Title page

Title
Activation of human liver sinusoidal endothelial cell by human platelets induces hepatocyte proliferation

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Short title
Platelets activate LSEC in liver regeneration

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List of abbreviations
LSECs, liver sinusoidal endothelial cells; IL-6, interleukin-6; VEGF, vascular endothelial growth factor; S1P, sphingosine 1-phosphate; HGF, hepatocyte growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; STAT3, signal transducer and activator of transcription 3; ERK1/2, extracellular signal-regulated protein kinase 1/2; NO, nitric oxide; IGF-1, insulin-like growth factor 1; I/R, ischemia/reperfusion; PRP, Platelet-rich plasma; ACD, acid citrate dextrose; HSA, human serum albumin; ANOVA, analysis of variance.

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Activation of human liver sinusoidal endothelial cell by human platelets induces hepatocyte proliferation

Abstract

Background/Aims: We previously reported that platelets promote hepatocyte proliferation. In this study, we focused on the role of platelets to liver sinusoidal endothelial cells (LSECs) in addition to hepatocytes in liver regeneration.

Methods: Immortalized human LSECs (TMNK-1) were used. The LSECs were cocultured with human platelets, and then the proliferation of LSECs and the excretion of growth factors and interleukin-6 (IL-6) were measured. The main factor of platelet which induced the excretion of IL-6 from LSECs was determined using inhibitors of each components contained in platelets. The necessity of direct contact between platelets and LSECs was investigated using cell culture inserts. Proliferation of human primary hepatocytes was measured after the addition of the supernatant of LSECs cultured with or without platelets.

Results: The number of LSECs cocultured with platelets significantly increased. Excretion of IL-6 and vascular endothelial growth factor (VEGF) increased in LSECs with platelets. JTE-013, a specific antagonist for sphingosine 1-phosphate (S1P) 2 receptors, inhibited the excretion of IL-6 from LSECs after the addition of platelets. When the platelets and LSECs were separated by the cell culture insert, the excretion of IL-6 from LSECs was decreased. DNA synthesis was significantly increased in human primary hepatocytes cultured with the

supernatant of LSECs with platelets.

Conclusions: Platelets promote LSEC proliferation and induce IL-6 and VEGF production. Direct contact between platelets and LSECs and S1P, that are contained in platelets, were involved in the excretion of IL-6 from LSECs. IL-6 from LSECs induced proliferation of parenchymal hepatocytes.

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1. Introduction

Liver regeneration is a critical issue related to clinical morbidity and mortality after an extended hepatectomy or other types of liver injury [1, 2]. The precise mechanism of liver regeneration is still unclear [3]. Hepatocytes have a mitogenic response to various growth factors and cytokines such as hepatocyte growth factor (HGF), interleukin-6 (IL-6), tumor necrosis factor alpha, epidermal growth factor (EGF), tissue growth factor beta and platelet-derived growth factor (PDGF) [4]. These factors stimulate their own receptors in the cell membrane and activate transcription factors and signal transduction pathways; i.e., nuclear factor kappa-B, signal transducer and activator of transcription 3 (STAT3), extracellular signal-regulated protein kinase (ERK) 1/2, protein kinase B (Akt) and activator protein-1. Subsequently, hepatocyte proliferation occurs [5, 6]. Kupffer cells, liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells are involved in the mechanism of liver regeneration [7]. LSECs have been reported to produce HGF, IL-6 and nitric oxide (NO), and to promote hepatocyte proliferation after

hepatectomy [8].

Platelets contain many growth factors, such as PDGF, VEGF, EGF and insulin-like growth factor 1 (IGF-1) in the alpha granules [9]. It was reported that platelets contribute to bone regeneration [10] and dermal wound healing [11]. Platelets also have an important role in liver regeneration [12]. However, few studies have investigated the relationship between platelets and LSECs. Recently, some studies have focused on the role of platelets in hepatic ischemia/reperfusion (I/R) [13]. Activated platelets have been considered to be involved in hepatic injury and contribute to the development of apoptosis in LSECs after I/R [14, 15]. In our previous studies we revealed that platelets stimulate hepatocyte proliferation in vitro and in vivo models [13, 16-23]. These results suggest that growth factors contained in the platelets are responsible for the activation of the Akt, ERK1/2 and STAT3 pathways. In this study, we focused on sphingosine 1-phosphate (S1P) in addition to growth factors. S1P is a lipid mediator contained in platelets and was reported to regulate a broad variety of cellular processes such as cell proliferation, apoptosis, calcium homeostasis, vascular maturation or angiogenesis [24].

