(Fig. 4C). This stimulatory effect of HSc025 on migration of fibroblasts was also observed in the presence of 2.5 ng/ml of TGF-β (Fig. 4D).

3.5. HSc025 enhanced migration of collagen-producing cells into granulation tissue in vivo

The stimulatory effect of HSc025 on migration of cultured dermal fibroblasts was further verified using an *in vivo* experimental system. For that purpose, an artificial dermis graft was embedded into a fresh wound made on the dorsum of transgenic COL/EGFP mice. The wound tissue including the dermal graft was excised at day 7 and subjected to fluorescent microscopic observation to detect collagen-producing cells migrating into the graft tissue. EGFP-positive cells were observed in both untreated (Fig. 5A) and HSc025-treated mice (Fig. 5B). However, the number of such collagen-producing cells was significantly higher in HSc025-treated mice than in control animals independently of the distance from the wound edge (Fig. 5C). These results confirmed that HSc025 increases the migration speed of dermal fibroblasts *in vivo* as well as *in vitro*.

3.6. Identification of pirin as a mediator of HSc25 in stimulating fibroblast migration

In order to elucidate the mechanisms responsible for the stimulatory effect of HSc025 on fibroblast migration, RNA samples were prepared from cultured dermal fibroblasts untreated or treated with HSc025 and subjected to cDNA microarray analyses (GEO ID: GSE53672). The results indicated an increase in *pirin* gene expression in cells treated with HSc025. This HSc025-induced pirin expression was confirmed at both mRNA and protein levels using real time RT-PCR assays (Fig. 6A and B) and Western blot analysis (Fig. 6C), respectively. HSc025 treatment caused a modest but significant increase in the amounts of *pirin* mRNA and protein in time- and dose-dependent manner. Similarly, real time RT-PCR assays of wound tissue samples showed an increasing tendency of *pirin* gene expression in HSc025-treated mice compared with

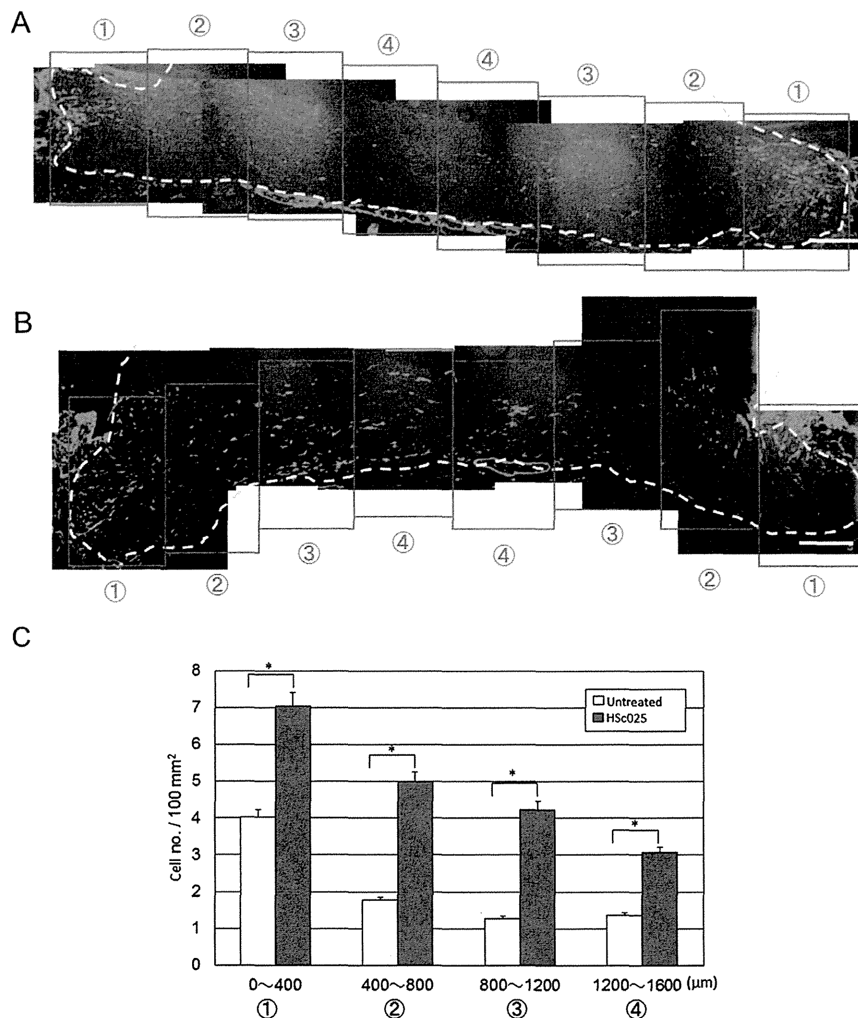

Fig. 5. Migration of collagen-producing cells into artificial dermis grafts. A full-thickness excisional wound made on the dorsum of female COL/EGFP mice (24 to 28 weeks of age) was embedded with an artificial dermis graft. Mice were then treated with daily local injections of 200 μl of either saline (A) or 100 μM of HSc025 (B) into the wounds. Dermal specimens were obtained at day 7 and analyzed under a fluorescent microscope to examine migration of EGFP-positive cells (*green*) into the artificial dermis graft. The *yellow hatched line* indicates the border between the autologous tissue and the embedded artificial dermis graft. The location of migrating EGFP-positive cells was divided into four categories (*red square boxes*) depending on the distance from the wound edge. The cell numbers were counted and compared between untreated and HSc025-treated mice (C). The values are expressed as means ± SE from eight wounds in each group. An *asterisk* indicates that the difference between the groups is statistically significant. *Scale bars*, 200 μm.

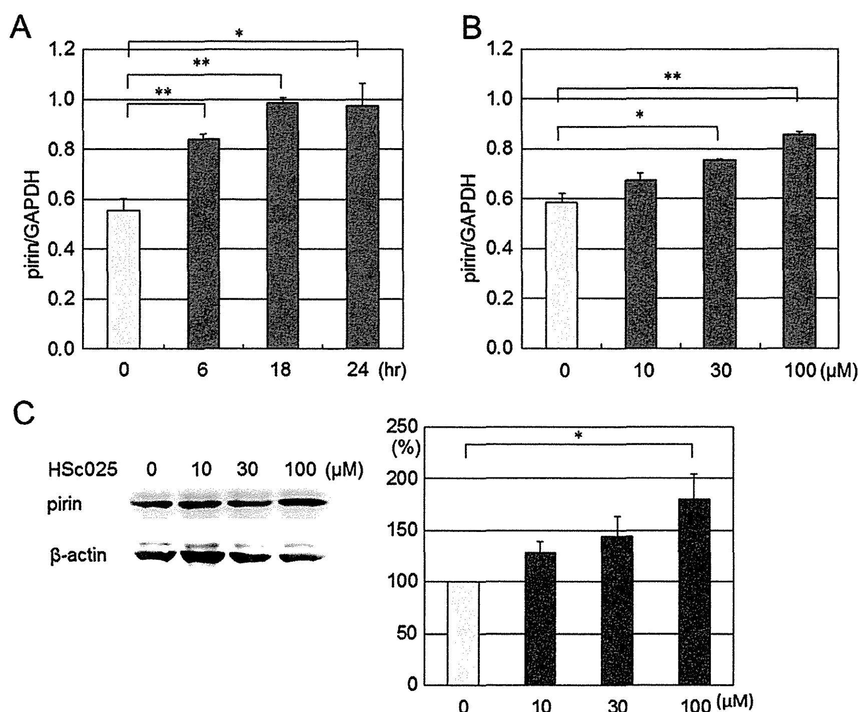

Fig. 6. Induction of pirin expression in HSc025-treated fibroblasts. Primary cultures of dermal fibroblasts were either untreated or treated with 30 μM of HSc025 for the indicated lengths of time (A), or with the indicated concentrations of HSc025 for 18 h (B) or 24 h (C). Total RNA and whole cell lysates were isolated and subjected to real time RT-PCR assays and immunoblot analyses, respectively. The relative expression levels of *pirin* mRNA were normalized against those of *GAPDH* gene in the same RNA preparation, and the values are expressed as means ± SE from one of the repeated experiments with three samples in each group (A and B). A representative picture of immunoblot analyses to detect expression of pirin and β-actin as an internal control is presented with the histograms showing the results of semi-quantitative analyses obtained from three independent experiments (C). An *asterisk* indicates that the value is significantly higher than that in pretreatment (A) or untreated control (B and C); * <0.05 and ** <0.01.

untreated animals at both day 2 (1.3-fold) and day 6 (1.6-fold) after the wounding (data not shown).

