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Inhibition of pancreatic stellate cell activation by the hydroxymethylglutaryl coenzyme A reductase inhibitor lovastatin

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Abstract

Pancreatic stellate cells (PSCs) play a key role in pancreatic fibrosis, a constant feature of chronic pancreatitis. PSC activation occurs in response to profibrogenic mediators such as cytokines and involves proliferation, transition towards a myofibroblastic phenotype and enhanced production of extracellular matrix proteins. Previously, we have shown that PSC activation correlates with the activity of the Ras-Raf-ERK (extracellular signal-regulated kinase) signalling cascade [Gut 51 (2002) 579]. Using a rat culture model of PSCs, we have now evaluated the effects of lovastatin, a hydroxymethylglutaryl coenzyme A reductase inhibitor that interferes with protein isoprenylation, on PSC viability and activation as well as on signalling through Ras proteins. Apoptotic cells were detected applying the TUNEL assay. Proliferation of PSCs was quantitated using the bromodeoxyuridine DNA incorporation assay. Expression of α-smooth muscle actin (an indicator of the myofibroblastic phenotype), ERK activation and membrane translocation of the Ras superfamily member RhoA were analysed by immunoblotting. Lovastatin inhibited serum- and platelet-derived growth factor-stimulated PSC proliferation in a dose-dependent manner. At drug concentrations above the level required for growth inhibition, a strong increase of apoptotic cells was observed. Furthermore, lovastatin inhibited induction of α-smooth muscle actin expression in the course of primary culture. Immunoblot experiments indicated that lovastatin suppressed both Ras-mediated ERK 1/2 activation and platelet-derived growth factor-induced membrane translocation of RhoA. Together, our data suggest that lovastatin, through the interruption of Ras signalling, interferes with PSC activation. The antifibrotic efficiency of statins should be tested in animal models of chronic pancreatitis.

Keywords: Pancreatic stellate cells; Chronic pancreatitis; Fibrosis; Lovastatin; Ras; RhoA

1. Introduction

Pancreatic fibrosis is a central pathological feature of chronic pancreatitis [1,2]. The molecular mechanisms and cellular interactions that underly the replacement of the pancreatic parenchyma by extracellular matrix-rich connective tissue are only partially understood. Significant progress, however, has been made in the past few years since the identification and partial characterisation of a pancreatic cell population with close morphological and biochemical similarities to hepatic stellate cells (HSCs): the pancreatic stellate cells (PSCs) [3,4]. PSCs are located in interlobular areas and interacinar regions of the pancreas and comprise about 4% of all pancreatic cells [3,4]. Like their hepatic counterparts [5], PSCs store retinoids in fat droplets [3,4]. In response to profibrogenic mediators such as ethanol and its metabolite acetaldehyde, oxidant stress and various cytokines PSCs undergo an activation process that mimics the activation of HSCs: the cells start to proliferate, develop features of myofibroblasts (such as expression of α-SMA), and secrete increased amounts of extracellular matrix proteins [3,4,6–9]. Studies using pancreatic sections from patients with chronic pancreatitis and from animal models of the disease have revealed the presence of activated PSCs in areas of pancreatic fibrosis, and activated PSCs were demonstrated to be the predominant

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Abbreviations: PSC, pancreatic stellate cell; HSC, hepatic stellate cell; α-SMA, α-smooth muscle actin; PDGF, platelet-derived growth factor; ERK, extracellular signal-regulated kinase; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; GTP, guanosine triphosphate; BrdU, 5-bromo-2′-deoxyuridine; FCS, fetal calf serum; TUNEL, terminal deoxynucleotidyl transferase-mediated X-dUTP nick end labeling; SDS, sodium dodecyl sulfate; PP, protein phosphatase.

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source of collagen in the fibrotic pancreas [10,11]. Together, these data support the hypothesis that PSCs play a key role in the development of pancreatic fibrosis [4,8,10,12].

Cultured PSCs respond to the cytokine PDGF by an enhanced proliferation [8,13], while transforming growth factor-β stimulates collagen synthesis [8,13] and is involved in autocrine control of PSC growth [14]. Furthermore, inflammatory cytokines such as tumor necrosis factor α and interleukin 6 have been implicated in PSC activation [15]. The intracellular signal transduction pathways that mediate cytokine receptor-derived activation signals in PSCs are only partially characterised so far. Recent data from our laboratory suggest an essential role of the proteins ERK 1 and 2 in the mediation of mitogenic signals in PSCs [16]. ERKs have also been implicated in the induction of HSC proliferation by PDGF, and acute liver damage (caused by CCl_4) has been shown to lead to a rapid ERK activation in HSCs *in vivo* [17].

