inhibiting some tumor-suppressor proteins.[6]

The accumulated data support the hypothesis that increased HTLV-1 proviral load and clonal expansion of HTLV-1-infected cells are key to the process of leukemogenesis in HTLV-1 carriers. However, proviral DNA levels and clonality of HTLV-1-infected cells have not been directly evaluated as predictors of the development of ATL in asymptomatic carriers. Given an ATL incidence rate as low as 1 case per 1,000 person-years among HTLV-1 carriers, such a study would require extensive follow-up of a relatively large number of HTLV-1 carriers.

The Miyazaki Cohort Study is a population-based prospective study of the natural history of HTLV-1, which was established in 1984.[13] During follow-up of the cohort through December 2000, 6 ATL cases were identified through an annual census or reports from next-of-kin.[14] Four of the 6 cases had available prediagnostic samples of PBMCs and were included in the present analysis (Table I). Diagnosis was confirmed by medical records for 3 of the 4 cases (cases 1, 3, 4). Lymph node biopsy for case 4 and the PBMCs at ATL diagnosis for cases 1 and 4 were available for analysis. Any symptoms suggestive of smoldering- or chronic-type ATL, such as skin lesions or lymphadenopathy, were not observed at the annual health examinations prior to the diagnosis of malignancy in the cases studied. Routine blood tests performed at the annual health examinations also did not reveal any abnormal findings suggestive of subclinical ATL. Therefore, it is unlikely that these cases had smoldering- or chronic-type ATL before the onset of acute- or lymphoma-type ATL. For comparison of HTLV-1 proviral load, age- and sex-matched HTLV-1$^+$ controls were randomly selected from asymptomatic carriers within the cohort. Informed consent was obtained from all study participants, and the study protocol was approved by the Human Subjects Committees of Miyazaki Medical College and Harvard School of Public Health.

HTLV-1 proviral DNA in PBMCs was quantitated using the AmpliSensor assay (AcuGen HTLV-1 Quantitation Test; Biotronics, Lowell, MA).[5,15] Prediagnostic HTLV-1 proviral loads of the 4 ATL cases are shown in Table I. Two cases were male (cases 1, 2), and 2 were female (cases 3, 4). Median age at onset of ATL was 73.5 years (range 64–83). Median HTLV-1 proviral load (copies per 100,000 PBMCs) at the earliest prediagnostic PBMC sample (collected 3–8 years prior to ATL onset) was 4,930 (range 830–10,560). Median proviral

Grant sponsor: National Institutes of Health; Grant number: CA38450; Grant sponsor: Ministry of Education, Science, Sports and Culture (Japan).

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Received 16 September 2003; Revised 17 December 2003; Accepted 7 January 2004

DOI 10.1002/ijc.20144
Published online 15 March 2004 in Wiley InterScience (www.interscience.wiley.com).

TABLE I – HTLV-I PROVIRAL DNA LOADS IN 4 CARRIERS WHO EVENTUALLY DEVELOPED ATL

	Case 1	Case 2	Case 3	Case 4
Year before onset				
8				6,690[1]
7			830	
6				
5	3,170		1,910	2,200
4			1,480	
3		10,560		
2			2,110	18,150
1			2,840	
ATL type at diagnosis	Acute	Not available	Lymphoma	Lymphoma
Age[2] (years), sex	83, male	64, male	76, female	70, female

[1]Copies per 100,000 PBMCs.–[2]Age at death.

