at E11.5 and E12.5 in *sek1*$^{-/-}$ and *c-jun*$^{-/-}$ livers, respectively. These results clearly indicate that impaired hepatoblast development certainly occurs in *sek1*$^{-/-}$ embryos and that *sek1* mutation is more severe than *c-jun* mutation in terms of hepatoblast development, which is consistent with the previous finding that *sek1*$^{-/-}$ embryos die earlier than *c-jun*$^{-/-}$ embryos.

SEK1-Deficient Livers Exhibit Normal Hepatic Gene Expression

A recent study has shown that *c-jun*$^{-/-}$ livers display the normal expression of the mRNAs, for albumin, keratin 18, hepatocyte HNF-1, β-globin, and erythropoietin, some of which are putative AP-1 target genes [30]. To confirm normal liver differentiation in *sek1*$^{-/-}$ embryos, the tissue-specific gene expression was measured by means of reverse transcription-polymerase chain reaction (RT-PCR). An early fetal hepatic marker, AFP, was expressed at the same level in wild-type and *sek1*$^{-/-}$ livers at E10.5 when cell numbers of Liv2-positive hepatoblasts were the same. Furthermore, a mature hepatic marker, albumin, was also expressed in both wild-type and *sek1*$^{-/-}$ fetal livers at E10.5 and E11.5. These results indicate that hepatic differentiation is not affected in *sek1*$^{-/-}$ fetal livers, similar to findings in *c-jun*$^{-/-}$ livers.

SEK1-Deficient Livers are Characterized as Showing Impaired Cell Growth of Hepatoblasts

We previously reported massive cell apoptosis in *sek1*$^{-/-}$ livers at E12.5 [15]. This apoptosis appeared to be associated with a decreased number of hepatoblasts at E11.5, without significant change in the gene expression of hepatic markers. Therefore, the growth capacity of *sek1*$^{-/-}$ hepatoblasts at the early stage of E10.5 was analyzed by the incorporation of bromodeoxyuridine (BrdU; Fig. 3). Interestingly, BrdU incorporation into Liv2-positive hepatoblasts was greatly reduced in *sek1*$^{-/-}$ livers compared with wild-type and *c-jun*$^{-/-}$ livers. These results suggest that *sek1* mutation may result in the impaired growth capacity of hepatoblasts at E10.5 when no other obvious defects in *sek1*$^{-/-}$ livers are observed.

Genetic Interaction Between *sek1* and *c-jun* in Murine Development

Because *sek1* and *c-jun* knockout mice display a similar phenotype of impaired liver formation [15], we further examined the genetic interaction between the two genes by preparing *sek1*$^{-/-}$ *c-jun*$^{-/-}$ double-mutant embryos. According to the Mendelian ratio (1:16), there was the expected number of the *sek1*$^{-/-}$ *c-jun*$^{-/-}$ genotype in E8.5 embryos, in addition to *sek1*$^{+/+}$ *c-jun*$^{+/+}$, *sek1*$^{-/-}$ *c-jun*$^{+/+}$, and *sek1*$^{+/+}$ *c-jun*$^{-/-}$ genotypes. No *sek1*$^{-/-}$ *c-jun*$^{-/-}$ embryos were, however, observed at E10.5, though embryos of the *sek1* or *c-jun* single mutant existed at the expected numbers. The loss of *sek1*$^{-/-}$ *c-jun*$^{-/-}$ embryos at E10.5 appeared to be due to resorption, because all the embryos at E8.5 were already dead. Thus, *sek1*$^{-/-}$ *c-jun*$^{-/-}$ double-mutant embryos died before E8.5 and underwent resorption through E10.5. These results indicate that the *sek1* and *c-jun* genes work synergistically during early embryonic development.