3.7. HSc025 enhanced migration of fibroblasts through induction of pirin expression

Since the induction of pirin expression by HSc025 was marginal as described above, we examined the effect of knockdown of pirin expression on migration of cultured fibroblasts. For that purpose, we employed the RNA interference technique using *pirin* siRNA. Transfection of dermal fibroblasts with the specific *pirin* siRNA caused knockdown of the endogenous pirin production nearly 90% at the protein level (Fig. 7A). Under this experimental condition, transfection with *pirin* siRNA, but not control siRNA, completely abolished the stimulatory effect of HSc025 on migration of cultured fibroblasts (Fig. 7B). These results therefore indicate that HSc025 does not simply increase the amount of pirin, but rather modifies its effect on fibroblast migration through unknown mechanisms.

4. Discussion

In this manuscript, we have shown that a novel small compound that was originally identified as an anti-fibrotic reagent suppresses macrophage infiltration and accelerates an early phase of wound healing after a full-thickness dermal excision. Experiments using primary cultures of keratinocytes and fibroblasts indicated that HSc025 acts on the two types of cells through different mechanisms. First, HSc025 stimulated proliferation and migration of primary keratinocytes mostly by antagonizing the TGF-β signal. Consistent with the results of an early study [21], incubation with 2.5 ng/ml of TGF-β suppressed both proliferation and migration of keratinocytes. HSc025 counteracted those inhibitory effects of TGF-β, but it exhibited no effects on the keratinocyte behaviors in the absence of TGF-β. Second, and in contrast to its action on keratinocytes, HSc025 stimulated migration, but not proliferation, of primary cultures of dermal fibroblasts both in the absence and presence of TGF-β. This indicates that the stimulatory effect of HSc025 on fibroblast migration is not a consequence of accelerated cell proliferation, and that HSc025 acts on dermal fibroblasts independently of TGF-β. The stimulatory effect of HSc025 on fibroblast migration was further confirmed by an *in vivo* experiment in which HSc025 treatment certainly increased the number of collagen-producing cells migrating into an artificial dermis graft (Fig. 5).

A comprehensive analysis of the gene expression profiles of untreated and HSc025-treated cultured fibroblasts has identified pirin as a critical mediator of HSc025 that stimulates cell migration. Pirin was originally reported as a highly conserved nuclear protein of unknown functions [22], and it has been reported recently that its interaction with Bcl3 regulates migration of melanoma cells [23]. In that experiment, siRNA knockdown of pirin expression or treatment with a small molecule inhibitor (triphenyl compound A) of the pirin–Bcl3 interaction suppressed migration, but not proliferation, of melanoma cells. Moreover, treatment of melanoma cells with triphenyl compound A, as well as knockdown of the endogenous pirin and Bcl3, down-regulated *SNAI2* expression [23]. Interestingly, SNAI2 (also known as SLUG) has been implicated in mobility of various cell types including keratinocytes [24], and SLUG is considered necessary for proper re-epithelialization during wound healing [25]. The results of the present study suggest that SNAI2/SLUG may also play an important role as a downstream effector of pirin in regulating fibroblast migration. This was supported by the *in vivo* findings that *pirin*

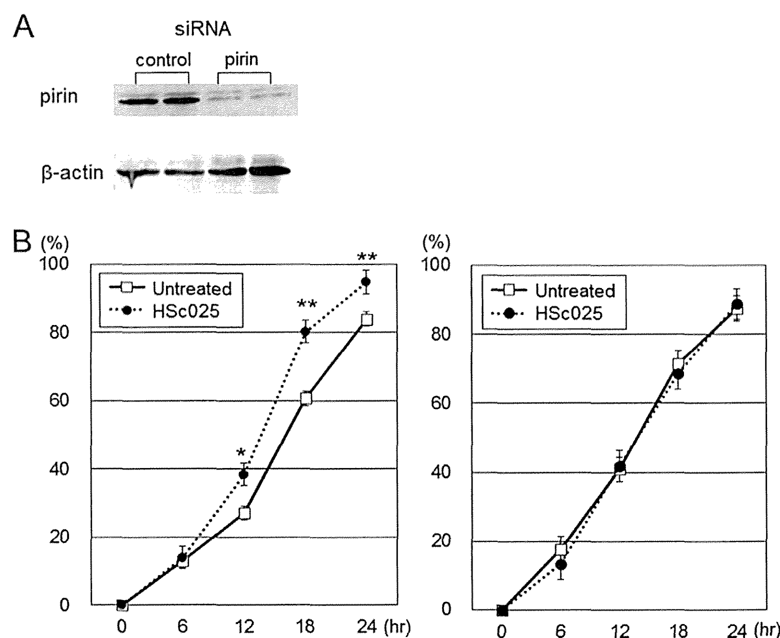

Fig. 7. Effects of knockdown of pirin expression on HSc025-stimulated fibroblast migration. Primary cultures of dermal fibroblasts were subjected to repeated transfection with 2 μg of control or *pirin* siRNA every 24 h for a total of three days. Efficient knockdown of the endogenous pirin expression was confirmed by immunoblot analyses using anti-pirin antibodies (A). Transfected cells with control (*left* in B) or *pirin* siRNA (*right* in B) were monitored for migration every 6 h. The values are expressed as means ± SE from 10 samples in each group. An *asterisk* indicates that the value of HSc025-treated sample is significantly higher than that in untreated cells at each time point; *<0.05 and **<0.01.

mRNA expression was up-regulated in the HSc025-treated granulation tissues following the excisional wounding.

It should be noted that, although only a marginal increase in pirin expression was induced by HSc025 treatment, knockdown of the endogenous pirin expression by siRNA transfection completely abolished the effects of HSc025 on fibroblast migration (Fig. 7). These findings may suggest that HSc025 does not increase the amount of pirin remarkably, but rather alters the intracellular localization of pirin and/or modifies its interaction with Bcl3 or other nuclear factors. Indeed, we have previously shown that HSc025 interferes with the interaction between YB-1 and its cytoplasmic anchor protein and accelerates nuclear translocation of YB-1, resulting in transcriptional regulation of target genes [13]. Further studies such as experiments using pirin knockout mice are necessary to clarify the detailed molecular mechanisms by which HSc025 enhances the action of pirin and accelerates dermal wound healing.

Early studies using TGF-β neutralizing antibodies [26] and a synthetic TGF-β antagonist [27] have shown that TGF-β is certainly a therapeutic target for accelerating wound healing as well as for reducing excessive scaring. This was confirmed by a study using Smad3-null mice, which clearly demonstrated accelerated wound healing characterized by reduced inflammatory monocyte infiltration and stimulated keratinocyte proliferation [6]. The results of the present study are consistent with those of the Smad3-null mouse experiments: HSc025 suppressed macrophage infiltration (Fig. 2) and accelerated the wound closure process (Fig. 1A and B). However, one of the concerns to use anti-fibrotic reagents for accelerating wound healing is that it may suppress the proper collagen expression and result in the decreased cutaneous tissue integrity. However, there was no significant deference in the overall collagen promoter activities in granulation tissues between untreated and HSc025-treated mice (Fig. 1C). Nor did Sirius red staining show any difference in the amounts of accumulated collagen between the two groups (Fig. 2E and F). This is probably because HSc025 increases the number of collagen-producing fibroblasts migrating into granulation tissue while decreasing the collagen expression levels in an individual cell. Alternatively, different cellular and molecular mechanisms govern the physiological wound healing and pathological dermal fibrosis [7,28], and fibroblasts present in normal and fibrotic dermal tissues respond differently to anti-fibrotic reagents such as HSc025.

It is also important to note that HSc025 accelerated an early phase of physiological healing after a full-thickness dermal excision, but did not shorten the overall period to re-epithelialization dramatically. It is of great worth to examine the effect of HSc025 in pathological conditions where dermal wound healing deteriorates significantly. Work is in progress in our laboratory to explore the detailed signaling pathway and the precise mechanisms responsible for such a beneficial effect of HSc025 on dermal wound healing, which eventually contributes to the development of a novel treatment strategy for intractable skin ulcer.