Growth factors such as PDGF induce the activation of ERK 1 and 2 through the binding to specific cell surface receptors and the initiation of a well-established signal transduction pathway that involves, among several other cytosolic proteins, the small G protein Ras and the serine/threonine-specific protein kinase Raf-1 [18–20]. Downstream targets of ERK signalling include for example the Ets family transcription factors Elk-1 and SAP-1, which regulate expression of target genes participating in cell growth control, e.g. c-*fos* [21,22].

The specific inhibition of growth-promoting intracellular signal transduction pathways is considered to be a promising approach for the treatment of diseases associated with pathological cell proliferation, including human malignancies [23]. Due to their well-established role as proto-oncogene products, Ras proteins are among the most interesting potential target molecules [24]. G proteins of the Ras superfamily, including Ras and Rho subfamily members, become membrane-associated after undergoing a series of post-translational modifications which involves isoprenylation [25–27]. Protein isoprenylation essentially depends on the availability of intermediates of the cholesterol biosynthetic pathway: while Ras proteins are generally farnesylated [26], Rho proteins such as RhoA, a GTPase participating in actin cytoskeleton organisation and the regulation of cell adhesion/motility [28], are geranylgeranylated [27]. The rate limiting step in the entire pathway is the biosynthesis of HMG-CoA [29]. HMG-CoA reductase inhibitors are widely used drugs in the treatment of hypercholesteremia. Recent studies, however, have indicated that they have also anti-proliferative, antiinvasive and antimetastatic properties which are related to the inhibition of the isoprenylation of Ras-superfamily G proteins [30–34]. Based on the observation of proapoptotic effects on lung fibroblasts *in vitro* and on granulation tissue formation *in vivo*, the use of HMG-CoA reductase inhibitors has also been suggested as a potential therapy for patients with fibroproliferative disorders [35]. In this study, we have analysed how viability and activation of cultured PSCs are affected by the HMG-CoA reductase inhibitor lovastatin. Furthermore, we have investigated the effects of lovastatin on Ras and Rho signalling in PSCs.

2. Materials and methods

2.1. Materials

Collagenase P and deoxyribonuclease were purchased from Roche Diagnostics, protease IX, mevalonic acid and the α-SMA antibody from Sigma-Aldrich, nitrocellulose and peroxidase-labelled antibodies from Amersham Pharmacia Biotech, Nycodenz from Nycomed, the phospho-ERK 1/2 (Thr 202/Tyr 204 of human ERK 1) antibody from New England BioLabs, and the antibodies to the ERK 1/2 protein and RhoA from Santa Cruz Biotechnologies. Hank's buffered salt solution, media and supplements for cell culture were obtained from Life Technologies and rat PDGF-BB from R&D Systems.

Lovastatin, a generous gift of Merck & Co., was delivered as a prodrug and acid-activated before use according to the manufacturer's instructions.

2.2. Isolation and culture of PSCs

Stellate cells were isolated from the pancreas of male LEW.1W inbread rats as previously described [16]. Briefly, the pancreas was digested with a mixture of collagenase P (0.05%), protease IX (0.02%) and deoxyribonuclease (0.1%) in Hank's buffered salt solution. After density gradient centrifugation (12% Nycodenz; centrifugation at 1400 *g* for 20 min), PSCs were collected from the top of the gradient, washed and resuspended in Iscove's modified Dulbecco's medium supplemented with 10% FCS, 1% nonessential amino acids (dilution of a 100× stock solution), 100 U/mL penicillin and 100 mg/mL streptomycin. Freshly isolated cells were cultured in 6-well culture plates at 37° in a 5% CO_2 humidified atmosphere. With the first two medium changes (24 and 48 hr after seeding), most of the contaminating cells were removed, and almost (>95%) pure PSC cultures (assessed by light, phase contrast, fluorescence and electron microscopy) were received.

After reaching confluency, cells were harvested by trypsination and replated at equal seeding densities according to the experimental requirements.

For fixation before microscopy, PSCs were rinsed with phosphate-buffered saline and exposed to methanol (100%) for 5 min.

2.3. Cell proliferation assay

To assess cell proliferation, incorporation of BrdU into newly synthesised DNA was quantitated using the BrdU labelling and detection enzyme-linked immunosorbent assay kit (Roche Diagnostics). Therefore, cells plated in

96-well plates and growing in FCS (10%)-supplemented Iscove's modified Dulbecco's medium were exposed for 2 days to the indicated agents. After 3 hr of labelling, BrdU uptake was measured according to the manufacturer's instructions.