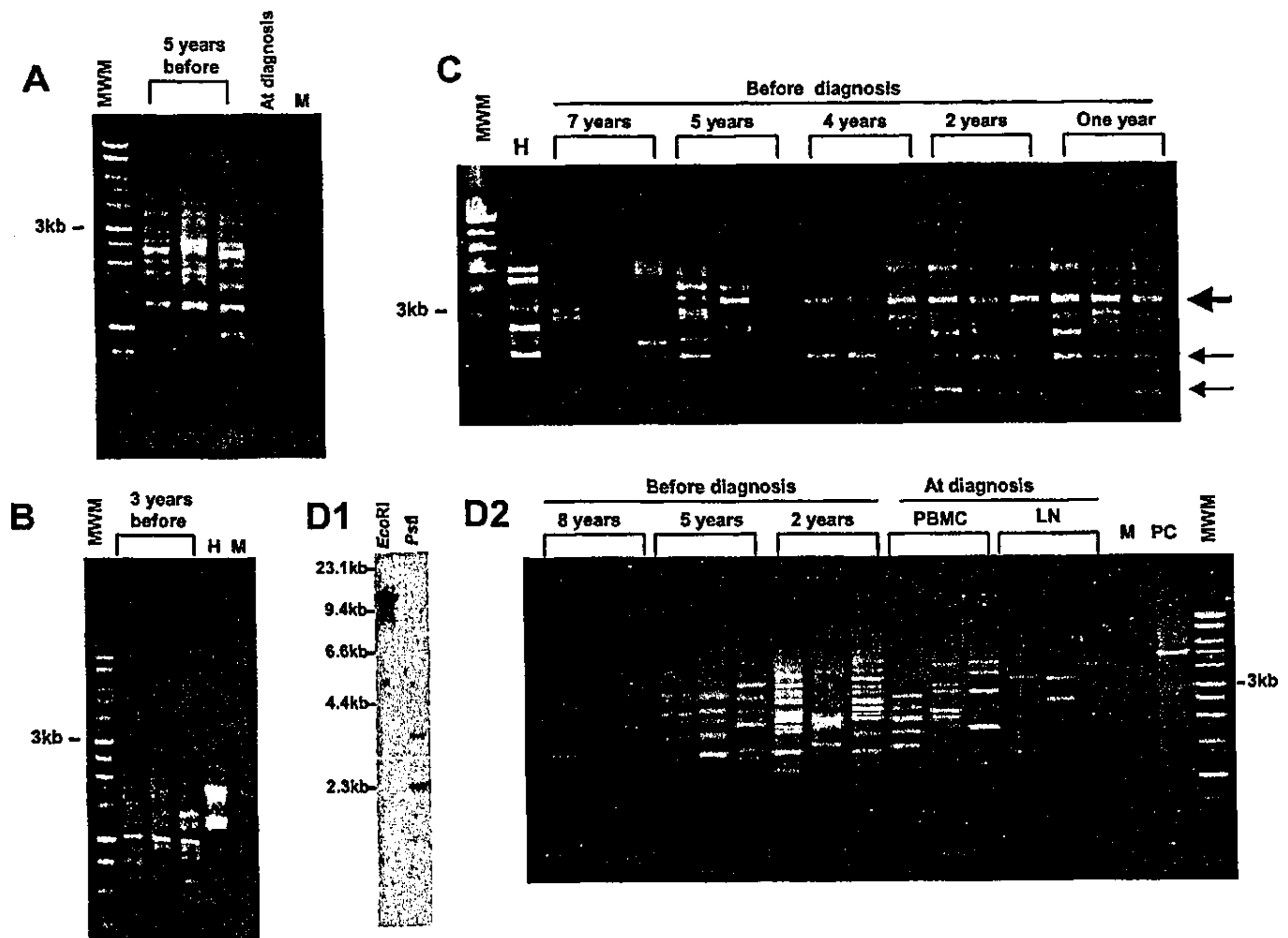

FIGURE 1 – Inverse-long PCR analysis of HTLV-1-infected cells in 4 cases before and at diagnosis of ATL. *(a)* Case 1. Triplicate analysis of PBMCs in the sample obtained 5 years before the onset of ATL and single analysis of ATL cells at diagnosis. *(b)* Case 2. Triplicate analysis of PBMCs in the sample obtained 3 years before the onset of ATL. *(c)* Case 3. Triplicate analysis of PBMCs in samples obtained 1–7 years before the onset of ATL. Arrows indicate major clones. *(d1)* Case 4. Southern blot analysis of lymph node biopsy specimen at diagnosis. EcoRI, EcoRI digestion; PstI, PstI digestion. *(d2)* Case 4. Triplicate inverse-long PCR analysis of PBMCs in samples obtained 2–8 years before the onset of ATL and from PBMC and lymph node (LN) samples obtained at diagnosis. MWM, m.w. marker (1 kb ladder); H, HUT102 cells as HTLV-1-positive control; M, Molt4 cells as HTLV-1-negative control; PC, leukemic cells obtained from an ATL patient, who was not a member of the study cohort, as a control.

load of the 37 age- and sex-matched HTLV-1$^+$ control subjects was 820 (10–9,790), which was significantly lower than the earliest prediagnostic proviral load of cases (Wilcoxon rank sum test, $p = 0.03$). In conditional logistic regression analysis, which accounted for the matched design (LogXact 4.1; Cytel, Cambridge, MA), there was a significant association between higher viral load and case status; the odds ratio of being an ATL case associated with each 1,000 copies (per 100,000 PBMCs) increase in viral load was 1.42 (95% exact confidence interval 1.04–2.10, $p = 0.03$).