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肝線維化改善の分子・細胞基盤

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索引用語：肝線維症, Matrix metalloproteinase (MMP), Tissue inhibitor of metalloproteinases (TIMP), 活性化星細胞, 線維化治療

1 はじめに

かつての病理学や肝臓病学の教科書を紐解くと，肝硬変は進行性かつ不可逆的な疾患であると記載されている．ところが，他稿で詳述されるように近年のウイルス性慢性肝炎に対する治療法の進歩は，進行した肝線維症といえども可逆的な病態であることを証明した．これまで，アルコール性肝硬変の禁酒症例など，日常臨床で感じていた肝線維化の可逆性が実証された意義は大きい．組織におけるコラーゲンの含量は，その合成とMatrix metalloproteinase (MMP)による分解とのバランスの上に成り立っており（図1），コラーゲン合成を促進させる原因を取り除くことによって，進行した肝線維症であっても組織学的に改善する．また，原因治療が困難な場合であっても，コラーゲンの合成を抑制する，あるいは分解を適切に誘導することで，肝線維症は治療可能な疾患である．「進行性かつ不可逆的」であったのは，肝線維症に対する効果的な治療法が存在せず，コラーゲン産生をきたす刺激が慢性的に反復していたからに他ならない．

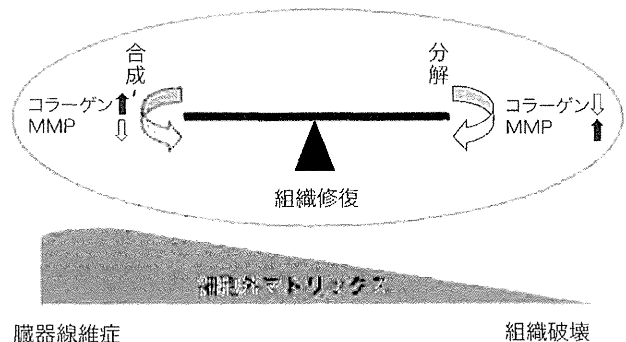

図1　コラーゲンの合成系と分解系

組織におけるコラーゲンの含量は，合成と分解のバランスの上に規定されている．その適切な発現は，組織修復や創傷治癒過程において重要な働きを演じているが，調節機構が破綻をきたすと組織に過剰のコラーゲンが沈着して諸臓器の線維化を引き起こす一方，分解系が優位に傾くと組織破壊や脆弱性が問題となる．

肝線維化の進展機序に関する研究の進歩と

Yutaka INAGAKI et al : Molecular and cellular basis for regression of liver fibrosis
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比較して，改善機序の研究はこれまで立ち遅れている感があったが，最近になって肝線維化の改善過程における活性化星細胞の動態について興味深い知見が得られるようになってきた．

本稿では，肝線維化改善の分子・細胞基盤に関する研究の現状を概説するとともに，硬変肝が正常肝にどこまで近づくかを規定する要因や，肝線維化改善機序の解明に立脚した新規治療法の開発に向けて克服すべき問題点についても言及したい．

2 肝線維化改善機序の研究の難しさはどこにあるのか

肝線維化の進展過程において中心的役割を担う星細胞の活性化機序や，活性化星細胞(筋線維芽細胞)によるI型コラーゲンの産生調節機構など，肝線維化の進展機序の研究は最近の20年余りで大きな進歩を遂げた．それと比較して，肝線維化の改善機序に関する研究，とりわけコラーゲン分解系の研究が立ち遅れているのには，いくつかの理由がある．

第一に，ヒトにおいては線維肝組織に増加したコラーゲン線維を分解する主要な間質性コラゲナーゼであるMMP-1が，ラットやマウスといった齧歯類には存在しないことがあげられる．齧歯類においてはMMP-1と相同性が高いMMP-13がこれに代わる間質性コラゲナーゼとされるが，ヒトにもMMP-13は存在している．ヒトにおけるMMP-1の発現が全身の臓器・組織に広範に認められるのに対して，MMP-13の発現は極めて限局的である．多くの実験的肝線維症モデルには齧歯類が用いられているが，種々の病態において齧歯類のMMP-13がヒトのMMP-1と全く同一の発現動態や働きを示すかは不明であり，齧歯類で得られた結果がそのままヒトの肝線維症に当てはまるかは即断できない．

次に，MMPが肝線維化の進展や改善の各病期において相反する病因的意義を有することを指摘しておきたい．例えば，後述するように肝線維化の改善過程においてはMMPの発現増加や活性化が重要な役割を演じるとされるが，MMP-13遺伝子を欠損させたマウスでは肝線維化の進展はむしろ改善していた[1]．これは，肝線維化の初期段階では既存の小葉構造を破壊してI型コラーゲンの沈着を促すうえでMMP-13が重要な働きを演じることを示唆しており，肝線維化の改善過程においてMMP-13が沈着したI型コラーゲンを分解してむしろ線維化改善に寄与する[2]のとは好対照である．

最後に，これまでにMMPの産生細胞と報告されている星細胞(MMP-2, MMP-13)やマクロファージ(MMP-9, MMP-13)は，まさにI型コラーゲン，あるいはその発現を促進するTransforming growth factor-β (TGF-β) やケモカインの産生細胞である．しかもMMPは単にコラーゲンを分解するのみならず，タンパク質分解酵素としてTGF-βの活性化反応にも深く関わっている．肝線維化の進展や改善過程において，同一細胞もしくは同一細胞集団内のコラーゲン合成と分解のシグナルがどのように相反的あるいは協調的に制御されているのかは興味深い問題である[3]．

3 肝線維化改善機序の研究はどこまで進んだか

1. 肝線維化改善過程におけるMMPとその抑制因子の発現動態

肝線維化の進展と改善におけるMMP研究は，線維肝組織中にコラーゲン分解活性を見いだした報告にその端を発する[4]．四塩化炭素の反復投与に代表される齧歯類を用いた実

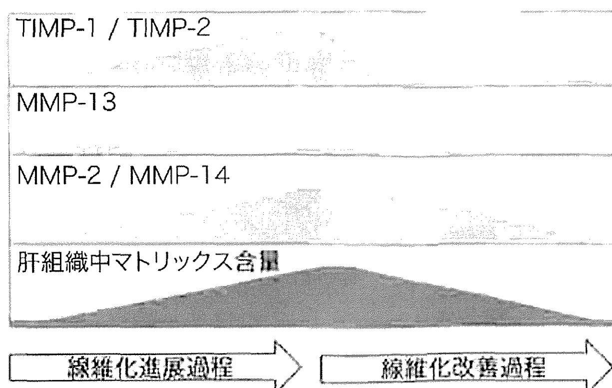

図2　肝線維化の進展ならびに改善過程におけるMMPとTIMPの発現動態

四塩化炭素の反復投与を中止すると，肝線維化の進行に伴って増加していたTIMP-1/TIMP-2の発現は速やかに低下する．この際，MMP-13は一過性に発現が増加し，その抑制因子であるTIMPの発現低下と相まってコラーゲン線維を分解する．また，MMP-2とその活性化を促すMMP-14は，TIMP-1/-2と同様に線維化進展に伴って発現が増加しているが，線維化改善過程においてはTIMPと比較してゆっくりと発現が低下するため，線維分解に寄与すると考えられている（文献2, 5, 10を統合）．

験的肝線維症の特徴は，線維化刺激がなくなると速やかにコラーゲン線維の分解と吸収がみられることである．そこで，このモデルを用いて肝線維化の改善過程におけるMMPやその抑制因子(Tissue inhibitor of metalloproteinases : TIMP)の発現動態や産生細胞が研究されてきた．

MMP-13は，先に述べたように齧歯類における主要な間質性コラゲナーゼである．その発現は正常肝ではほとんど認められず，線維化の進展過程においてもわずかな発現増加にとどまる．しかしながら，四塩化炭素の反復投与を中止するとMMP活性を抑制するTIMP-1およびTIMP-2の発現が急激に低下するため，MMP活性が増加する(図2)．しかも後述するように，この時期に一致して活性化星細胞がアポトーシスに陥るために，新たなコラーゲン産生も抑制されて線維化の改善に至るというモデルが提唱された[5]．TIMP-1の線維化改善における重要性は，TIMP-1トランスジェニックマウスを用いた研究により直接的に証明された．すなわち，TIMP-1を過剰発現すると四塩化炭素投与中止後の活性化星細胞のアポトーシスと肝組織中のMMP-2活性が抑制され，肝線維化の改善が有意に阻害された[6]．