2.4. Analysis of apoptosis

PSCs were grown to subconfluency and exposed for 2 days to the indicated agents. Afterwards, adherent cells were trypsinised and combined with detached cells collected by culture medium centrifugation before they were fixed with paraformaldehyde at a final concentration of 2%. Next, cells with DNA strand breaks (caused by endonuclease activation; a key step in the induction of apoptosis) were labelled with the TUNEL technique, using the *in situ* Cell Death Detection Kit, Fluorescein (Roche Diagnostics) according to the manufacturer's instructions. Finally, apoptotic cells were quantitated by flow cytometry (FACSort; Becton Dickinson). If labelling solution without terminal transferase was used instead of the TUNEL reaction mixture (negative control), less than 1% of the cells were detected as TUNEL-positive.

2.5. Immunoblotting

Protein extracts of PSCs (pretreated as indicated) were prepared and adjusted to identical protein concentrations as previously described [16]. Afterwards, proteins (15 μg per sample) were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and blotted onto nitrocellulose filters. Next, membranes were blocked with 1% bovine serum albumine and incubated with the indicated protein-specific antibodies overnight at 4°. After a final incubation with a horseradish peroxidase-labelled anti-rabbit- or anti-mouse Ig antibody, blots were developed using the enhanced chemoluminescence Plus kit (Amersham Pharmacia Biotech). For reprobing with additional antibodies, blots were stripped by incubation in stripping buffer (62.5 mM Tris–HCl, pH 6.7, 2% SDS, 100 mM 2-mercaptoethanol) at 50° for 30 min.

2.6. Separation of the membrane fraction

The membrane fraction was separated following a protocol of Kusama *et al.* [33]. Therefore, subconfluent PSCs pretreated as indicated were washed with cold phosphate-buffered saline and lysed by freeze-thawing in ice-cold lysis buffer (50 mM HEPES, pH 7.5, 50 mM NaCl, 2 mM EDTA, 1 mM $MgCl_2$, 10 mM sodium fluoride, 1 mM dithiothreitol, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride). After a centrifugation at 100,000 *g* for 30 min at 4°, the membrane pellet was resuspended in lysis buffer supplemented with 1% Triton X-100, and protein concentrations were determined by the method of Lowry *et al.* [36] using bovine serum albumine as the standard. Samples with adjusted protein concentrations were subjected to immunoblotting.

2.7. Quantitation of band intensities

Chemoluminescence signals on X-ray films were quantitated by scanning densitometry using an imaging densitometer (Bio-Rad).

2.8. Statistical analysis

Results are expressed as means ± SEM for the indicated number of separate cultures per experimental protocol. The data were analysed using Wilcoxon's rank sum test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of lovastatin on PSC morphology

HMG-CoA reductase inhibitors have previously been shown to affect cytoskeletal organisation through preventing the isoprenylation of proteins involved in this process [37–39], resulting in cell rounding. Therefore, we first analysed the effects of lovastatin on PSC morphology. PSCs after 6 days in culture (1 passage) were either stellate-like with long cytoplasmic extensions or spindle shaped (Fig. 1A). Application of lovastatin (3 μM) for 24 hr caused a retraction of the cytoplasmic extensions and a cell rounding (Fig. 1B). At higher concentrations of lovastatin (≥5 μM) a strong increase of detached cells was observed (data not shown).

3.2. Lovastatin-induced apoptosis of PSCs

Because previous studies have shown an increased rate of apoptosis in lovastatin-treated lung fibroblasts [35], we sought to determine if PSCs would also manifest susceptibility to lovastatin-induced apoptotic cell death. Apoptotic cells in lovastatin-exposed cultures (including detached cells collected from the supernatant) or corresponding controls were labelled with fluorescein using the TUNEL assay and quantitated by flow cytometry. As shown in Fig. 2, lovastatin induced a dose-dependent and, at 5 μM, statistically significant increase of TUNEL-positive cells. Simultaneous application of mevalonic acid (250 μM), the intermediate whose production is blocked by HMG-CoA reductase inhibitors, antagonised the induction of apoptosis by lovastatin.

3.3. Effects of lovastatin on PSC proliferation and expression of α-SMA

To study the effects of lovastatin on PSC growth, the cells were exposed to concentrations of the drug below the level