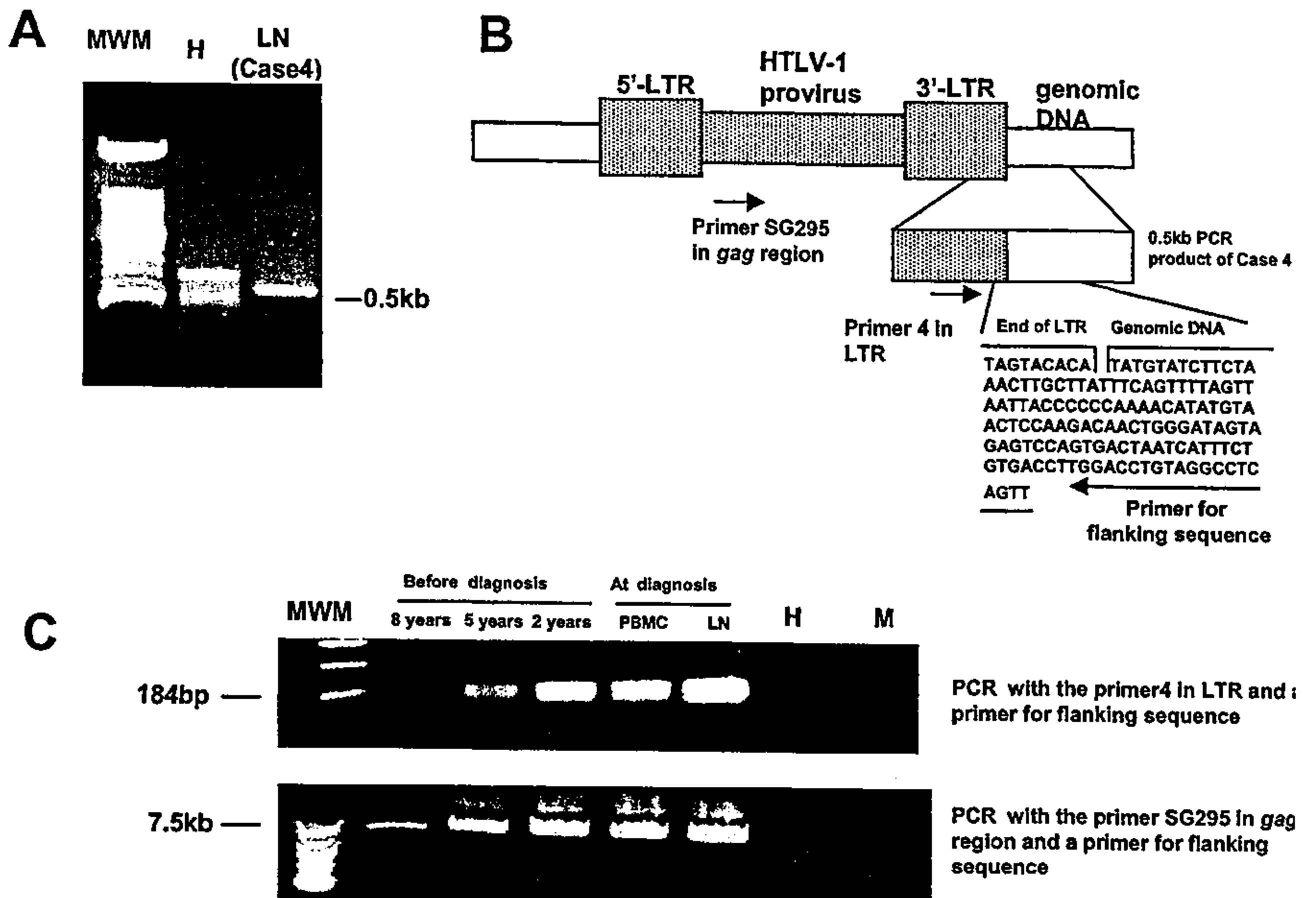

FIGURE 2 – Detection of the preleukemic clone in PBMC samples obtained from case 4 before diagnosis of ATL. *(a)* Inverse PCR analysis of lymph node (LN) specimen obtained at ATL diagnosis. *(b)* Schema showing subcloned and sequenced DNA of the inverse PCR amplicon derived from case 4 and the locations of primers used for leukemic clone-specific PCR. *(c)* Leukemic clone-specific PCR analysis using primer 4 of the LTR (upper panel) and primer SG295 (lower panel) before and at diagnosis of ATL. MWM, m.w. marker; H, HUT102 cells as HTLV-1-positive control; M, Molt4 cells as HTLV-1-negative control.