また，同じく四塩化炭素投与モデルを用いた研究により，肝線維化の回復過程に一過性にMMP-13発現が上昇すること，その産生細胞の一部がα-smooth muscle actin（αSMA）陽性の活性化星細胞であることが報告された(図2)[2]．MMP-13の由来としては，線維組織に浸潤したマクロファージとする報告もある[7]．筆者らは，骨髄をEnhanced green fluorescent protein (EGFP)陽性細胞で置換したマウスに四塩化炭素を投与して，骨髄細胞の線維肝組織への浸潤とMMP産生について検討した．その結果，肝線維化の改善過程において骨髄から流入・生着した未分化な幹前駆細胞がMMP-13を発現しており，これらは肝組織中のMMP-13産生細胞の約半数を占めていた[8]．また，MMP-13の下流に位置し，MMP-13によって活性化されるMMP-9についても，既知の好中球やマクロファージに加えて骨髄由来の未分化な前駆細胞による産生が確認され，肝線維化の改善における骨髄と末梢の臓器相関が示された[9]．

MMP-2は，その上流に位置する活性化因子MMP-14とともに，肝線維化の進展に伴って発現が著しく増加する．その主たる基質は基底膜の構成成分であるⅣ型コラーゲンであるが，Ⅰ型コラーゲンや部分的に分解されたゼラチンに対する分解活性も有するため，TIMP発現が低下した状況下ではMMP-13と

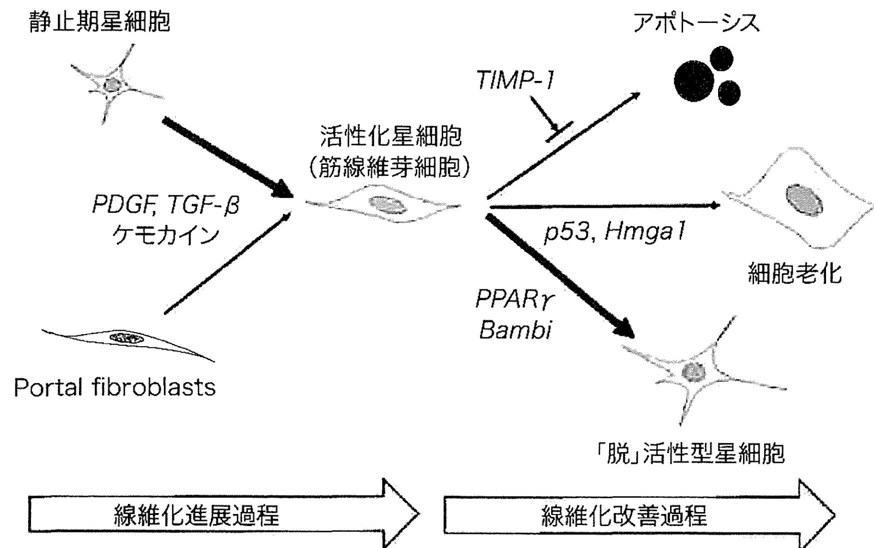

図3　コラーゲン産生細胞の活性化と「脱」活性化

肝実質内の星細胞や門脈域に存在するportal fibroblastsは，肝線維化刺激によって筋線維芽細胞(様細胞)に形質転換して，活発にコラーゲンを産生する．一方，線維化の改善過程においては，活性化した星細胞はアポトーシスや細胞老化に陥ることで排除されると考えられていたが，最近になってその約半数が非活性型に移行(「脱」活性化)することが報告された．

協調して肝線維化の改善に寄与する可能性が考えられる(図2)[10]．

2. 肝線維化改善過程における活性化星細胞の変容

前述したように，四塩化炭素の反復投与を中止して肝線維化所見が改善する際に活性化星細胞数は急激に減少し，アポトーシスに陥ると考えられてきた[5]．しかも，筋線維芽細胞のアポトーシスはMMP-14を介してMMP-2活性を増加させることから，いっそう線維化を改善させる[11]．加えて，肝線維化の改善には活性化星細胞の老化(senescence)が深く関わることが報告された[12]．細胞老化に陥った活性化星細胞はNK細胞の標的となって排除されることで，線維化の改善が促進されるという(図3)．ところが，活性化星細胞のアポトーシスや細胞老化は線維化刺激により増加し，線維化改善過程においてのみ見られる現象ではない．しかも，線維化改善過程においてアポトーシスや細胞老化に陥る細胞数は限られている[5,12]．

最近，実験的肝線維症の改善過程において活性化した星細胞の約半数は非活性型に戻る(「脱」活性化)という報告が相次いでなされた(図3)[13,14]．これまでも，培養星細胞の活性型と非活性型との相互移行については脂肪細胞の分化・脱分化過程と類似してperoxisome proliferator-activated receptor γ (PPARγ)などの転写因子による制御が報告されていたが，この可逆性が*in vivo*においても証明された意義は大きい．しかしながら，線維化改善に伴って脱活性化した星細胞は線維化刺激を全く受けていない静止期の星細胞と同一とはいえず，再度の線維化刺激に対する反応性も高いという．星細胞が有するこの可逆性を肝線維症の治療に応用するには，さらに詳細なメカニズムの解明が必要である．また，活性化星細胞以外にも，門脈域に存在するportal fibroblastsは胆管結紮モデルに代表される胆汁うっ滞に伴う肝線維症の発症と進展におい

て重要な役割を演じている．脱活性化が星細胞のみに認められる現象か，portal fibroblastsから派生した筋線維芽細胞においても共通してみられるかは，肝線維症に対する原因別テーラーメード治療の必要性を考えるうえでも重要な問題である．

4 硬変肝はどの程度まで正常肝に近づくのか

肝線維症の可逆性を論じる場合には，“Reversal”と“Regression”という2つの用語を意識して使い分ける必要がある．Reversalとは進行した肝線維症であっても正常な肝小葉構造に復する意味合いが強く，これに対してRegressionは線維化の程度に一定の改善が認められる場合を指す[15]．完成された硬変肝は，本当に正常肝に戻るのであろうか．細胞外に分泌されて組織に沈着したコラーゲン分子は，その成熟の過程で共有結合を介した架橋形成(cross-link)が完成するとマトリックス構成タンパク質としての安定性が増し，MMPによる分解を受けづらくなる[16]．また，進行した線維肝組織が正常の小葉構造に回復しうるかは，線維化進展に伴う血管走行の不可逆的変化によっても左右されるが，その詳細については他稿に譲る．ヒトの肝硬変を対象とした病理組織学的研究では，micronodular cirrhosisは線維化の改善に伴ってmacronodular cirrhosis，次いで不完全な線維性隔壁へと変化するという[17]．

近年，C型慢性肝炎の治療法がいっそう進歩するに伴って，血中からウイルスが消失してもかつて報告されたような肝線維化の改善が認められない症例が経験されるようになってきた．肥満やアルコール多飲などの他の要因が存在する症例は別としても，抗ウイルス治療法の適応拡大に伴って肝硬変を含むより進行した肝線維化症例を治療対象としていることに留意すべきである．肝線維化の進行症例では，その罹病期間の長さとコラーゲン線維の蓄積量の両面から見てもマトリックスの架橋形成の程度や血管走行の変化が著しく，たとえ原因治療が奏効しても組織に蓄積したコラーゲン線維の分解に長期間を要することは容易に想像できる．

5 コラーゲン分解機序の解明をいかに肝線維化治療につなげるか

肝線維化の進展がコラーゲン合成と分解の不均衡によってもたらされるものならば，その治療は合成系と分解系のバランスの是正に他ならない(図1)．実際，ヒトMMP-1遺伝子[18]もしくはMMP-13遺伝子[19]を，アデノウイルスベクターを用いて線維肝組織に過剰発現させることで実験的肝線維症の改善が報告されている．しかしながら，コラーゲンは組織や臓器の形態保持とともに創傷治癒や組織修復においても重要な役割を担っており，MMPの過剰な発現は組織の脆弱性や関節軟骨の破壊などを引き起こすことが懸念される．アデノウイルスは肝細胞に高い親和性を有することから他臓器への影響は少ないが，これに代わる低分子化合物などを薬剤として投与する際には，線維肝組織に選択的に運搬・作用させる工夫が必要となる．

一方，実際の臨床試験に目を向けると，薬効自体の問題や副作用の懸念のみならず，臨床研究デザインの限界が抗線維化治療薬の開発において大きな障害になっている．すなわち，さまざまな進行速度を有する多くの慢性肝疾患患者の中から比較的均一な対象集団を設定し，通常10年単位の長期経過をたどる肝線維症に対する薬物の投与効果を，1年前後という短期の介入試験で評価することの困