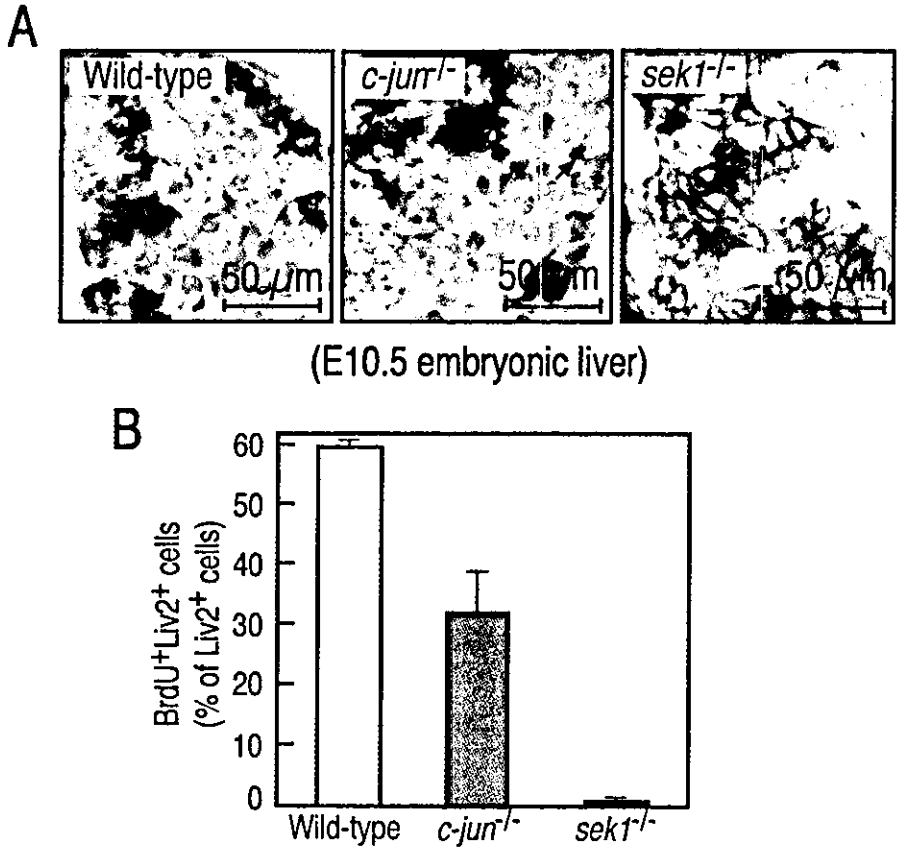

Fig. 3A,B. Impaired hepatoblast growth observed in *sek1*$^{-/-}$ livers. Pregnant mice bearing E10.5 embryos were injected with 0.3 ml of 50 mg/ml bromo-deoxyuridine (*BrdU*). After 4 h, the embryos were isolated, fixed with 4% paraformaldehyde, and genotypes of the embryos were determined by polymerase chain reaction (PCR). A Transverse sections were prepared from the paraffin embedding of wild-type (*left*), *c-jun*$^{-/-}$ (*middle*), and *sek1*$^{-/-}$ (*right*) embryos and stained with anti-Liv2 (*brown*). *Arrows* indicate BrdU-incorporated (*blue colored*) Liv2-positive hepatoblasts. B The BrdU-incorporated cells were counted in the three embryos (wild-type, *c-jun*$^{-/-}$, and *sek1*$^{-/-}$), and the values are expressed as percentages of the total of Liv2-positive hepatoblasts

No Requirement of Hematopoiesis for Hepatoblast Growth in Early Hepatogenesis

To examine the relationship between hepatoblast growth and hematopoiesis in early fetal liver, we measured the number of Liv2-positive cells at E11.5 in *AML1*$^{-/-}$ embryos, which lack definitive hematopoiesis. The total numbers of hepatic cells *plus* blood cells in wild-type and *AML1*$^{-/-}$ livers at E11.5 were counted, at 3.0×10^5 and 2.2×10^5 cells, respectively. When paraffin sections were prepared from E11.5 fetal livers and stained with anti-Liv2 or anti-TER119, there were 44% and 79% of Liv2-positive cells in wild-type and *AML1*$^{-/-}$ livers, respectively. *AML1*$^{-/-}$ fetal livers at E11.5 contained approximately 80% of Liv2-positive cells and 20% of TER119-positive primitive erythrocytes. Therefore, the numbers of Liv2-positive cells were calculated to be 1.3×10^5 and 1.7×10^5 in wild-type and *AML1*$^{-/-}$ livers, respectively. Thus, the cell numbers of hepato-

blasts were higher in *AML1*$^{-/-}$ livers than in wild-type ones. These results indicate that hematopoiesis and growth factors from blood cells are not essentially required for hepatoblast growth in early hepatogenesis.

Impairment of HGF-Induced SAPK/JNK Activation in Fetal Livers Lacking SEK1

To elucidate the biochemical interaction between SEK1 and SAPK/JNK, we first investigated whether HGF, which is produced by nonblood cells, could activate SAPK/JNK in fetal liver cells expressing the HGF receptor, c-Met. In a previous report [15], we could not detect any SAPK/JNK activation by HGF in a primary cell culture prepared from fetal livers. Therefore, in this study, we screened liver cells in an intact condition and found that strong SAPK/JNK activation was observed in response to HGF by using whole livers at E10.5, where about 60% of cells are Liv2-positive hepatoblasts. There was maximally a more than 25-fold increase in SAPK/JNK activity at 10 min, and the activity decreased rapidly. Such marked activation of SAPK/JNK was, however, greatly reduced in *sek1*$^{-/-}$ fetal livers. Interestingly, another member of the stress-activated mitogen activated protein (MAP) kinase family, p38, was constitutively phosphorylated without stimulation by HGF in fetal livers, and this phosphorylation was still observed in *sek1*$^{-/-}$ fetal livers. These results indicate that SEK1 is required for HGF-induced full activation of SAPK/JNK but not for p38 activation, and that HGF is one of the growth factors that regulate hepatoblast growth in early fetal liver development.

Conclusion

Embryonic liver formation is, genetically, a crucial checkpoint in fetal hematopoiesis and development. Although hematopoiesis has been characterized at the cellular and molecular levels, hepatogenesis and liver formation are just beginning to be characterized. To analyze early liver development, we first screened monoclonal antibodies that specifically recognized murine fetal livers by using transverse sections of E11.5 embryos. One of the antibodies, anti-Liv2, is applicable to paraffin sections and whole mount embryos, and the Liv2 antigen appears to be localized in the cell membrane (Fig. 1). The ratios of Liv2-positive cells from E9.5 to E12.5 were consistent with those of hepatic cells defined as hepatoblasts. Using anti-Liv2, we measured the exact cell numbers of hepatoblasts in fetal liver development in wild-type, *sek1*$^{-/-}$, and *c-jun*$^{-/-}$ mice. Thus, our experiments proved anti-Liv2 to be a useful tool for identifying murine hepatoblasts in early fetal livers, though the Liv2 antigen and its physiological role have not been determined yet.

Previously, we and another group reported that *sek1*$^{-/-}$ embryos died between E10.5 and E12.5, with a decreased number of cytokeratin-positive hepatocytes at E11.5, and massive hepatocyte apoptosis at E12.5 [15,17]. To extend the above results, we examined an interesting question: whether the hepatic apoptosis and embryonic lethality observed in *sek1*$^{-/-}$ mice are rescued by the introduction of *tnfr1* gene mutation (Table 1 and Fig. 2). As shown in Fig. 4, TNFR1 relays TNF-α stimulation to three separate pathways, which include the induction of apoptosis, NF-κB activation, and SAPK/JNK activation [20]. Activation of NF-κB protects against TNF-α-induced apoptosis.