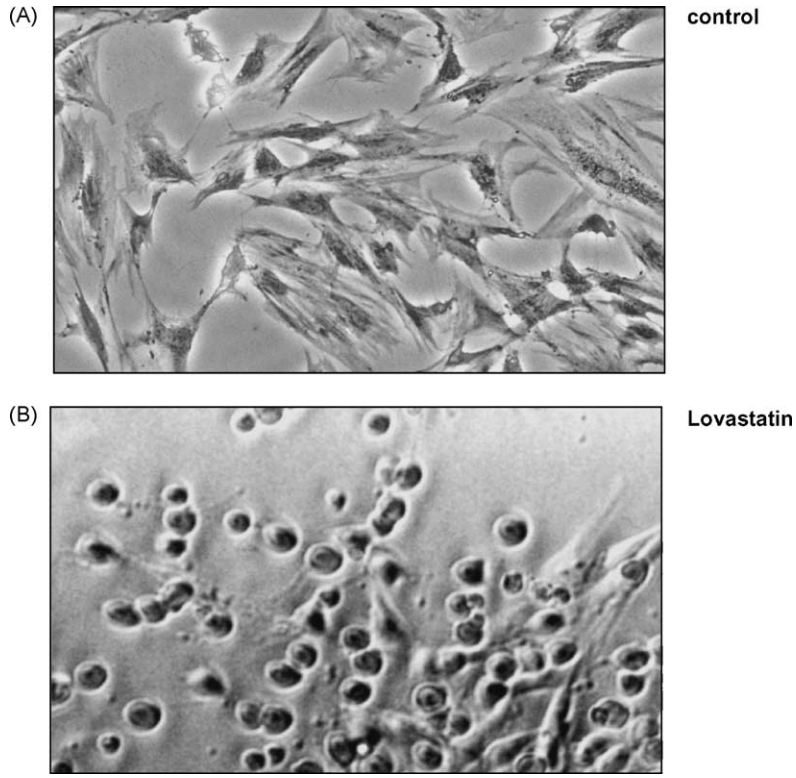

Fig. 1. Lovastatin-induced changes of PSC morphology. PSCs of the second passage (day 6 after isolation) were either left untreated (A) or incubated with lovastatin at 3 μM (B). Twenty-four hours later, the cells were fixed with methanol and examined by phase contrast microscopy (original magnification 320×).

inducing a high rate of apoptosis, and proliferation was assessed using the BrdU DNA incorporation assay (Fig. 3A). Lovastatin significantly inhibited DNA synthesis in a concentration range from 0.3 to 1 μM. Application of mevalonic acid (250 μM) did not affect PSC growth in the absence of lovastatin but completely eliminated the antiproliferative effect of the drug (Fig. 3A). A strong, dose-dependent growth inhibition by lovastatin was also observed when proliferation of PSCs (growing in culture medium with 10% FCS) was further stimulated by PDGF-treatment (Fig. 3B).

The conclusion that lovastatin-induced growth suppression is related to the inhibition of HMG-CoA reductase was supported by the observation that simvastatin, another HMG-CoA reductase inhibitor, displayed similar antiproliferative effects on PSCs (data not shown).

To address the question if lovastatin is also able to interfere with the development of a myofibroblastic phenotype, freshly isolated PSCs were exposed to lovastatin (0.1–1 μM) for 7 days, and expression of α-SMA, a protein typical for myofibroblasts, was analysed by immunoblot-

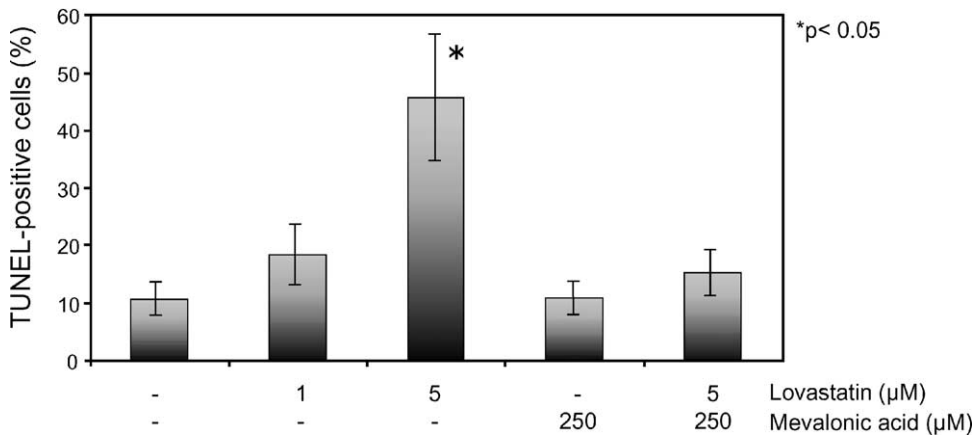

Fig. 2. PSCs are susceptible to lovastatin induced-apoptosis. PSCs of the second passage were exposed to lovastatin and mevalonic acid for 48 hr as indicated. Afterwards, cells with DNA strand breaks were labelled using the TUNEL assay and quantitated by flow cytometry (N = 6 independent experiments). $^{*}P < 0.05$ vs. untreated cells.