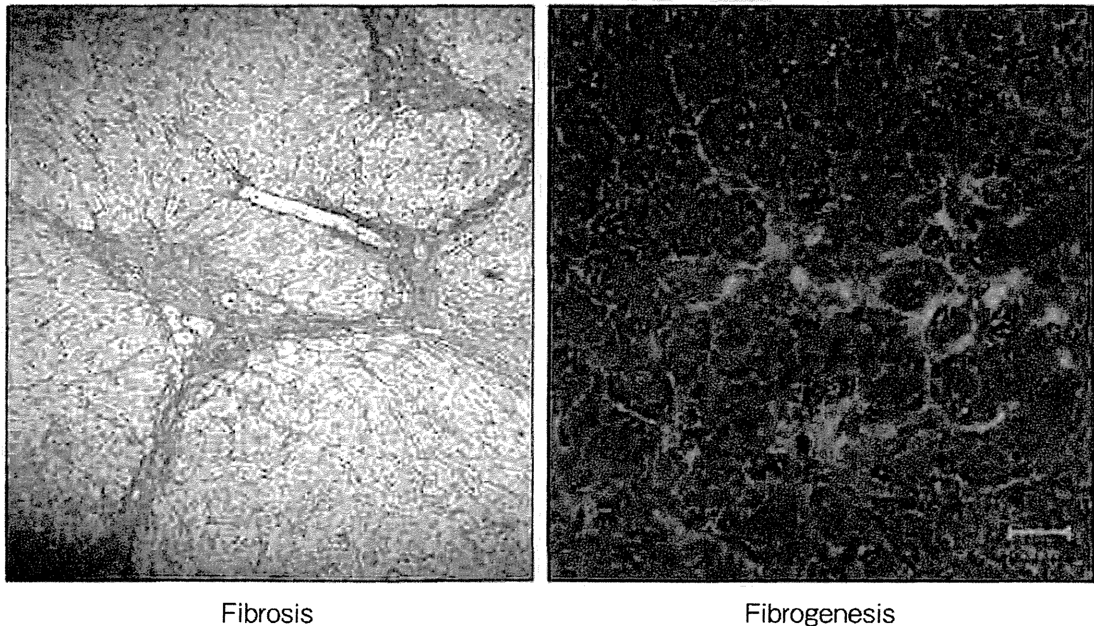

図4　FibrosisとFibrogenesis/Fibrolysis

臓器線維症(fibrosis)は，組織学的に細胞外におけるコラーゲン線維の蓄積を確認することで診断される静的な病態である(左図)．これに対して，右図は筆者らが開発したコラーゲン産生細胞をEGFP蛍光で可視化するレポーターマウス[24]に四塩化炭素を投与した際の蛍光観察像で，ここでは細胞のI型コラーゲン産生(fibrogenesis)を同遺伝子プロモーターの活性化として動的に捉えている．抗線維症治療薬の開発においても，線維の合成(fibrogenesis)と分解(fibrolysis)をリアルタイムで捉えるようなマーカーが必須である．

難さである．肝組織生検は今なお最も信頼できる肝線維症の診断手段であるが，全症例に対して治療前後で実施することは現実的でない．また，肝生検のstage分類で得られる線維化の情報は組織に沈着したコラーゲン線維の半定量化であり，静的な指標である[20]．近年開発された超音波装置やMRを用いた肝の弾性度診断も，その低侵襲性に大きな利点があるものの，基本的には組織のコラーゲン量を反映している．感度および特異度に優れた肝線維化の非侵襲的診断方法，しかも線維化の程度(fibrosis)ではなく，コラーゲンの合成系(fibrogenesis)や分解系(fibrolysis)の動的な評価系が熱望されるゆえんである(図4)．最近，血清タンパク質の糖鎖構造の変化が線維化の進展と改善を鋭敏に反映することが報告された[21]．新たな肝線維化の動的マーカーとして期待したい．

6 おわりに

Pérez-Tamayo[22]やRojkind[23]といった肝線維化研究の先達が，臨床例における肝線維症の可逆性を指摘して以来，すでに35年が経過した．この間のウイルス性肝炎に対する治療法の進歩は，ヘモクロマトーシスやウイルソン病といった特殊な代謝性疾患でなくとも肝線維化の改善が起こりうることを実証した．近年の肝線維症治療薬の開発に対する産学の関心の高まりは著しい．培養細胞を用いた試験や動物実験によって同定された数多くの抗線維化作用物質をいかに臨床応用に結びつけるか，どのような患者を線維化治療の対象に選んでどのような評価系を構築すべきか，線維肝組織に選択的に薬剤を到達させて副作用を軽減するにはどうしたらよいのか，肝線維症治療薬の一刻も早い臨床応用に向け

てさらなる研究と工夫が求められている．

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Review

Fibrogenesis and Carcinogenesis in Nonalcoholic Steatohepatitis (NASH): Involvement of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinase (TIMPs)

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Abstract: Nonalcoholic steatohepatitis (NASH) is emerging worldwide because life-styles have changed to include much over-eating and less physical activity. The clinical and pathophysiological features of NASH are very different from those of HBV- and

HCV-chronic liver diseases. The prognosis of NASH is worse among those with nonalcoholic fatty liver diseases (NAFLD), and some NASH patients show HCC with or without cirrhosis. In the present review we discuss fibrogenesis and the relationship between fibrosis and HCC occurrence in NASH to clarify the role of MMPs and TIMPs in both mechanisms. Previously we proposed MMP and TIMP expression in the multi-step occurrence of HCC from the literature based on viral-derived HCC. We introduce again these expressions during hepatocarcinogenesis and compare them to those in NASH-derived HCC, although the relationship with hepatic stem/progenitor cells (HPCs) invasion remains unknown. Signal transduction of MMPs and TIMPs is also discussed because it is valuable for the prevention and treatment of NASH and NASH-derived HCC.

Keywords: hepatocellular carcinoma (HCC); nonalcoholic steatohepatitis (NASH); nonalcoholic fatty liver disease (NAFLD); matrix metalloproteinase (MMP); tissue inhibitor of metalloproteinase (TIMP); cancer invasion; cancer metastasis; bone marrow-derived stem cell; hepatic progenitor cell (HPC); cancer stem cell

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1. Introduction

Simple hepatic steatosis, or fatty liver, is often seen in patients with obesity, diabetes mellitus, dyslipidemia and metabolic syndrome in the developed and developing countries (reviewed in [1–5]). Among them, the patients who never drink alcohol, or drink less than 70 g/week for women and 140 g/week for men are defined as nonalcoholic fatty liver disease (NAFLD) if the patients do not have any drug history, negative HBV- or HCV-related markers or negative markers for autoimmune liver diseases, and fatty liver is observed by hepatic echography, CT or MRI (reviewed in [1–6]). NAFLD is seen in 9% to 37% of the general population, and is currently emerging in both developed and developing countries due to changing life-styles (reviewed in [1–6]).

Nonalcoholic hepatitis (NASH) reported by Ludwig *et al.* in 1980 [7], has been clarified to be a severe form of NAFLD; 13% to 31% cases of NAFLD progress to NASH (reviewed in [4,5]). NASH is very similar to alcoholic hepatitis (ASH) in pathology (reviewed in [1–7]). Its prognosis is somehow better than that of ASH (reviewed in [1]), but 9% to 20% of NASH patients usually progress to liver cirrhosis (reviewed in [3,4–6,8]). NASH is seen in 2% to 5% of the general population (reviewed in [4,5]). NAFLD include a large spectrum of chronic liver diseases from simple hepatic steatosis or fatty liver, through NASH, to cirrhosis post-NASH. As NASH progresses to cirrhosis, steatosis progressively disappears with the development of fibrosis to cirrhosis, a phenomenon known as "burn-out NASH" [8], and such cases may present as cryptogenic cirrhosis ([9], reviewed in [1–8]). As it is difficult to discriminate NASH among NAFLDs, scoring of histological findings by liver biopsy has been developed [10].

Some patients with NASH show hepatocellular carcinoma (HCC) with or without liver cirrhosis [11]. Hepatocellular carcinoma (HCC) ranks third in cancer mortality and annual deaths number over 600,000 [12]. Studies have shown that HCCs are due mainly to hepatitis B virus (HBV) infection (50% to 80%) and hepatitis C virus (HCV) infection (10% to 25%) (reviewed in [13]). Geographical endemic

infection in the World varies: in China nearly 99% HCC are reported to be HBV-related, while 12% is HCV-related in another report; in Gambia HBV-related HCC is 61%, while HCV-related HCC is 19%; in Japan HBV-related HCC is 15% and HCV-related HCC 61%; in the USA HBV-related HCC is 16% and HCV-related 36% (reviewed in [8,13]). HCC patients with HBV (with HIV) or HCV have shown HBV-related or HCV-related chronic hepatitis and/or liver cirrhosis prior to development of HCC (reviewed in [13,14]). In the USA 22% of HCC cases is associated with alcohol-induced liver disease and more than 40% associated with diabetes, NAFLD and NASH (reviewed in [13]). In Japan 15% of HCC is associated with diabetes, metabolic syndrome, NAFLD and NASH (reviewed in [13]). In developed countries (Japan, USA, Europe) HCV-related HCC has decreased, but a new trend in HCC development is emerging with changes in environment and lifestyles, e.g., the growing burden of diabetes, metabolic syndrome and obesity (reviewed in [13]).