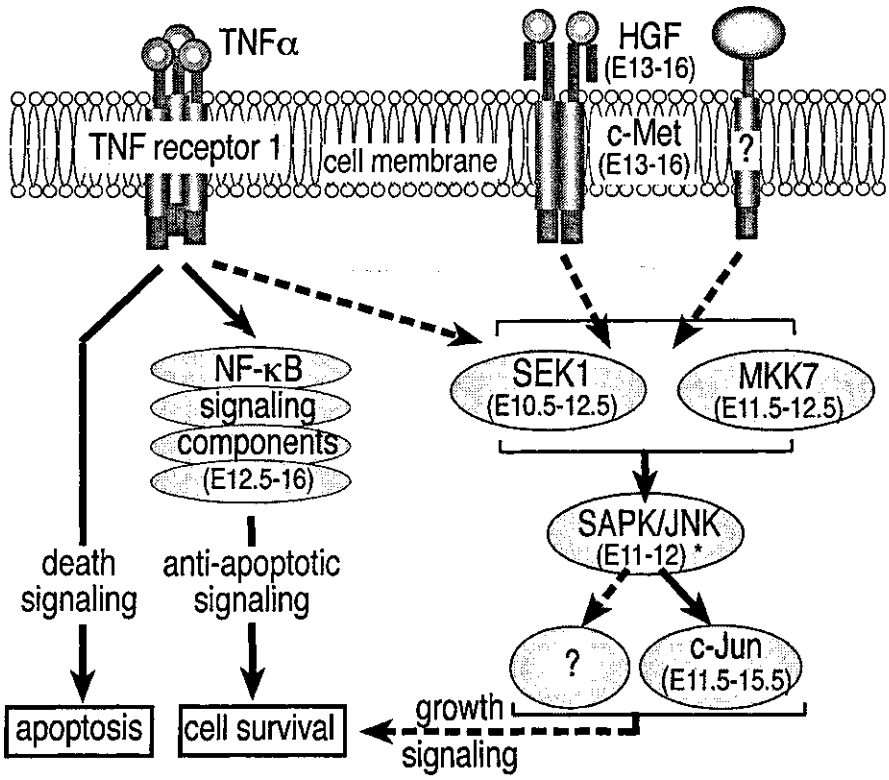

Fig. 4. Proposed model for SEK1-associated signaling pathways in hepatoblasts. The *numbers in parentheses* are days of embryonic lethality reported in previous studies and in the present study. *Solid* and *broken lines* show signaling pathways reported in previous studies and the present study, respectively. *Asterisk* indicates that *Jnk1*$^{-/-}$ *Jnk2*$^{-/-}$ mutant mice die between the eleventh and twelfth days of gestation [42] *TNF* α, tumor necrosis factor alpha, *HGF*, hepatocyte growth factor; *NF*-κB, nuclear factor kappa B; *SAPK/JNK*, stress-activated protein kinase/c-Jun N-terminal kinase

Therefore, mice lacking components of the NF-κB signaling pathway, such as RelA, IKK2, NEMO, or T2K show embryonic lethality, with massive liver apoptosis, and are rescued by the introduction of *tnfr1* mutaion. However, the physiological role of SEK1 and/or MKK7-mediated SAPK/JNK activation in response to TNFR1 stimulation remains to be resolved. In the present study, we examined the phenotype of body sizes and fetal livers of both SEK1 and TNFR1 double-knockout mice. Interestingly, embryo resorption was partially inhibited in *sek1*$^{-/-}$ *tnfr1*$^{-/-}$ mice, although the liver defect induced by *sek1* mutation was not rescued (Table 1 and Fig. 2). These results clearly show that the SEK1-mediated signal plays an important role, which is apparently different from the NF-κB signal, in fetal liver formation. Another important conclusion from the results with the *sek1*$^{-/-}$ *tnfr1*$^{-/-}$ mice is that TNFR1 plays a role in embryo resorption. Some studies have suggested the involvement of TNF-α in embryo loss and resorption by showing changes in its expression [31–33]. Our genetic experiments showing partial rescue of embryo resorption by the inactivation of TNFR1 function are consistent with the idea that TNFR1-mediated death signaling plays a role in the clearance of abnormal embryos. Thus, TNF-α elicits a wide spectrum of cellular responses, including apoptosis and cell growth. TNFR1 may relay its stimulation to SEK1-mediated cell growth in hepatoblasts, resulting in cell survival. The balance of

three separate pathways may be important for eliciting various cellular responses, depending on the type of cell and its developmental stage (Fig. 4).

To find the primary cause of the liver defect caused by *sek1* mutation, we analyzed *sek1*$^{-/-}$ livers using anti-Liv2 and found decreased numbers of hepatoblasts in the mutant embryos at E11.5. Furthermore, we found impaired BrdU incorporation into Liv2-positive hepatoblasts in *sek1*$^{-/-}$ livers at E10.5 (Fig. 3). Because liver-specific gene expression was normal in *sek1*$^{-/-}$ fetal livers, the liver defects in *sek1*$^{-/-}$ embryos are very likely due to the impaired cell growth of hepatoblasts in early hepatogenesis, resulting in decreased numbers of hepatoblasts and massive hepatoblast apoptosis. A recent report showing the maturation of SEK1-null embryonic stem cells into a hepatic lineage in vitro also indicates that the liver defect caused by *sek1* mutation is not due to impaired differentiation or maturation of hepatocytes [34]. Thus, SEK1 may provide crucial and specific growth and survival signals for hepatoblasts.

Interestingly, the findings of impaired SAPK/JNK activation in *sek1*$^{-/-}$ fetal livers extend our recent report that the synergistic activation of SAPK/JNK is impaired in SEK1-deficient murine embryonic stem cells and MKK7-deficient murine mast cells [35,36]. Furthermore, mice lacking MKK7 also show embryonic lethality between E11.5 and E12.5, with impaired liver formation and a decreased level of SAPK/JNK activation (our unpublished data). Thus, the synergistic activation of SAPK/JNK by SEK1 and MKK7 seems to occur in fetal livers and seems to be crucial for hepatoblast growth in mouse development (Fig. 4).