Although the number of cases analyzed was small, the difference in proviral load between cases and controls was striking and statistically significant. Our prospective analysis, thus, demonstrates an association between higher proviral DNA load and the subsequent development of ATL. HTLV-1 proviral DNA levels were stable over several years in asymptomatic carriers.[5] In cases 3 and 4, who had PBMC samples from more than 5 years, proviral levels were increased in samples obtained closest to the time of ATL diagnosis (1 and 2 years before onset, respectively). These increased proviral loads may reflect the clonal expansion of certain HTLV-1-infected cells.

Because the primers of the *pol* region were used for the quantitative assay of proviral DNA load, HTLV-1-infected cells with *pol*-defective provirus could not be detected by the current method. It is possible that the HTLV-1-infected cells with such defective virus were seen more commonly in carriers who developed ATL rather than in the control group because the defective provirus is sometimes found in patients with ATL.[16] As a result, the difference in proviral DNA load between cases and controls might have been larger if we had used a more conserved region, such as *tax*, for the primers.

We also examined whether the preleukemic HTLV-1-infected clone can be found prior to diagnosis in the peripheral blood of carriers who go on to develop ATL. To amplify the genomic DNA adjacent to the integration sites of the HTLV-1 provirus, inverse long-PCR was used.[12] All 4 cases had clonal bands of various sizes and intensities, indicating oligoclonal or polyclonal expansion of HTLV-1-harboring cells prior to diagnosis (Fig. 1). For case 1, a single band indicating the leukemic clone was detected in the peripheral blood by inverse long-PCR at diagnosis (Fig. 1*a*). Case 3, a carrier who developed lymphoma-type ATL, had several bands that were consistently strong over several years (arrows in Fig. 1*c*). These bands appear to represent major clones, consisting of large numbers of HTLV-1-infected cells with the same proviral integration site. One of the bands increased in intensity in the samples obtained close to the onset of ATL (large arrow in Fig. 1*c*). However, it was not possible to determine whether this clone was the preleukemic one because a sample from the time of ATL diagnosis for this carrier was not available.

For case 4, monoclonal integration of the HTLV-1 provirus in the leukemic cells from the lymph node was demonstrated by Southern blot assay at diagnosis (Fig.1*d1*). However, the

leukemic clone was undetectable by inverse long-PCR, and only some faint bands were seen in the lymph node sample obtained at the time of ATL diagnosis (Fig. 1*d2*). This observation is not surprising since leukemic clones are reported to be detectable only in about half of patients by inverse long-PCR due to the lack of adequate sites for restriction enzymes or to sequence deficiencies of the HTLV-1 provirus in leukemic cells.[12]

To identify the preleukemic clone in the peripheral blood of case 4 prior to diagnosis, the nucleotide sequence of the 3′ flanking region of the integrated provirus in the ATL cells first was determined using the inverse PCR method (Fig. 2*a,b*).[17] We then synthesized a primer (5′-AACTGAGGCCTACAG-GTCCA-3′) that would specifically amplify the junction site of the 3′ long terminal repeat (LTR). Using this leukemic clone-specific primer, the HTLV-1-infected cell clone with the same integration site of the provirus as the leukemic cells was detectable 2, 5 and 8 years before the onset of ATL, with 2 different primers for the HTLV-1 LTR (primer 4 used in inverse PCR) and *gag* sequences (primer SG295) (Fig. 2*c*).[18] The intensity of this clone was increased at 2 years prior to ATL diagnosis compared to the intensity at 5 and 8 years before diagnosis (Fig. 2*c*).

In addition, case 4 was diagnosed as lymphoma-type, and leukemic cells were rarely observed in the peripheral blood smear at diagnosis. However, clone-specific PCR was strongly positive not only in the lymph node but also in the peripheral blood of case 4. This observation suggests that preleukemic cells without morphologic abnormality, which belonged to the original clone, existed in the peripheral blood even at diagnosis of ATL. At some point during the 8 years before ATL onset in this case, a subclone could have acquired additional malignant potential, presumably due to genetic and epigenetic abnormalities. Indeed, the presence of multiple clones of ATL cells based on the methylation patterns of the *CDKN2A* gene, despite having the same HTLV-1 integration site, has been reported.[19] It also has been reported that multiple clones with simple chromosomal abnormalities already exist in HTLV-1 carriers and that selected clones with more complex chromosomal abnormalities can be found in progressed chronic ATL, based on the clonal culture method.[20] Therefore, HTLV-1 carriers are hypothesized to vary with respect to the genetic and epigenetic abnormalities of their HTLV-1-infected cells. The risk of developing ATL might be related to these differences among HTLV-1 carriers.