We have investigated fibrogenesis and fibrolysis in liver diseases ([14–28], reviewed in [29–31]) as well as the occurrence and stromal invasion of hepatocellular carcinoma (HCC) from the viewpoint of MMP science ([32–34], reviewed in [35]). The present review discusses the fibrogenesis of NASH, the relationship between fibrosis and HCC occurrence in NASH, and finally introduce past, present and future prospects of HCC carcinogenesis and its relation to MMPs and TIMPs for application to HCC treatment.

2. Method for Selection of References

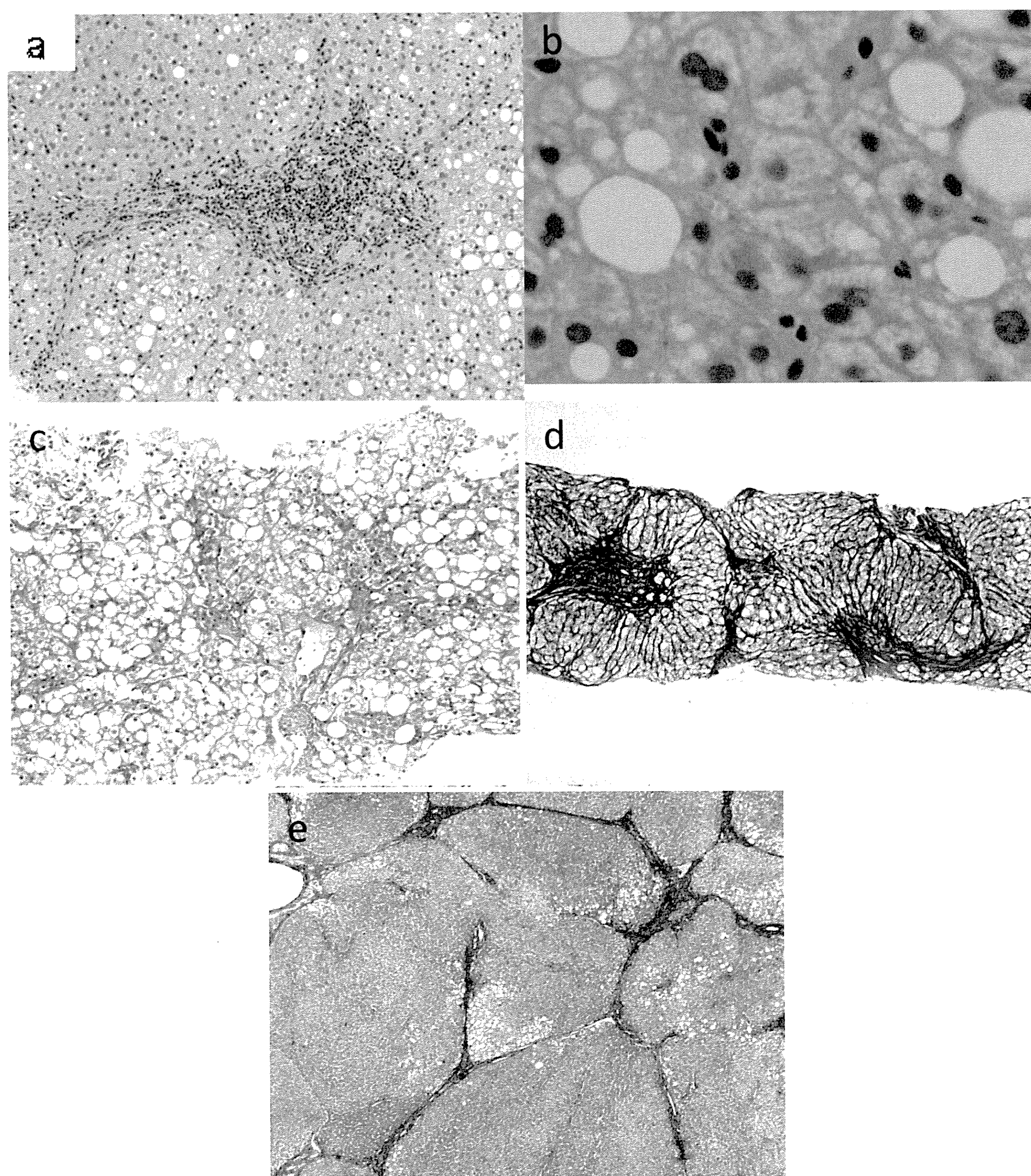
The papers listed in this review dated before 2000 were selected from review articles previously published by the present authors [29–31,35]. Those published after 2000 were chosen from PubMed. The keywords used in the search were as follows: NASH, fibrogenesis and MMPs; NASH, carcinogenesis and MMPs; hepatitis B or C virus-derived chronic hepatitis and MMPs. We selected those papers published after 2000 that we considered to be most important and appropriate. The purpose of this review is not to extract any conclusive research result as in a meta-analysis. We introduce basic and novel science reports focusing on fibrogenesis and carcinogenesis and their relation with MMPs and TIMPs for this review.

3. Pathophysiology of NASH

NAFLD comprises the following three groups: simple steatosis or fatty liver (FL), few inflammatory lesions around the portal triad (FL-IN) and NASH [3]. In NASH, four histological features are observed, *i.e.*, steatosis, lobular inflammation, hepatocellular ballooning and fibrosis [3,5–7]. A NAFLD activity score (NAS) has been developed to classify NAFLD cases into "NASH", "borderline" and "not NASH" [10]. Balooning and Mallory bodies (mitochondrial degeneration of hepatocytes) are considered to be necrotic processes in NASH [3,5]. These characteristic alterations followed by fat deposition in hepatocytes may cause more severe, chronic liver inflammatory lesions (Figure 1a,b). In the early stage of NASH slight fibrosis is seen around the tributaries of central veins. Fine fibrous bands are formed around hepatocytes in zone 3, known as "wire-meshed", "pericellular" and "chicken-wire" fibrosis (Figure 1c). The second stage, "bridging fibrosis", is the connecting fibrosis between zone 3 and the fibrosis around the portal area including arteries, veins and bile ducts (Figure 1d). The fibrosis

progresses into the nodule, and circles the newly formed regenerative nodule, resulting in liver cirrhosis (Figure 1e).

Figure 1. Histological features of the different steps of fibrosis in NASH. (**a**) NASH: Steatosis, hepatocyte balooning degeneration, and inflammatory cells composed predominantly of lymphocytes in the portal area (Hematoxylin-eosin stain, 200× magnification); (**b**) Mallory body in NASH showed staghorn pattern (Hematoxylin-eosin stain, 400× magnification); (**c**) NASH: Detail of wire-mesh fibrosis and steatosis (Azan stain. 100× magnification); (**d**) Bridging fibrosis: Portal-central fibrous septa linking portal tracts and central veins (Reticulin silver stain, 40× magnification); (**e**) NASH-derived cirrhosis: Larger nodules with thin fibrous septa and steatosis (Azan stain. 40× magnification).



These progressions are also seen in alcoholic fatty liver to alcoholic cirrhosis, but are very different from hepatitis B virus-derived chronic hepatitis to cirrhosis and hepatitis C virus-derived chronic hepatitis to cirrhosis. Pericellular fibrosis and/or perivenular fibrosis around the central vein tributaries in zone 3 is observed in the early stage of NASH, while the fibrosis around the portal or bile ducts is seen in chronic viral hepatitis [3,5].