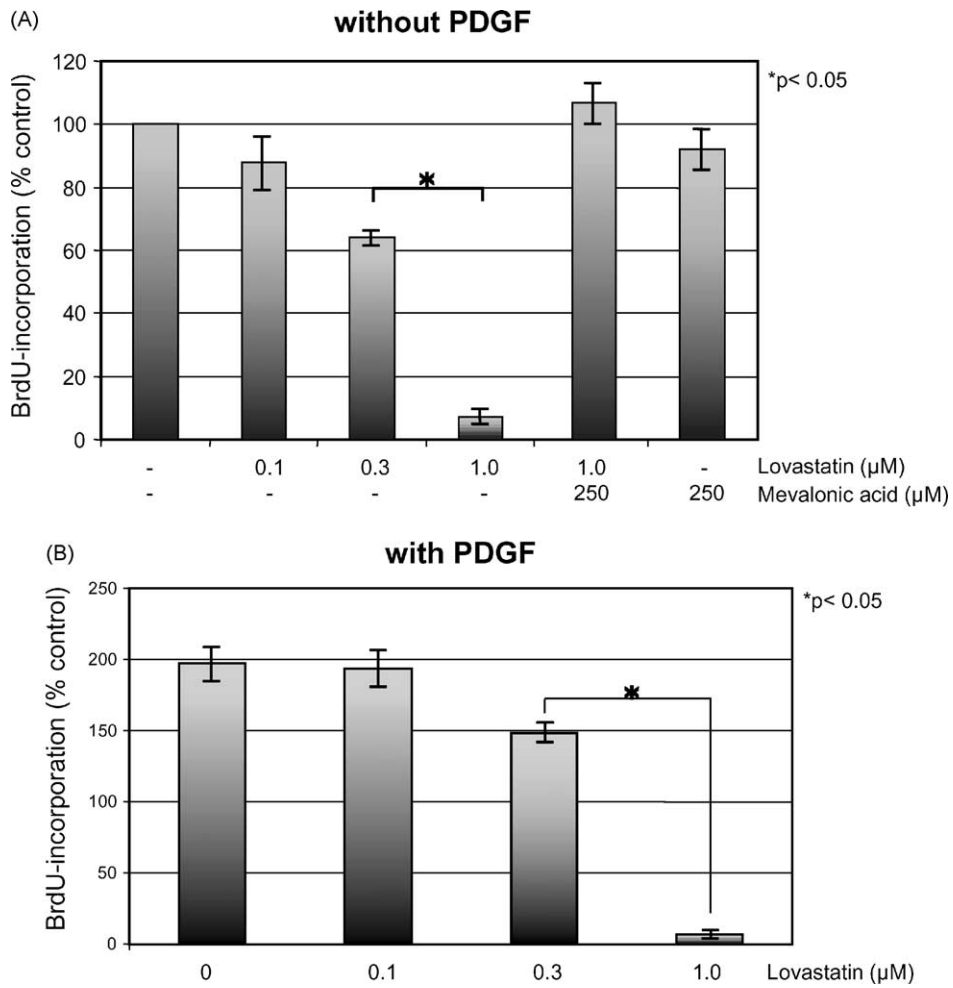

Fig. 3. Lovastatin inhibits proliferation of PSCs. PSCs of the first passage were harvested, resuspended in complete culture medium (A) without or (B) with PDGF (10 ng/mL) and replated at equal seeding densities in 96-well plates. Afterwards, cells were treated with lovastatin and mevalonic acid at the indicated concentrations for 48 hr, the time when the fastest-growing cultures were almost confluent. Cell proliferation was assessed with the BrdU DNA-incorporation assay (N = 6 separate cultures). One hundred percent cell proliferation corresponds to untreated PSCs. $^{*}P < 0.05$ (A) vs. untreated cells (B) vs. cells exposed to PDGF only.

ting (Fig. 4A). In the absence of lovastatin, a strong increase of α-SMA expression from days 2 to 7 was observed (lanes 1 and 2). Lovastatin not only suppressed cell proliferation but also prevented the increase of α-SMA expression in a dose-dependent manner (lanes 3–5). Again, mevalonic acid (at 250 μM) antagonised lovastatin action (lane 6). To exclude a nonspecific effect, expression of the intracellular proteins ERK 1 and 2 was analysed and found to be not diminished by lovastatin treatment (Fig. 4B). While lovastatin efficiently inhibited the induction of α-SMA expression in the course of primary PSC culture (Fig. 4A), it had no effect on α-SMA protein levels when already activated cells (day 7 after isolation) were exposed to the drug (Fig. 4C).