As described above, hepatoblast development in early hepatogenesis does not require definitive hematopoiesis. Therefore, growth factor(s), produced by nonblood cells, together with their receptor(s) on hepatoblasts, could relay SEK1 activation and promote cell growth and cell survival (Fig. 4). This idea is supported by a recent report showing that vasculogenic endothelial cells and nascent vessels are critical for the earliest stages of hepatogenesis, prior to blood-vessel function [37]. Various growth factors, such as HGF, epidermal growth factor, IL-1, and TNF-α have been implicated in hepatogenesis [38]. However, mice lacking the *TNF-α*, *TNFR1*, *IL-1*, or *IL-1R* genes did not have any defects in liver formation [39]. By contrast, HGF-deficient mice die between E13 and E16 with liver failure, and the embryonic livers are reduced in size and show extensive loss of hepatocytes [40,41]. We found that HGF is capable of stimulating SAPK/JNK activity in wild-type fetal livers at E10.5 and that the activation is markedly impaired in *sek1*$^{-/-}$ fetal livers. Thus, the HGF receptor, c-Met, is one of the candidates for relaying SEK1 activation and promoting hepatoblast growth and survival. However, *hgf*$^{-/-}$ mice die later than *sek1*$^{-/-}$ mice and have an additional defect in placental development [40,41]. Therefore, another factor(s), which could also induce SEK1 activation, may be more essential for hepatoblast growth in early hepatogenesis (Fig. 4). Thus, SEK1 may receive various signals from cell-surface receptors to regulate hepatoblast growth and survival in murine embryogenesis.

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Strategy for the Development of Cell Therapy Using Bone Marrow Cells to Repair Damaged Liver

Shuji Terai, Isao Sakaida, Naoki Yamamoto, Kaoru Omori, and Kiwamu Okita

Summary. Recently, the plasticity of stem cells in bone marrow to differentiate into other cell lineages has been reported. If we can use stem cells in bone marrow, we can easily isolate hepatic stem cells to regenerate liver. In particular, cell therapy using autologous bone marrow cells to regenerate blood vessels has already been done clinically. To develop cell therapy using bone marrow cells to repair damaged liver, we have to understand the mechanism by which bone marrow cells differentiate into hepatocytes. In this chapter, we report a strategy for the development of cell therapy for liver regeneration.

Key words. Bone marrow cell, Stem cell, Cell therapy, Liver regeneration

Introduction

Although liver transplantation is an established therapy for fulminant hepatitis and other severe liver diseases, the scarcity of brain-dead donors, particularly in Japan, limits the use of transplantation [1]. Regenerative medicine using stem cells will be an attractive therapy for patients suffering from severe liver failure. Regenerative medicine can be divided into three approaches. One approach is cell therapy, using special cells which have plasticity, such as embryonic stem (ES) cells and tissue-specific adult stem cells [2–4]. A second approach is therapy using tissue engineering techniques [5,6]. The third approach is therapy using bioartifical devices (Fig. 1) [7]. Among these three approaches, we would like to develop a new cell therapy using autologous bone marrow cells (BMCs) to repair damaged liver. Compared with other tissue-specific stem cells, the isolation of stem cells from bone marrow is easier than that of other tissue-specific stem cells [3,8–11]. Cell therapy using autologous BMCs presents few ethical problems and will have many medical applications for treating severe liver, pancreatic, and intestinal disease.

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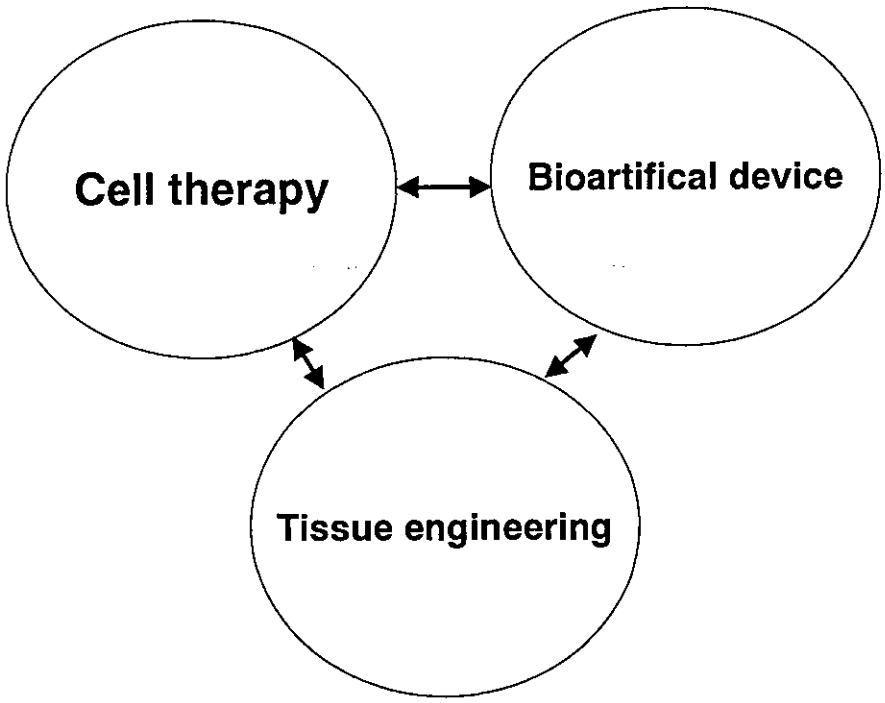

Fig. 1. Approaches to regenerative medicine

Hepatic Stem Cells are Present in Bone Marrow

Peterson et al. [8] and Theise et al. [12] showed the existence of hepatic stem cells in bone marrow by transplanting BMCs from male rats into females, then analyzing the recipients' hepatocytes for Y chromosome markers by fluorescent in situ hybridization (FISH). In human studies, the transdifferentiation of BMCs into hepatocytes was demonstrated by detection of the presence of Y chromosome markers in autopsied liver specimens from male-to-female bone marrow transplant recipients [9,13]. These results show the possibility of new cell therapy using BMCs [14]. On the other hand, after reports that spontaneous cell fusion is an important mechanism for the transdifferentiation of BMCs and tissue-specific stem cells, many people cannot accept the existence of cells in adult BMC that have multipotenty capacity for differentiation [15,16]. Although Laggase et al. [17], using a fumarylacetate hydrolase (FAH)-deficient model, reported that purified hematopoietic stem cells could become transdifferentiated into hepatocytes and compensate liver function, recently they reported that the mechanism of transdifferentiation of BMCs into hepatocytes arose from cell fusion between the BMCs and hepatocytes. Wagers et al. [18] also reported that there was little evidence of plasticity of adult hematopoietic stem cells. In a human study, Tran et al. [19] reported that cell fusion was a rare event, by analyzing for Y chromosome makers, using a FISH technique, in buccal epithelial cells after male-to-female BMC transplantation. On the other hand, Schwarz et al. [20] and Jiang et al. [21] established multipotent adult progenitor cells (MAPCs) in bone marrow with a culture system; these cells were derived from mesenchymal stem cells. At present, the mechanism for the plasticity of (BMCs) is unknown. Moreover, although there are various kinds of

cells in bone marrow, we could not confirm which of the cells in bone marrow are the real hepatic stem cells. To develop effective cell therapy with BMCs, we need a better understanding of the regulatory mechanisms that control BMC plasticity and BMC transdifferentiation into nonhematopoietic cell lineages.