The whole spectrum of NAFLD occurs mostly in patients with obesity (60% to 95%), type 2 diabetes mellitus (28% to 55%), and dyslipidemia (27% to 92%) [36]. In NASH, the first hit is the occurrence of NAFLD, and a second hit may cause more severe inflammation and fibrosis [37]. One of the second hits shows a pattern of fat distribution, the accumulation of visceral fat [38]. Moreover, abnormal glucose regulation causes increased risk for NASH [38]. Increase in BMI is a significant risk [39]. BMI and diabetes have also been found in cross-sectional studies to be associated with advanced fibrosis in patients with NASH [39]. TG accumulation in the liver is usually a result of *de novo* lipogenesis in increased free fatty acids derived from diet and peripheral lipolysis on adipocyte tissue. Among those suffering from obesity *de novo* lipogenesis is markedly increased, contributing to promote hepatic steatosis. These processes may be due to "insulin resistance," *i.e.*, insulin inhibits glucose production and promotes lipogenesis. Hepatic insulin resistance was also caused by increased methylation of the gene promoter region in the catalytic subunit of protein phosphatase 2A [40]. PNPLA3 and other genes may be implicated in promoting TG deposition in the liver (reviewed in [1,2,41]).

4. Mechanism of Fibrogenesis in NASH

4.1. Fibrogenesis in the Liver: Cells Responsible for ECM Formation, Cytokines, Signal Transduction, Role of Bone Marrow (BM)-Derived Cells

The deposition of ECM in the liver is based on the balance of ECM formation and degradation. Hepatic stellate cells (HSCs; Ito cells; fat-storing cells) localized in the perisinusoidal space have been clarified to be the most important producer of ECM. HSCs have several unique components such as vitamin A-related components and lipids, and cytoskeletal markers (vimentin, desmin) in normal liver. HSCs transform into proliferative, fibrogenic and contractile myofibroblasts following liver injuries (reviewed in [42]). This phenotypical transdifferentiation of HSCs is known as activation of HSC transferred from quiescent HSC under a physiologically normal condition. The activated HSCs (myofibroblasts) lose retinoid storage fat and express unique cytoskeletal markers (α-SMA, GFAP, nestin), cytokine receptors (PDGF-R, TGFβ-R type I, II, and III, ET-R, EGF-R, VEGF-R), other receptors [integrin, DDRs (discoidin domain receptors), thrombin-R, mannose-6-phosphate-R, uPA-R], signaling components (raf and MAP kinase), and transcription factors (Sp1, NFκB, Z19/KLF6). HSCs produce very important cytokines, growth factors and inflammatory mediators (prostanoids, leukocyte mediators (M-CSF, MCP-1, PAF), acute phase components (α_2-macroglobulin, IL-6), mitogens (HGF, EGF, PDGF, SCF, IGF-I, -II, αFGF), adhesion molecules (I-CAM-1, V-CAM-1, N-CAM), vasoactive mediators (ET-1, NO), fibrogenic cytokines (TGF-β1, -β2, β-3, CTGF), IL-10, C1NC), extracellular matrix such as collagen (type I, III, IV, V, VI, XIV) and proteoglycans (heparin, dermatan and chondroitin sulfates, perlecan, syndecan-1, biglycan, decorin), glycoproteins (cellular

fibronectin, laminin, merosin, tenascin, nidogen/entactin, undulin, hyaluronic acid), proteases (MMP-1, -2, -3, -14) and protease inhibitors (TIMP-1, -2, PAI-1) (reviewed in [43,44]).

Among them, mitogens such as PDGF, TGFα and EGF stimulate mitosis in HSCs, creating an *autocrine* loop for cellular proliferation. Pinzani and Mara revealed that PDGF is the most potent mitogen for cultured HSC isolated from rat, mouse, or human liver, and sequential signal transmission followed by phosphatidylinositol 3-kinase (PI 3-K) activation is necessary for both mitogenesis and chemotaxisis induced by PDGF in human HSC cultures (reviewed in [45]). HGF causes hepatocyte mitosis followed by the activation of HSCs. The injured hepatocytes, the activated Kupffer cells, and the activated endothelial cells can produce and secrete cytokines which bind to the receptors of HSCs mentioned above, and HSCs are activated to become myofibroblasts in a *paracrine* manner followed by wound healing. DDRs may mediate interactions between stellate cells and the surrounding interstitial matrix during progressive liver injury (reviewed in [43,44]). The different phenotypes of HSCs in normal and injured liver are regulated as the result of interactions with neighboring cells through *paracrine* and *autocrine* pathways as well as the interactions between HSCs and changes in ECM. This process is called the "*initiation*" phase of HSC activation. The second phase of HSC activation is "*perpetuation*", that is, myofibroblats proliferate, migrate to the site of the liver injury and produce excessive amounts of ECM resulting in scarring of the liver (reviewed in [43–46]).

The fate of the activated HSC is not only to progress to apoptosis but also to regress to quiescent HSC. Tsukamoto's group has reported dynamic phenotype reversibility regarding this problem. Twenty years ago they found PPARγ to be a regulator for the activation of HSC, similar to adipogenic transdifferentiation (reviewed in [47]). Very recently they found that HSC-derived Delta-like Homology 1 (DLK1) protein activates HSC via epigenetic repression of the master adipogenic gene *Pparγ* in a manner dependent on canonical Wnt [48]. Up-regulation of DLK1 participates in liver regeneration after partial hepatectomy followed by hepatocyte proliferation and liver growth. They also demonstrated that DLK1 knockdown reverses activated HSCs to fat-storing quiescent cells via epigenetic derepression of *Pparγ*. Moreover, their group [49] showed that myofibroblasts revert to an inactive phenotype in the recovery phase from liver fibrosis. However, inactivated HSCs are sensitive to the stimulator to transdifferentiate again to the active HSC. The cell markers of sinusoidal HSCs have been reported to be different to those of portal myofibroblasts. Vimentin, desmin, HSP47 and αSMA are expressed by both HSCs and portal myofibroblasts. CD95L, α2-macroglobulin, p100, reelin, fastin [50] and cytoglobin [51] are expressed in HSC, NCAM in human HSCs in the periportal area, whereas fibulin-2 is expressed in myofibroblasts.

Although the cells responsible for the increased synthesis and deposition of ECM during fibrogenesis in the liver are not only HSCs but also endothelial cells and other mesenchymal cells ([52], reviewed in [53]), BM-derived cells migrating into fibrotic tissue of the liver have recently been noted [54–56]. BM-derived cells exhibit the features of collagen-producing cells such as HSC, myofibroblasts, and fibrocytes and seem to participate in the progression of liver fibrosis. These findings were observed by BM transplanted with sex-mismatched cells [54,55] and BM-derived marked cells with enhanced green fluorescent protein (EGFP) [56–58]. When α-SMA-positive myofibroblasts are observed, there is no clear evidence that these cells are producing collagen and contributing to the progression of liver fibrosis (reviewed in [59]). Thus the present author's group sought to evaluate the direct contribution of BM-derived cells to collagen production by using the following specific and

quantitative methods ([27], reviewed in [59]). Wild-type mice were irradiated and their BMs destroyed. The irradiated mice were then transplanted by BM from transgenic animals having tissue-specific enhancer/promoter sequences of α2(1) collagen gene (COL1A2) linked to EGFP or firefly luciferase (LUC) gene. We observed a large number of EGFP-positive collagen-expressing cells in liver tissue of transgenic COL1A2/EGFP mice in both liver fibrosis models, introduced by repeated CCl_4 injections or ligation of the common bile duct. However, we observed few EGFP-positive BM-derived collagen-producing cells in the two liver fibrosis models of recipient mice. Luciferase assay also confirmed that BM-derived cells produced little collagen in response to fibrogenic stimuli [27]. Although BM-derived cells may differentiate into collagen-producing cells depending on the etiology and the extent and timing of tissue injury, we showed negligible contribution of BM-derived cells to collagen production during hepatic fibrogenesis in mice using very sensitive and specific methods [27].

Macrophages have recently been shown to play a role in the resolution [60] as well as in the fibrogenesis of experimental mouse fibrosis [61]. Hepatic macrophages (HM)-derived IL-1 and TNF-α activated NF-κ and prevented HSC death in *in vivo* and *in vitro* studies. IL-1 and TNF-α did not promote HSC activation but promoted survival of activated HSCs *in vitro* and *in vivo* and thus increased liver fibrosis. Xie *et al.* [62] showed that the signals of both Notch and Hedgehog pathways affect key cells participating in the tissue repair in adult liver and modulate epithelial-to-mesenchymal-like/mesenchymal-to-epithelial-like cell translations.