In case 1, a clone with the same integration site of the provirus in the leukemic cells was not detectable in the PBMC sample obtained 5 years before the onset of ATL (data not shown). This finding could be due to insufficient sensitivity of the PCR assay to detect that particular clone.

In conclusion, the present prospective study provides evidence that a higher proviral DNA load strongly predicts the development of ATL in asymptomatic HTLV-I carriers. Moreover, the preleukemic clone can be observed in peripheral blood as many as 8 years before onset of the malignancy. This particular finding supports the belief that persistent clonal expansion is important in leukemogenesis. Thus, measuring the proviral DNA load and determining the clonality of HTLV-1-infected cells may provide important information for identifying carriers at increased risk of ATL. Further studies to characterize the level of genetic and epigenetic abnormalities accumulated in HTLV-1-infected cells will be necessary for the development of prophylactic therapies.

Yours sincerely,

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HGFによる肝再生医療の現況

HGF hepatic regeneration therapy

Key Words ☞ゲノム用語解説105ページ～

⇒劇症肝炎
⇒トランスレーショナルリサーチ
⇒生体部分肝移植過小グラフト

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はじめに

肝細胞増殖因子(hepatocyte growth factor；HGF)は，1986年に筆者らによって劇症肝炎患者血漿から単離・精製された，肝細胞の増殖を強力に促進する増殖因子である．HGFは肝細胞のみならず種々の上皮細胞に対して増殖促進，細胞遊走促進，形態形成，抗アポトーシスなど多彩な作用を誘導し，多くの組織の再生・修復因子と考えられている[1-4]．筆者らはこれまでに製薬会社と共同して遺伝子組換え型ヒトHGFの医薬品化を進めてきたが，2004年にはGMPに準拠した遺伝子組換え型ヒトHGFが供給される見通しである．このような背景から，現在，劇症肝炎，成人生体部分肝移植，肝硬変を対象とした**トランスレーショナルリサーチ**「肝再生医療プロジェクト」を京都大学付属病院探索医療センターで展開している．本稿では種々の肝疾患モデルを用いた*in vivo*実験系におけるHGFの有効性および遺伝子組換え型ヒトHGFの臨床応用を目的とした薬理動態・安全性試験について述べる．

肝疾患モデルにおけるHGFの有効性

1. 急性肝障害モデル

劇症肝炎患者血清中の著しいHGF上昇は，肝組織におけるHGF産生増加と標的細胞である肝細胞の減少によると考えられる[5,6]．急性肝障害ラットでも内因性HGFが増加しているが，それに加えて外因性HGFを投与するとHGF非投与ラットに比べてDNA合成活性が増加する[7]．また，ジメチルニトロサミン(dimethylnitrosamine；DMN)によって誘導した肝障害ラットに10日間遺伝子組換え型ヒトHGFを静脈内投与すると，アルブミンが有意に増加し，肝予備能も改善する(図1)．一方，アゴニスティックな抗Fas抗体(Jo2抗体)で誘導される肝不全モデルでは肝細胞にアポトーシスが誘導され，マウスは

24～48時間以内に死亡する．この致死的な肝不全マウスに遺伝子組換え型ヒトHGFを単回前投与すると生存率は著明に改善し，肝臓のアポトーシス細胞は減少する．このようなHGFの抗アポトーシス作用はBcl-xLを介したものとされており，Dガラクトサミン(D-galactosamine；D-GalN)，リポポリサッカリド(lipopolysaccharide；LPS)，四塩化炭素などによる肝障害モデルでも確認されている(表1)．またHGFの抗アポトーシス作用にはPI-3K/Aktシグナル伝達系やc-Met/Fas複合体形成が関与していることも報告された[8,9]．これらの肝障害モデルにおけるHGFの効果から，劇症肝炎患者に対する遺伝子組換