Inflammation Signals and Liver Development

Hepatic stem cells can differentiate into hepatocytes under several different conditions [22]. For example, induction of liver damage in rats with 2-acetylaminofluorene and partial hepatectomy (AAF/PH) is one model system for the differentiation of hepatic stem (oval) cells [23]. Some inflammation signals, such as tumor necrosis factor (TNF)-alpha and the nuclear factor (NF)-κB pathway, are crucial for liver regeneration and hepatogenesis, as shown in studies of knockout mice [24–26]. These signals might also regulate the commitment of BMCs to a hepatocyte lineage. If inflammation signals have an important role in the differentiation of BMCs and hepatocytes, we have to analyze the interaction between these signals and the cells. The inflammation signal might create a specific microenvironment "niche" which regulates the differentiation and proliferation of cells [26] (Fig. 2).

Green Fluorescence Protein (GFP)/CCl4 Model: New in vivo Model to Monitor the Transdifferentiation of BMCs into Hepatocytes

To analyze the mechanism of the transdifferentiation of BMCs, we developed a new in vivo model, different from the FAH model, to monitor the differentiation of BMCs into functional hepatocytes [27]. In this model, we transplanted BMCs without adding prior culture. For the liver cirrhosis model in the recipient, we selected a model produced by the injection of carbon tetrachloride (CCl4), carried out for 4 weeks; this model is similar to liver cirrhosis in humans. In this study we used transgenic mice expressing GFP as a source of BMCs to explore the process of differentiation into

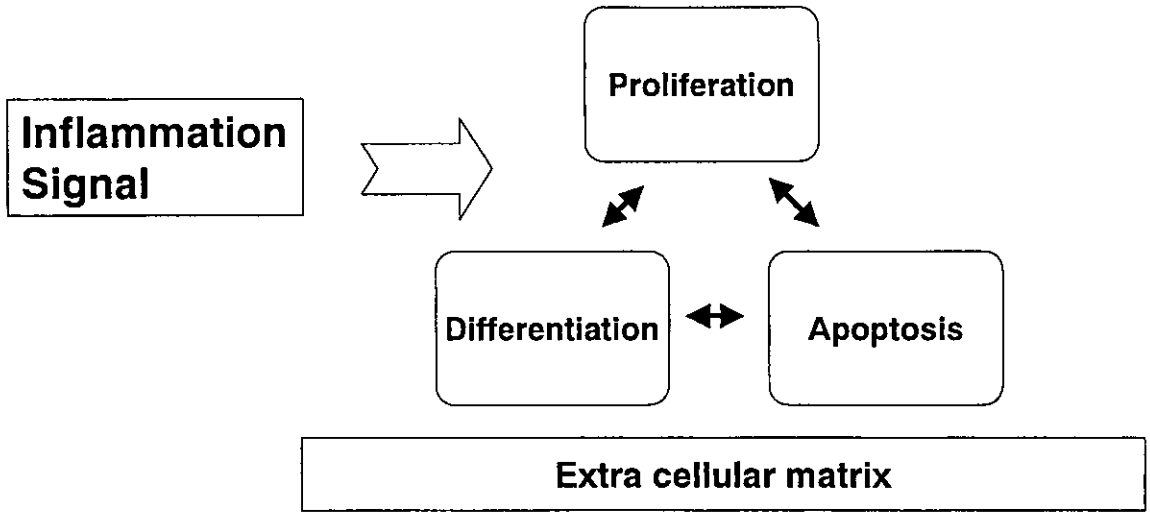

Fig. 2. Differentiation "niche"

hepatocytes [28]. We isolated GFP-positive BMCs and transplanted these GFP-positive BMCs into mice with liver cirrhosis. Mice with liver cirrhosis induced by CCl4 were injected with 1×10^5 nontreated GFP-positive BMCs via the tail vein. In these mice, the transplanted GFP-positive BMCs efficiently migrated into the periportal area of liver lobules after 1 day, and had repopulated one-fourth of the recipient liver by 4 weeks under conditions of persistent liver damage [27]. In contrast, no GFP-positive BMCs were detected following transplantation into control mice with undamaged livers. The BMCs transdifferentiated into functional mature hepatocytes via immature hepatoblasts. Serum albumin was significantly elevated by BMC transplantation to compensate for chronic liver failure. In our model, the transdifferentiation of BMCs into hepatocytes occurred under the conditions of persistent liver damage. These results suggested that the inflammation signal was also important for the transdifferentiation of BMCs into hepatocyte. Inflammation signals such as TNF-alpha and the NF-κB pathway are crucial for liver regeneration and hepatogenesis, as shown in studies of knockout mice [24–26]. The differentiation processes in BMC transdifferentiation and liver development are similar.

Discussion

The result with the GFP/CCl4 model showed that the condition of the recipient and the microenvironment are key factors for successful cell therapy using BMCs. If we consider the use of cell therapy with BMCs to repair damaged liver, we must evaluate the condition of the recipient. If the condition of the recipient is adequate for the transdifferentiation of BMCs into hepatocytes, we will succeed in repairing the damaged liver. To analyze the regulatory mechanism of the transdifferentiation system, our GFP/CCl4 model will be useful. Based on the analysis of this model, we will be able to evaluate the mechanism of transdifferentiation, using the analysis of gene expression and proteomics. By analyzing the mechanism, we will be able to develop a new cell therapy using BMCs to repair damaged liver.