Chemical reagents, such as acetaldehyde, metabolized after alcohol consumption, have been considered to stimulate the *initiation* phase of HSCs activation in alcoholic liver diseases (ALD). Rapid transcriptional changes in HSCs induce cell response to cytokines and other stimuli, such as acetaldehyde. The next problem is "*reactive oxygen species (ROS)*" generated either from metabolically impaired hepatocytes or activated Kupffer cells seen in steatosis and steatohepatitis. In ALD, even though ROS can by itself activate HSC, cytokines or lipid oxidation products may also be involved in the activation. Abundance of proinflammatory cytokines, including TNF-α and IL-1 or IL-6, induces and perpetuates HSC activation followed by the release of neutrophils and monocyte chemoattractants and upregulation of adhesion molecules. Gut-derived endotoxin, endotoxin-recognizing receptors (CD14, TRL4, MD2), and endotoxin-induced activation pathways of NFκB and JNK have been observed in HSC activation in ALD. Augmented ethanol-induced ROS production in CYP2E1-overexpressing HSC leads to enhanced collagen α(1) gene expression [63]. ROS modulates the activity of transcription factors involved in HSC activation and fibrogenesis, e.g., c-Jun/AP-1, NF-κB, SP1 or c-Myb [63]. Lactic acid resulting from the cellular redox state in the liver by the ratio of NAD/NADH and NADP/NADPH, has been known to induce HSC activation and fibrogenesis. NADPH oxidase, mainly expressed in activated Kupffer cells, may activate HSC by generating H_2O_2, which induces collagen α(1) gene upregulation [64]. Hypoxia-inducible factor-1 is also seen in ALD and upregulates the transcription of VEGF. Acetaldehyde can directly upregulate collagen genes (reviewed in [46]).

4.2. Fibrogenesis in NASH

Progression of fibrosis was noted in 26% to 37% of NASH patients during a follow-up period of up to 5.6 years, with up to 9% progressing to cirrhosis (reviewed in [4]). The reason why the deposition of TG causes inflammation in the liver has been investigated [41]. Increased exposure of hepatocytes to

saturated fatty acids can trigger inflammation by interacting with TLRs (described later) and apoptosis by activating death receptors [65] (Table 1). Saturated fats can also inhibit mitochondrial function and induce the ER stress pathway [65]. Diet-induced weight loss with increased physical activity has been shown to be associated with improvement of liver pathology [66]. It is known that NASH-related fibrogenesis develops from NAFLD due to multiple factors, such as insulin resistance, oxidative stress, pro-inflammatory cytokines and adipokines and innate immune responses. HSC is the main player in development of fibrogenesis in NASH and the activation mechanisms of HSC have been investigated in experimental studies ([67–71], reviewed in [72–74]) and human studies ([75–78], reviewed in [72–74,80]). Paradis *et al.* [67] revealed that high glucose and hyperinsulinemia stimulate connective tissue growth factor expression, and showed increased type I collagen expression in HSCs. In human studies insulin resistance is closely associated with the advanced stage of liver fibrosis in NASH patients, and the fibrosis is partially reversed by treatment with insulin sensitizers, such as pioglitazone, rosiglitazone and metformin ([69,75–77], reviewed in [72–74]).

Table 1. Reported factors involved in fibrogenesis of NASH.

Factors	Reference No.
Apoptosis of hepatocytes due to the deposition of TG	[41]
due to activated death receptors	[65]
Insulin resistance	[69,72–78]
Oxidative stress	[68,69,73,75,79]
Pro-inflammatory cytokines	[67–78,80–83]
Adipokines including leptin	[70,72–74,80]
Innate immune responses including TLRs	[73,74,84–90]
Connective tissue growth factor due to high glucose level	[67]
due to hyperinsulinemia	[67]
Liver fatty acid binding protein (L-Fabp)	[91]
Farnesoid X receptor (FXR)	[73,79]
Peroxisome proliferator-activated receptors (PPARγ)	[47,74,77,92]
MCP-1, CCR2	[93]
Bone-marrow-derived macrophages (Ly6C)	[93,94]
Hepatic stem/progenitor cells (HPCs)	[95–99]

Liver fatty acid binding protein (L-Fabp) has recently been noted. L-Fabp modulates HSC fatty acid utilization and regulates the fibrogenic genes. L-Fabp deletion inhibits HSC activation and attenuates both diet-induced hepatic steatosis and fibrogenesis [91]. L-Fabp appears to be useful in differentiating NASH patients from patients with simple steatosis.

Oxidative stress in NAFLD based on lipid peroxidation in mitochondria and peroxisomes induces activation of HSC. Ikeda *et al.* [68] showed the relation between ROS seen in a NASH model and HSC activation, increased mRNA expression of type I collagen and MMP-2 through the p38/MAPK signaling pathway. Li *et al.* [69] reported that ROS derived from hypoxic hepatocytes regulates MMP-2 expression in HSC. CYP2E1, as in ALD, generates oxidative stress in NAFLD, and activates HSC with the increased secretion of type I collagen, and this process was blocked by anti-oxidants and CYP2E1 inhibitors (reviewed in [73]). Sanyal *et al.* [75] showed effective results of vitamin E as an anti-oxidant in NASH patients, and McCarty [76] reported the useful effect of astaxanthin [76].

In patients with NAFLD daily fructose ingestion is associated with increased fibrosis (reviewed in [74,80]). Mice maintained on a high-fat and high-fructose diet in addition to developing obesity also showed increased hepatic ROS formation and a NASH-like phenotype with significant fibrosis (reviewed in [74,80]). Recently Inagaki's group succeeded in demonstrating the direct contribution of mitochondrial oxidative stress to hepatic fibrogenesis using "Tet-mev-1 mouse" in which a mitochondrial reactive oxygen species can be induced by deoxycycline-regulable expression of mutant succinate dehydrogenase [79].

Mari *et al.* [81] found that loading of free cholesterol (FC) on mitochondria sensitizes cells to TNF- and Fas-induced steatohepatitis, but this mechanism was not observed with the loading of free fatty acids or triglycerides. Free cholesterol accumulated in hepatocytes exacerbated LPs-stimulated acute liver injury followed by apoptosis through TNF-α. Teratani *et al.* [82] pointed out that TNF-α mediated hepatocytes apoptosis was not involved in the progression of liver fibrosis. They observed that a high cholesterol diet aggravated a mouse liver fibrosis model induced by BDL or CCL_4. Exacerbation of liver fibrosis was clearly caused by HSC accumulated in FC which sensitized HSC to TGFβ-induced activation.

As noted above, NAFLD is frequently found among patients with obesity, type 2 diabetes and metabolic syndrome. The adipocytes, inflammatory cells including macrophages and other monocytes secrete adipokines and pro-inflammatory cytokines. Adipokines include adiponectin, leptin, resistin, TNF-α, IL-6, visfatin, chemerin and vaspin. Adipokine receptors, AdipoR2 and AdipoR1, are present in the liver and skeletal muscle. AdipoR2 is known to play an important role in NAFLD because AdipoR2 expression decreased in a rodent NAFLD model fed a high-fat and cholesterol-rich diet followed by inflammation and fibrosis. Adiponectin has antifibrogenic effects in liver injury, and may act to reverse HSC activation and abrogates TGF-β1 signal transduction (reviewed in [72–74,80]). Adiponectin knockout mice showed more severe pericellular fibrosis compared with WT mice [70]. The advanced stage of NASH with fibrosis to cirrhosis sometimes showed reduction of hepatic fat (burn-out NASH) as described above. Liver fat loss often accompanies advanced fibrosis and cirrhosis. Van der Poorten *et al.* [9] clarified that the circulating adiponectin levels have an inverse correlation with hepatic fat content. As hepatic fat declines with advanced fibrosis, adiponectin levels progressively rise, independent of insulin resistance, leptin, BMI and waist/hip ratio. Adiponectin, in part, signals through phosphorylation of activated protein kinase and acetyl-CoA carboxylase to reduce lipogenesis. Increased levels of bile acids are seen in late-stage NASH, and bile acids act directly to regulate adiponectin synthesis in adipocytes [9]. As new anti-fibrogenic factors the liver X receptor (LXR) ligands, the farnesoid X receptor (FXR) and GW4064 (agonist of FXR) have recently been reported (reviewed in [73,80]). FXR is a bile acid sensor that functions to protect the liver and the intestine against bile acid toxicity and regulate synthesis, uptake and excretion of bile acids. As the bile acid pool size can affect lipid metabolism, FXR is considered to play a key role in lipid homeostasis by reducing both hepatic lipogenesis and plasma triglyceride and cholesterol levels (reviewed in [80]). The activity of peroxisome proliferator-activated receptors (PPARs) is involved in HSC activation, and PPARs play a key role in fibrogenesis of NASH (reviewed in [47,100]. PPARγ maintains the quiescent state of HSC phenotype (reviewed in [47]), and PPARγ agonists such as pioglitazone and rosiglitazone recovered to the quiescent phenotype from myofibroblast-like cells of HSC followed by