3.4. Effects of lovastatin on PDGF-induced ERK activation and RhoA translocation to the cell membrane

Ras superfamily G proteins have been suggested to be key mediators of HMG-CoA reductase inhibitor-effects on cell growth and survival [30-32,35]. We therefore characterised signal transduction through Ras proteins in lovastatin-exposed PSCs.

First, phosphorylation of ERK 1 and 2, two well characterised downstream targets of Ras signalling, in response to stimulation of PSCs with the mitogen PDGF was analysed (Fig. 5). As previously shown [16], PDGF-BB treatment of cultured PSCs induced a rapid increase of ERK 1 and 2 phosphorylation, suggesting an activation of the Ras-Raf-ERK signal transduction pathway in response to the growth factor (lanes 1–3). In the presence of lovastatin (1 μM, lanes 4–6), two distinct effects were detected: an increase of the basal (PDGF-independent) ERK 1 and 2 phosphorylation, and a reduced peak of the phospho-ERK 1 and 2 level in response to PDGF stimulation. While the latter observation supports the hypothesis that mitogenic signalling through the Ras-Raf-ERK signal transduction pathway is impaired in lovastatin-exposed PSCs, the increase of basal ERK phosphorylation may reflect a Ras-independent additional lovastatin-effect,

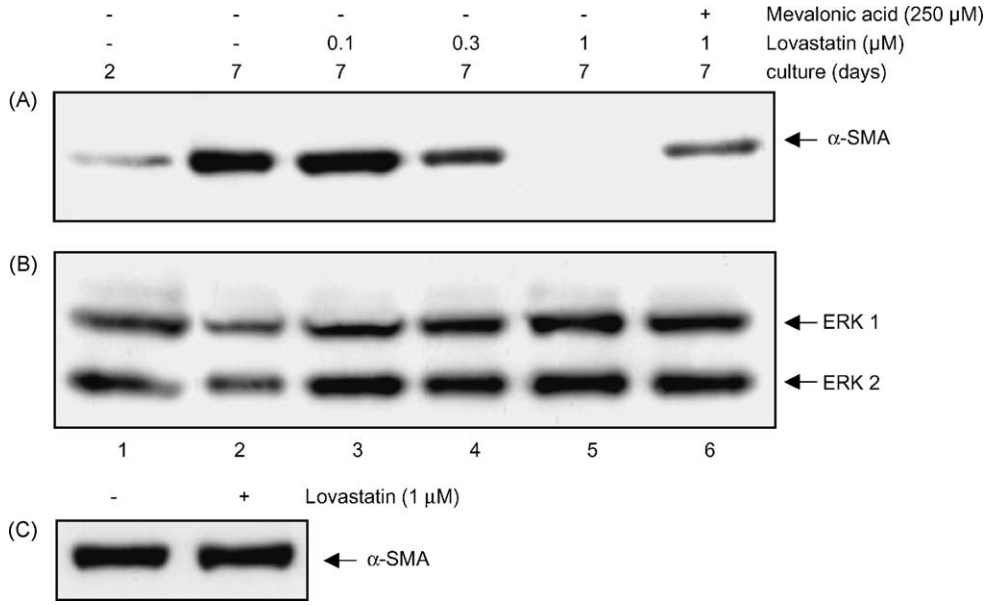

Fig. 4. Expression of α-SMA (panels A and C) and ERK 1/2 (panel B) in lovastatin-treated PSCs. Freshly isolated PSCs (panels A and B) and cells at day 7 after isolation (panel C) were treated with the indicated drugs for the entire time of primary culture (panels A and B) or 48 hr (panel C). Cell lysates were resolved by 8% SDS–PAGE. After blotting, the membrane was incubated with an antibody to α-SMA (panels A and C). Afterwards, the membrane shown in panel A was stripped and reprobed with an ERK 1/2 protein-specific antibody (panel B). Results are representative of three independent experiments.