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癌細胞のアミノ酸代謝

―ことに肝細胞癌を中心に―

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アークメディア

癌細胞のアミノ酸代謝

―ことに肝細胞癌を中心に―

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索引用語：amino acid, hepatocellular carcinoma, methionine, glutamine

1 はじめに

肝臓が営む機能は多岐にわたっており，肝硬変症ではさまざまな代謝・栄養障害がみられる(表1).

中でも蛋白質・アミノ酸代謝は重要で，分枝鎖アミノ酸(Branched chain amino acid; BCAA)を除く大部分のアミノ酸は肝臓で代謝される．一方，BCAAは肝臓以外の臓器(筋肉，脂肪組織，腎臓，脳)において肝臓には存在しない酵素(分枝鎖α-ケト酸デヒドロゲナーゼ複合体)により異化され，エネルギー源となる．したがって肝機能の低下した肝硬変症患者ではエネルギーを獲得するためBCAAの利用が促進し，その結果，血中のBCAA濃度は低下し，芳香族アミノ酸(Aromatic amino acid; AAA)やメチオニンは増加する．しかし同じ肝硬変症でも肝細胞癌を合併した患者では血中のメチオニンやチロシンの増加はそれほど顕著ではないとの報告があり[1]，このことからも肝細胞癌ではメチオニンなどのアミノ酸需要が大きいことが示唆される．本稿では，肝細胞癌のアミノ酸代謝についてこれまでの報告を総説するとともに，なかでも比較的報告の多いメチオニンとグルタミン(グルタミン酸とアンモニアの化合物)について概説する．

表1 肝硬変症患者にみられる代謝・栄養障害

①糖質代謝異常
②蛋白質・アミノ酸代謝異常
③脂質代謝異常
④ビタミン・微量元素異常
⑤骨代謝異常
など

2 肝細胞癌合併患者のアミノ酸分析

1. 血漿中アミノ酸分析

Watanabeらは肝性脳症のない肝硬変症患者群25名(肝細胞癌非合併群11名，肝細胞癌合併群14名)と健常人群10名の血漿中のアミノ酸を分析報告している[1]．これによると肝硬変症群では，アミノ酸総量は健常人群より少ないものの，メチオニンやチロシンは増加しており，BCAA/AAAモル比の低下

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表２　血漿中のアミノ酸分析（文献１表３を一部改変引用）

アミノ酸	健常群 (10) (μ mol/l)	肝癌非合併肝硬変 (11) (μ mol/l)	肝癌合併肝硬変 (14) (μ mol/l)
Taurine	150 ± 54	69 ± 18 ‖	63 ± 27 #
Aspartic acid	26 ± 9	12 ± 8 #	32 ± 28
Threonine	119 ± 32	119 ± 17	95 ± 25 §
Serine	149 ± 37	121 ± 35	110 ± 20 ‡
Asparagine	49 ± 12	60 ± 17	43 ± 12 §
Glutamine	161 ± 74	59 ± 27 ‡	92 ± 58 *
Glutamine	544 ± 174	668 ± 122	523 ± 205 †
Proline	166 ± 62	219 ± 217	116 ± 48
Glycine	234 ± 67	177 ± 40 *	171 ± 29 ‡
Alanine	341 ± 62	290 ± 59	266 ± 78 *
Valine	193 ± 44	121 ± 34 #	140 ± 32 ‖
Methionine	22 ± 14	46 ± 30 *	15 ± 8 ¶
Isoleucine	61 ± 13	38 ± 11 #	37 ± 10 #
Leucine	114 ± 28	65 ± 18 #	67 ± 19 #
Tyrosine	56 ± 15	123 ± 31 #	76 ± 22 *,**
Phenylalanine	64 ± 13	80 ± 28	62 ± 21
Ornithine	95 ± 35	74 ± 24	73 ± 16
Lysine	172 ± 53	124 ± 34 ¶	126 ± 39 *
Histidine	79 ± 17	74 ± 10	48 ± 20 ‖,**
Arginine	64 ± 12	76 ± 20	51 ± 12 *,**
BCAA/AAA	3.1 ± 0.4	1.2 ± 0.5 #	1.6 ± 0.4 #,**
Total amino acid	2,890 ± 451	2,573 ± 347	2,246 ± 523 ‡

*，‡，‖，#：健常群 vs 肝癌非合併肝硬変
†，§，¶，＊＊：肝癌合併 vs 肝癌非合併肝硬変
*，†：P＜0.05；‡，§：P＜0.02；‖，¶：P＜0.01；#，＊＊：P＜0.001