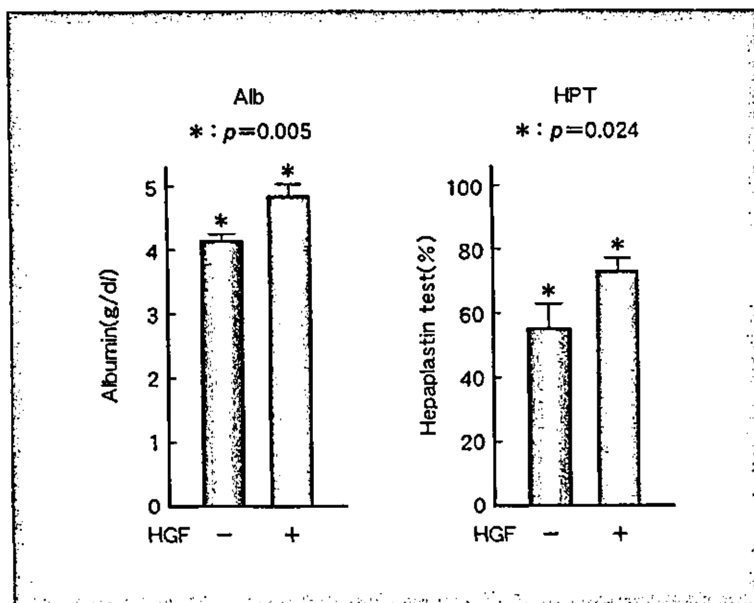

図1　ジメチルニトロサミン肝硬変モデルに対するHGFの肝再生促進効果
ジメチルニトロサミン(10 mg/kg)を2日間投与して誘導した肝硬変ラットにHGF(1 mg/kg/day)を10日間静脈内投与したところ，血清アルブミン(Alb)およびヘパプラスチンテスト(HPT)が改善した．

表1　肝障害モデル動物に対するHGFの *in vivo* 効果

動物モデル/方法	投与法	効　果	報告者(文献)
マウス/GalN	トランスジェニックマウス	生存率改善	Okano J(Hepatology 26：1241-1249，1997)
マウス/抗Fas抗体	腹腔内投与	生存率改善	Kosai K(Biochem Biophys Res Commun 244：683-690，1998)
		生存率改善	Masunaga H(Eur J Pharmacol 342：267-279，1998)
マウス/GalN＋LPS	腹腔内投与	生存率改善	Kosai K(Hepatology 30：151-159，1999)
マウス/抗Fas抗体	静脈内投与	生存率改善	Mori I(Med Sci Res 27：355-359，1999)
マウス/GalN＋LPS	遺伝子導入(アデノウイルス)	生存率改善	Nomi T(Biochem Biophys Res Commun 278：338-343，2000)
マウス/四塩化炭素	電気遺伝子導入(前頸骨筋)	肝障害軽減	Xue F(Gut 50：558-562，2002)

HGF蛋白投与または遺伝子導入は種々の急性肝不全動物モデルにおいてその生存率を改善し，肝障害を軽減する．

え型ヒトHGF投与は肝再生促進に加えて抗アポトーシス作用を介した病態進展阻止にも効果を発揮することが期待される.

2. 肝移植モデル

ラット肝移植モデルにHGFを投与すると肝再生促進のみならず，グラフト肝におけるIL-1β，TGF-β，caspase-1が減少し，肝線維化および慢性拒絶反応が抑制される[10,11]. また，グラフト肝容量を60％に減少させたモデルでは免疫抑制剤にHGFを併用することで，肝再生促進のみならず急性拒絶反応からの保護作用が誘導される[12]. 一方，虚血・再灌流による肝障害は肝切除および肝移植などで問題となる病態で，特に肝虚血時間は術後合併症の最も重要な危険因子と考えられている．HGFはこのような肝虚血・再灌流モデルに対しても肝臓における壊死領域を縮小し，肝再

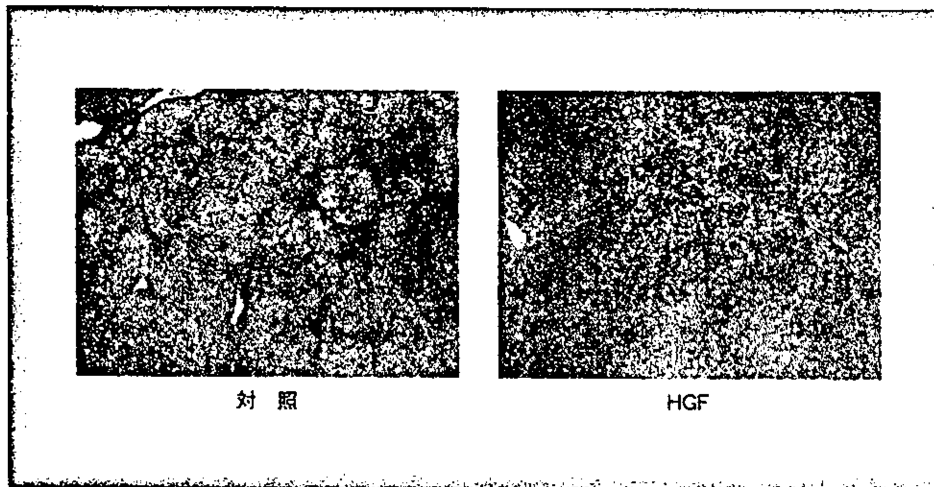

図2 DMN肝硬変モデルに対するHGFの抗線維化作用

DMN(10 mg/kg)を4週間投与して誘導した肝硬変ラットにHGF(0.3 mg/kg/day)を静脈内投与したところ線維化を抑制した．(Azan染色 40×)