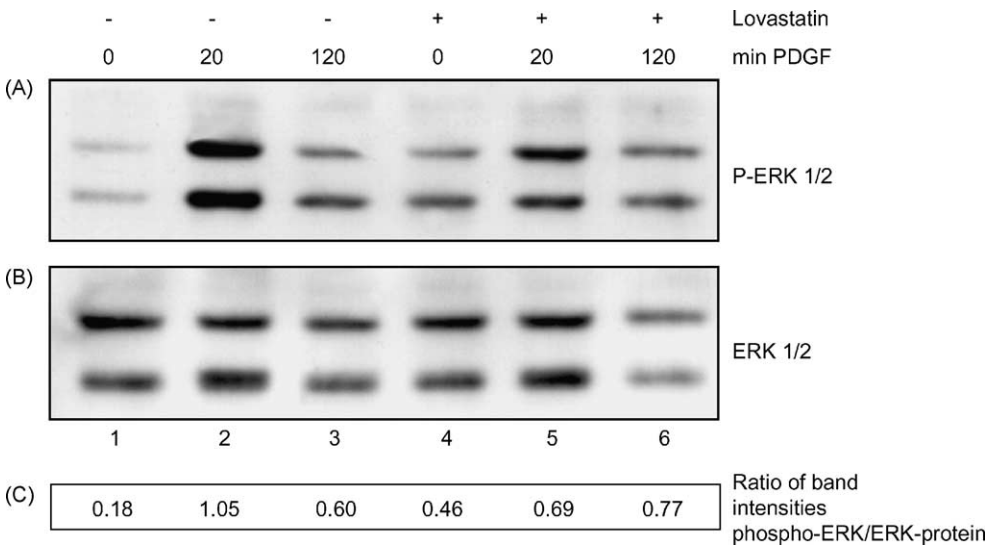

Fig. 5. Lovastatin inhibits ERK 1/2 activation in PDGF-stimulated PSCs. PSCs of the second passage were preincubated with lovastatin (1 μM) for 18 hr or left untreated. Next, the cells were washed free of serum, and lovastatin pretreatment continued for additional 6 hr. Afterwards, a stimulation with PDGF-BB (10 ng/mL) was performed as indicated. Cell lysates were resolved by 8% SDS–PAGE. After blotting, the membrane was incubated with a phospho-ERK 1/2 (P-ERK 1/2)-specific antibody (panel A) before it was stripped and reprobed with an ERK 1/2 protein-specific antibody (panel B). Phospho-ERK levels were further analysed by scanning densitometry and related to the ERK 1 and 2 protein levels (panel C). Therefore, the sum of the band intensities of phospho-ERK 1 and 2 (panel A) as well as the sum of the corresponding ERK-protein band intensities (panel B) were determined, and the ratio phospho-ERK/ERK-protein was calculated. Results shown are representative of three independent experiments.

such as inhibition of protein phosphatases PP-1/PP-2A [40], two potential negative regulators of ERK phosphorylation in PSCs.

In a second series of experiments, the effect of lovastatin on the mitogen-induced translocation of the Ras superfamily member RhoA to the cell membrane was analysed (Fig. 6). After PDGF-stimulation of PSCs, a rapid increase of membrane-bound RhoA protein was observed (lanes 1–3). In lovastatin-pretreated cells, the PDGF-effect was strongly reduced (lanes 4–6).

4. Discussion

To date, no specific therapies are available to inhibit pancreatic fibrosis, a constant feature of chronic pancreatitis.

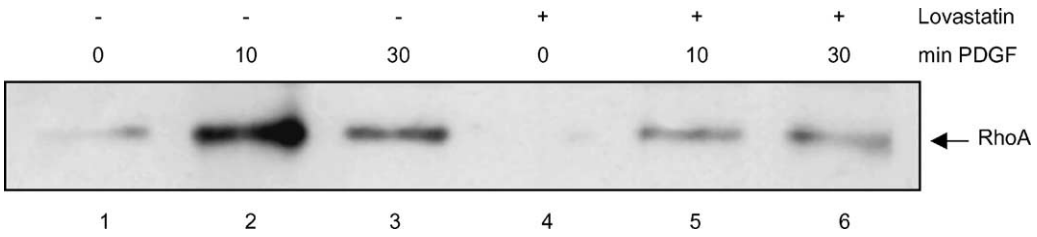

Fig. 6. Lovastatin interferes with PDGF-induced translocation of RhoA to the PSC membrane. PSCs pretreated by lovastatin exposure (1 μM) and serum withdrawal as described in Fig. 5 were stimulated with PDGF-BB (10 ng/mL) as indicated. Afterwards, the membrane fraction was isolated, and samples with adjusted protein concentrations were subjected to SDS–PAGE. Detection of RhoA was performed by immunoblotting. Results are representative of three independent experiments.

In the past, the development of strategies for the treatment of pancreatic fibrosis has been hampered by the poor knowledge of the molecular mechanisms that underly this pathological process. However, the identification of PSCs [3,4] and subsequent studies showing that PSCs play a key role in pancreatic fibrogenesis [10,11] have encouraged the search for new therapeutic options aimed at the inhibition of PSC activation. For example, ligands of the peroxisome proliferator-activated receptor-γ, a member of the nuclear hormone receptor superfamily that has previously been shown to play a key role in adipocyte differentiation [41], have recently been suggested as potent antagonists of the PSC activation process [42].