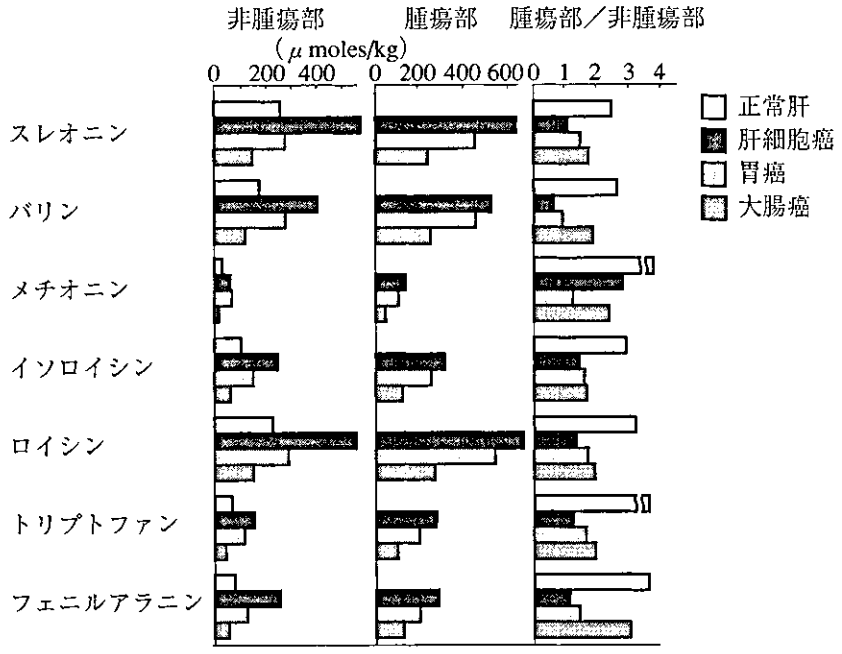

図１　固形癌のアミノ酸分析（文献１図３を一部改変引用）

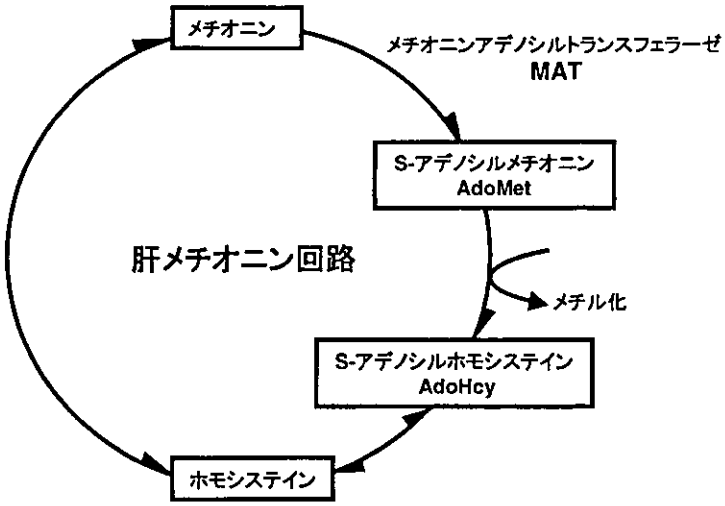

図2　メチオニン回路

が特徴である．しかし肝細胞癌合併群におけるメチオニンやチロシンの増加は，肝細胞癌非合併群より少なかった(表2)．

2．肝細胞癌部と背景肝のアミノ酸分析

多くの肝細胞癌患者は肝硬変症を合併しているため，全体でみると肝硬変症と同様の栄養状態といえ，BCAA/AAA モル比の低下がみられる．しかし，肝細胞癌そのものの含有アミノ酸を分析してみると，周囲の背景肝よりアミノ酸濃度は増加していた．中でもメチオニン[1,2]，チロシン[1]，フェニルアラニン[1]の増加がみられ，この傾向は他の癌(胃癌や大腸癌)でも同様に認められた(図1)[1]．一方，グリシンやシスチンは減少していた[2]．

さらに肝発癌動物実験ラットモデル(0.25％エチオニン含有食)での検討では，メチオニン，フェニルアラニン，アルギニン，プロリン，スレオニン，セリン，アスパラギン，オルニチンの増加が肝細胞癌部で認められ，メチオニンや芳香族アミノ酸の要求性が高いことがわかる[1]．

これらヒト臨床検体や動物モデルによる解析からも，肝細胞癌部ではアミノ酸需要が大きく，血液中や周囲肝から奪取したアミノ酸を原料にして増殖に必要な蛋白質を生合成していると考えられた[1,3]．このような状態を「窒素奪取(Nitrogen trap)の現象」という．

3　肝細胞癌とメチオニン

メチオニンは，S-アデノシルメチオニン(S-adenosyl-methionine; AdoMet)を経て3個のプロピオニル CoA とプロピオン酸へと最終的には分解される．中間産物の AdoMet は不安定なスルホニウムイオン(メチル基)をもっており，これが多くの生合成反応において強力なメチル化剤として働き(メチオニン回路)(図2)，肝臓で全メチル化反応の約85％が行われている．

肝硬変症ではこのメチオニン代謝に変調があることが古くから知られており[4]，これまでにも様々な動物実験モデルを通して解析されてきた．エタノールや四塩化炭素などによる肝障害時には，AdoMet が減少し，S-アデノシルホモシステイン(S-adenosyl-homocysteine; AdoHcy)が増加する．一方，AdoMet を補充することで肝障害が改善したとの報告が多くある[5~8]．さらに Pascale らは，ラット肝発癌モデル(部分肝切除，1,2-dimethylhydrazine, orotic acid 投与モデル)に AdoMet を投与すると，肝細胞癌の発生率は低下し，

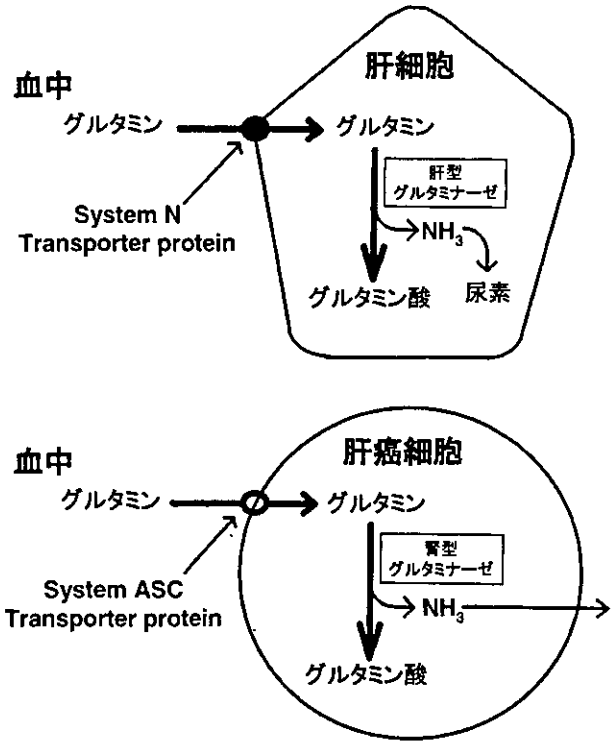

図3　肝細胞と肝癌細胞のグルタミン代謝
（文献16　図9を一部改変引用）

肝細胞癌中のapoptosisの頻度が増加していた，と報告している[9]．

メチオニンは肝癌細胞の要求性が高く，肝癌細胞中に多く含まれているにもかかわらず，肝発癌抑制効果をもつAdoMetは減少していた．これは，AdoMetはメチオニンアデノシルトランスフェラーゼ（methionine adenosyl transferaze; MAT）によりメチオニンから生成されるが，肝硬変や肝癌細胞ではMATのプロモーター領域がメチル化されているためMATの発現が減弱しているためである[10]．このようにメチオニン代謝に関与する酵素の発現低下が，肝硬変や肝細胞癌でのAdoMet減少，メチオニン増加につながっていると考えられる．