表2 肝硬変モデル動物に対するHGFの *in vivo* 効果

動物モデル/方法	投与法	効 果	報告者(文献)
ラット/DMN	静脈内投与	抗線維化作用	Yasuda H(Hepatology 24 : 636-642, 1996)
ラット/ブタ血清，DMN，四塩化炭素	静脈内投与	抗線維化作用	Matsuda Y(Hepatology 26 : 81-89, 1997)
ラット/DMN	遺伝子導入(HVJリポソーム)	抗線維化作用	Ueki T(Nat Med 5 : 226-230, 1999)
ラット/TAA	静脈内投与	抗線維化作用	Sato M(Biochem Pharmacol 59 : 681-690, 2000)
ラット/TAA	腹腔内投与(ゼラチンマイクロスフェア)	抗線維化作用	Oe S(J Control Release 88 : 193-200, 2003)
マウス/四塩化炭素	電気遺伝子導入(前頸骨筋)	抗線維化作用	Xue F(Gut 52 : 694-700, 2003)
ラット/DMN	電気遺伝子導入(肝臓)	抗線維化作用	Matsuno Y(Gene Ther 10 : 1559-1566, 2003)

HGF蛋白投与または遺伝子導入は種々の肝硬変動物モデルにおいて抗線維化作用を発揮する.

生を促進する[13-15]．これらの成績は生体部分肝移植成人レシピエント過小グラフトに対して遺伝子組換え型ヒトHGFを投与すると肝再生のみならず，その抗アポトーシス作用を介して虚血・再灌流や拒絶反応による肝障害が軽減されることも期待される．

3．肝硬変モデル

肝硬変では肝再生が障害されており，HGF活性化障害や硬変肝の低酸素状態によってHGFおよびc-Met発現が低下することがその一因と考えられている[16,17]．また，四塩化炭素によって誘導した肝硬変モデルに部分肝切除を加えると早期にTGF-β発現が誘導されて肝細胞のDNA合成が抑制される[18]．一方，DMNで誘導した肝硬変ラットに遺伝子組換え型ヒトHGFを静脈内投与すると線維化が改善する(図2)．このようなHGFの抗線維化作用は四塩化炭素やチオアセタミド(thioacetamide；TAA)による肝硬変モデルでも確認されており，I型コラーゲンやTGF-βの発現抑制やアルブミンの発現増強が認められる(表2)．これらの肝硬変モデルを用いた実験結果から，肝硬変患者に対する遺伝子組換え型ヒトHGF投与は肝再生促進や抗線維作用による肝予備能の改善が期待される．

HGFによる肝再生医療の対象疾患

1．劇症肝炎

劇症肝炎は急激に起こる肝の広範性壊死に基づいて意識障害(肝性昏睡)を主徴とする急性肝不全症状を呈する疾患である．劇症肝炎の生命予後は不良で，肝移植非実施例(内科的治療)における救命率は初発症状から脳症発現までの日数が10日以内の急性型で48.8％，11日以降の亜急性型で17.0％である[19]．救命が困難と考えられる症例では早期に肝移植を前提とした治療体制をとることが救命につながることから，全国集計で得られた劇症肝炎155例を用いて脳症発現時における予後予測式(武藤ら)が作成された(表3)[20]．この予測式の正診率は75.5％で，死亡確率(P)≦0.20では90％の症例が生存したが，死亡確率(P)≧0.80では約10％の症例しか救命されていない．一方，0.20<死亡確率(P)<0.80の生存率は43.0％であったが，この群においてステロイド，グルカゴン・インスリン療法，血漿交換などの各種治療法による生存率を比較しても，単独で有意に生存率を向上させる治療法は見出せていない[20]．これらの解析結果は，予後予測が可能となっても肝移植以外に有効な治療法が確立されていない現状を反映している．したがって，まず死亡確率(P)>0.20で肝移植が実施されない劇症肝炎症例を遺伝子組換え型ヒトHGFを用いた治療の対象にしたいと考えている．