Using rat PSCs, we show here for the first time that the HMG-CoA reductase inhibitor lovastatin suppresses the proliferation of these fibroblast-like cells and interferes with the induction of α-SMA expression in the course of primary culture. The effect of lovastatin on α-SMA expression in PSCs is probably indirect and reflects an inhibition of the transition towards a myofibroblastic phenotype, because the drug did not cause a reduction of α-SMA protein levels in already activated cells. The latter observation also suggests that lovastatin does not induce a reversion of the phenotype once the cells are activated.

HMG-CoA reductase inhibitors block the synthesis of mevalonic acid, a precursor of lipid moieties required for isoprenylation of Ras superfamily G proteins [26,27]. A growing body of evidence suggests that inhibitors of the HMG-CoA reductase are not only efficient in lowering serum cholesterol, but also display antiproliferative and/or proapoptotic effects on several types of tumor cells [30,32,39,43,44] and nontransformed cells (such as renal myofibroblasts [45]) which are related to the inhibition of protein isoprenylation. Furthermore, recent studies using tumor-bearing animals have shown that HMG-CoA reductase inhibitors decrease the metastasis of, for example, breast cancer, melanoma and pancreatic cancer cells, suggesting a possibility of useful clinical applications [33,46,47]. The antiproliferative and proapoptotic efficiency of HMG-CoA reductase inhibitors has also already encouraged a first study aimed at elucidating the therapeutic potential of these drugs for nonmalignant fibroproliferative diseases: Using fibroblasts derived from normal and fibrotic lungs as well as an animal wound chamber model, Tan *et al.* showed that lovastatin treatment can induce fibroblast apoptosis *in vitro* and *in vivo* [35]. Our data indicate that cultured PSCs, like lung fibroblasts, are highly susceptible to a treatment with HMG-CoA reductase inhibitors. Significantly, inhibitory effects of lovastatatin on PSC proliferation and transition towards a myofibroblastic phenotype were detected at concentrations of only 1 μM (or even less), a level that is clinically achievable [48]. Furthermore, cell detachment and a strong increase of apoptotic cells were observed when the lovastatin concentration was further increased.

Proteins of the Ras superfamily are membrane-bound small GTPases activated by GTP binding. They are involved in the regulation of a variety of fundamental cellular processes. Ras, for example, plays a central role in the regulation of cell proliferation, survival and differentiation through the activation of signal transduction pathways such as the Raf-ERK signaling cascade [19,20,25]. Members of the Rho subfamily, on the other hand, have emerged as key regulators of the actin cytoskeleton and, by interacting with multiple target proteins, of additional cellular activities including adhesion, motility and invasion [28]. The results of this study suggest that distinct molecular mechanisms may be involved in the mediation of lovastatin effects on PSCs.

First, an inhibition of PDGF-induced Ras signalling through ERK proteins was observed. ERKs have previously been shown to play a central role in the transduction of mitogenic signals in HSCs and PSCs [16,17]. In the course of PSC activation induced by sustained culture, activation of ERK 1 and 2 is an early event that precedes enhanced expression of α-SMA and correlates with the induction of cell proliferation [16]. Like lovastatin, the compound PD98059, a specific inhibitor of ERK activation, is a potent suppressor of PDGF-stimulated PSC growth [16]. Together, these data suggest that inhibition of mitogenic signalling through the Ras-Raf-ERK pathway is an important principle of lovastatin action in PSCs.

Secondly, lovastatin interfered with the membrane translocation of RhoA in response to mitogen stimulation; an effect that has previously been linked to HMG-CoA reductase inhibitor-induced changes of cell morphology and to the antiinvasive/antimetastatic efficacy of these drugs [33,38].

Furthermore, additional mechanisms of lovastatin action are likely to exist. Thus, we found an increased basal (mitogen-independent) ERK 1/2 phosphorylation which may be related to a previously reported inhibition of PP-1 or PP-2A [40]. Apparently, the molecular effects of HMG-CoA reductase inhibitors are also to some degree cell type-specific. Particularly, inhibition of Ras-mediated ERK activation is a frequent but not a constant finding [30,32,33]. Here, it seems likely that diverse effects of HMG-CoA reductase inhibitors, such as inhibition of Ras isoprenylation on one hand but inactivation of potential negative regulators (e.g. PP-1 and PP-2A) on the other one, affect ERK phosphorylation; with the net effect being dependent on the specific cellular background.

Together, our data provide evidence that the HMG-CoA reductase inhibitor lovastatin interferes with Ras signalling in PSCs and acts as a potent inhibitor of PSC activation *in vitro*. Currently, we are planning to evaluate the potential of HMG-CoA reductase inhibitors to suppress PSC activation and pancreatic fibrogenesis *in vivo*, using a rat model of chronic pancreatitis and pancreatic fibrosis [49].

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