4　肝細胞癌とグルタミン

グルタミンはグルタミン酸と有毒なアンモニアとの化合物であり，組織中のアンモニア

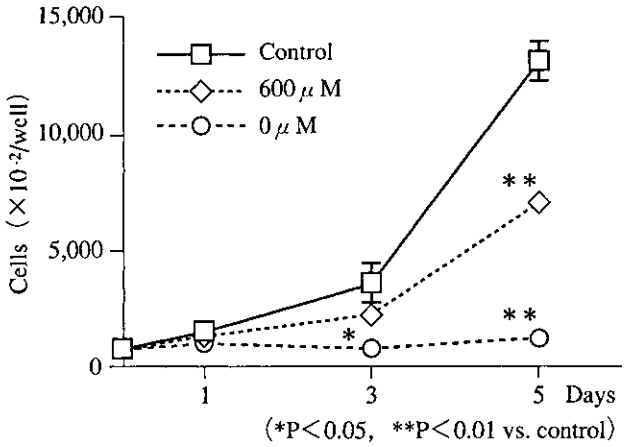

図4　グルタミン濃度と細胞増殖速度（HepG2）
（文献14図1Fより引用）

はグルタミンとして肝臓まで輸送される．輸送されたグルタミンは，肝ミトコンドリアにおいてグルタミナーゼによりグルタミン酸とアンモニアに再分解され，アンモニアは尿素サイクルを経由し無害な尿素となる．グルタミンは非必須アミノ酸であるが，腫瘍細胞にとっては蛋白や核酸合成，エネルギー源として大変重要なアミノ酸である．そのため肝癌細胞膜には，担癌の低栄養状態下でもその高い需要をみたすために特別な細胞膜トランスポートシステムが備わっている．グルタミンは正常肝細胞では，主にSystem Nと呼ばれるナトリウム依存性トランスポーターにより細胞内に輸送され[11,12]，肝型グルタミナーゼによりグルタミン酸となる．しかし肝癌細胞では，System Nとは異なる10～20倍の輸送速度をもつ細胞膜トランスポーター（System ASC）により細胞内へ輸送される[13~15]．さらに，グルタミンからグルタミン酸への分解反応も正常細胞の肝型ではなくグルタミンに対する親和性がより高い腎型グルタミナーゼにより行われ，これらの機構により肝癌細胞の要求はみたされている（図3）[16]．

また細胞内に取り込まれたグルタミンは，

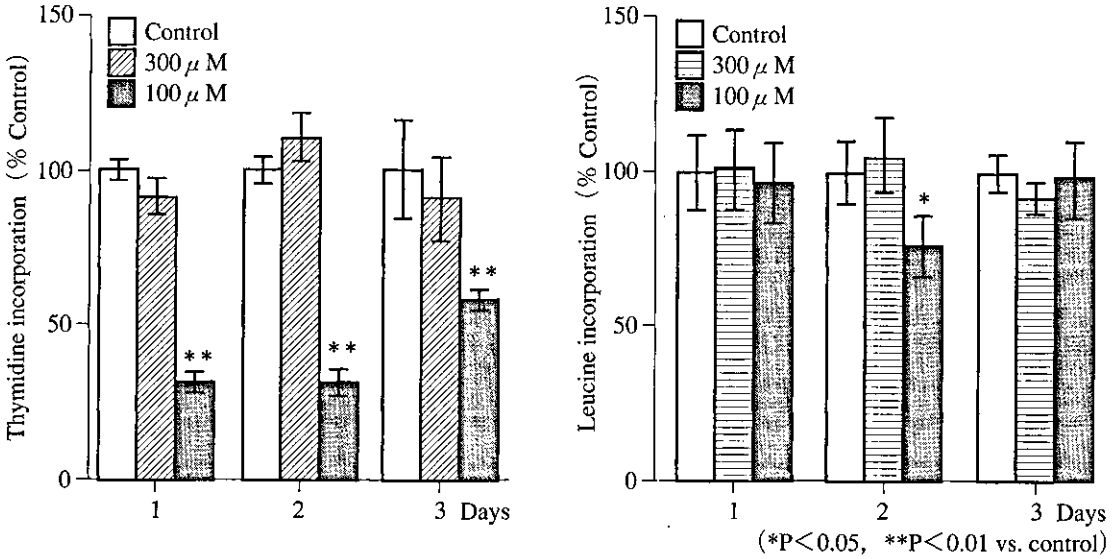

図5　グルタミン濃度とSK-Hep細胞におけるDNA，蛋白質合成
a：DNA合成，^{3}Hチミジンの DNA への取り込み
b：蛋白質合成（文献14　図4D, 5Eより引用），^{3}Hロイシンの蛋白質への取り込み

HepG2やSK-Hep細胞株を用いた検討では，濃度依存的に細胞増殖速度は増加しており，細胞増殖促進作用を持っている[16~18]（図4）．さらに，DNAや蛋白質合成においてもグルタミンは重要な調節因子である[14,17]（図5）．

5　おわりに～治療への応用～

このように肝細胞癌はアミノ酸需要が大きいため，癌細胞の蛋白や核酸代謝を選択的に抑制する目的で，特にメチオニン，チロシン，フェニルアラニンを欠乏させたアミノ酸インバランス療法が試みられた．肝細胞癌合併肝硬変患者では一般に免疫系の低下がみられ，チロシンやフェニルアラニンのアミノ酸インバランス状態では免疫賦活作用があるなど[19~22]，肝細胞癌への効果が期待できる．しかし，低栄養や脂肪肝などの副作用があり，さらなる検討が必要である．近年では，アミノ酸インバランスと抗癌剤を併用した治療も試みられている．

最近われわれは，アミノ酸の中でシステインに抗線維化作用があることを見出した[23]．

肝細胞癌は，言うまでもなく肝硬変を背景に発生することが多く，肝細胞癌対する治療のみならず，慢性肝疾患の進行阻止にもアミノ酸が有用である可能性があり，今後詳細にメカニズムを探っていくことが必要である．

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