2．成人生体部分肝移植

近年，右葉グラフトを用いる方法が確立され，成人をレシピエントとした生体肝移植症例の2年生存率も良好な成績が得られるようになった．しかし，成人生体部分肝移植ではドナーから得られるグラフト肝の大きさに限界があるため，過小グラフトに起因する術後肝障害と肝合成能低下，遷延する黄疸，さらにはグラフト肝壊死といったsmall-for-size(SFS)症候群を呈することが，解決すべき大きな課題となっている[21]．過小グラフトのレシピエントに遺伝子組換え型ヒト型ヒトHGFを投与すると，肝再生促進による合成能と黄疸の改善，抗アポトーシス作用による肝障害の軽減が期待される．しかし，SFS症候群発症には，単にグラフト肝の容量過小だけではなく，レシピエントの年齢や病状(腹水や肝硬変・側副血行路の有無)，ドナーの年齢や血管構造の解剖学的差異，手術にともなう虚血・再灌流による肝障害など，多くの因子が関与している．HGF投与がこれらの要因をすべて解決するわけではないのでどの程度の効果が期待できるかは推測できないが，少なくともSFS症候群の発症に対しては抑制的に作用するものと考えられる．

3．肝硬変

本邦ではB型肝炎ウイルスやC型肝炎ウイルスに

表3　脳症発現時における劇症肝炎の予後予測

予測式(武藤ら)：$\mathrm{logit}(\lambda) = -0.0649 \times \mathrm{PT}(\%) + 0.0357 \times 年齢 - 2.81 \times \mathrm{D.Bil./T.Bil.} + 0.703 \times \log_e \mathrm{T.Bil.} + 1.04 \times \mathrm{OCD}$

OCD：発症-昏睡期間　0：≦10日，1：>11日

死亡確率(P)＝$1/(1+e^{-\lambda})$

死亡確率(P)	≦0.20	0.20<P<0.80	0.80≦
症例数(急性：亜急性)	10(10：0)	86(56：30)	59(8：51)
生存率(%)	90.0	43.0	10.2

劇症肝炎の予後予測式(武藤ら)は正診率75.5％で，0.20<死亡確率(P)<0.80の群(生存率43.0％)における各種治療法による生存率を比較したところ，単独で有意に生存率を向上させる治療法は見出せなかった．この結果は，予後予測が可能となっても肝移植以外に有効な治療法が確立されていない現状を反映しているものと考えられる．

(文献20より改変引用)

よる肝硬変を背景とした肝細胞癌患者数が増加している．特にＣ型肝炎ウイルスによる肝硬変における肝細胞癌の発生率は年率７％に達する．しかし，最新のインターフェロンと抗ウイルス剤の併用療法を行ってもＣ型肝炎ウイルスが排除される症例は約50％で，ウイルス排除できなかった多くの慢性肝炎患者が肝硬変に進展している．また，原発性胆汁性肝硬変や原発性硬化性胆管炎が進行し非代償性肝硬変となれば，唯一肝移植しか治療法がないのも現状である．このような進行した肝硬変患者に対して抗線維化および肝再生を目的とした有効な治療法はいまだ確立されておらず，抗線維化作用および肝再生促進作用をもった遺伝子組換え型ヒトHGFを用いた治療は新たな肝硬変治療法として期待される．しかし，肝硬変に対する治療は長期投与が必要なため，HGFの発癌性の検討や投与方法の工夫が重要である．

遺伝子組換え型ヒトHGFの前臨床試験と問題点

1. 薬理動態と投与方法

遺伝子組換え型ヒトHGFを単回静脈内投与するとその半減期は2.4分と短いが，肝臓におけるc-Metチロシンリン酸化は誘導される．静脈内投与された遺伝子組換え型ヒトHGFの最大の標的臓器は肝臓であるが，副腎，脾臓，腎臓などにも移行する．特に腎臓への移行は後述する遺伝子組換え型ヒトHGFの腎毒性とも関連しているため，肝臓選択的なドラッグデリバリーシステムの開発が望まれる．一方，門脈内投与では遺伝子組換え型ヒトHGFの肝外組織への移行が減少するため，より肝臓選択的な投与方法として腎毒性軽減が期待される．しかし，門脈内投与は内科的疾患においてはアプローチが困難であるため，肝移植レシピエントへの応用を考えている．

2. 一般毒性

遺伝子組換え型ヒトHGFを反復静脈内投与すると蛋白尿が出現し，腎臓のメサンギウム細胞増生および免疫複合体の沈着が認められた．これらの腎毒性は動