No previous studies have mentioned the relationship between platelets, human LSECs and hepatocytes in liver regeneration. The purpose of the present study was to clarify the role of platelets on the stimulation of LSECs and the effect of activated LSECs by platelets on hepatocyte proliferation.

2. Materials and methods

2.1. Preparation of platelets

Blood was obtained from healthy volunteers after obtaining their signed informed consent. Platelet-rich

plasma (PRP) was obtained by centrifuging anticoagulated blood containing acid citrate dextrose (ACD) in a 1:4 volume ratio at 200 x g at 4°C for 10 min. The platelets were washed twice by centrifugation of PRP at 1000 x g at 4°C for 15 min and resuspended in citrate buffer (120 mM NaCl, 4.26 mM NaH_2PO_4, 5.5 mM glucose, 4.77 mM sodium citrate, and 2.35 mM citric acid) adjusted to pH 6.5. Finally, the platelets were suspended in 0.5% human serum albumin (Wako Pure Chemical Industries, Tokyo, Japan)-PBS (HSA-PBS).

2.2. Liver sinusoidal endothelial cell line (TMNK-1)

TMNK-1 is a human liver sinusoidal endothelial cell line established by the transfection of SV40T and hTERT that proliferates in vitro [25]. TMNK-1 cells were cultured in a humidified atmosphere containing 5% CO_2 at 37°C in D-MEM medium (Wako) supplemented with 10% FBS (Hyclone, Road Logan, UT) and 1% penicillin and streptomycin (Invitrogen, Grand Island, NY).

2.3. Isolation and culture of human hepatocytes

Adult human normal liver samples were obtained during partial hepatic resection performed at University of Tsukuba Hospital for primary or secondary liver tumors. These patients were seronegative for hepatitis B and C with normal liver function and none had received chemotherapy just before operation. All sampled specimens of liver tissues were pathologically normal. All samples were collected with the informed consent of the patients. Furthermore, analysis of the samples was approved by the ethics committee and the institutional review board of University of Tsukuba Hospital. Hepatocytes were prepared using the Seglen collagenase perfusion technique [26]. The isolated parenchymal cell suspension had viability between 90% and 95% on the trypan blue exclusion test.

The hepatocytes were cultured in a humidified atmosphere containing 5% CO_2 at 37°C in Williams' E medium (Sigma, St. Louis, MO) supplemented with 200 μM L-glutamine (Invitrogen), 10% FBS (Hyclone), 100 M dexamethasone (Sigma), 1g/mL insulin (Sigma), 1% penicillin and streptomycin (Invitrogen).

2.4. Effects of platelets on TMNK-1 proliferation

The effect of platelets on TMNK-1 proliferation was evaluated counting TMNK-1 cells cocultured with platelets. Briefly, the TMNK-1 cells (3×10^4 in 1ml D-MEM medium supplemented with 10% FBS) were seeded in 6-well plates. After incubation at 37°C for 24 h, the medium was changed to the serum-free D-MEM medium (1mL/well) and cultured for 2 h. Then, platelets suspended in 0.5% HSA-PBS (100μL/well) at different densities (1×10^7/mL, and 1×10^8/mL) were added to each well. After incubation at 37°C for 24 and 48 h, the TMNK-1 cells were counted.

2.5. Western blot analysis of transcriptional factor in TMNK-1

To investigate the mechanism of platelet-induced TMNK-1 proliferation, we analyzed the TMNK-1 signals that were activated by platelets. TMNK-1 cells were precultured under confluent conditions in 6-well culture plates, and the medium was changed to the serum-free D-MEM medium (2mL/well) and cultured for 2 h. Then, platelets suspended in 0.5% HSA-PBS at 1×10^9/mL density were added to each well (200μL/well). TMNK-1 cells were harvested at 30, 60, 90, 120, and 180 min after the addition of platelets. For Western blot analysis, TMNK-1 lysates were subjected to 10% SDS-PAGE and transferred to a PVDF membrane. The following antibodies were used as primary antibodies: STAT3 (9132), phospho-STAT3 (9131), Akt (9272), phospho-Akt (9271), ERK1/2 (9102),

phospho-ERK1/2 (9101), GSK3β (9315) and phospho-GSK3β (9336)(Cell Signaling, Beverly, MA).

2.6. Assay of HGF, IL-6, VEGF and IGF-1 in the supernatant of cultured TMNK-1

TMNK-1 cells were precultured under confluent conditions in 6-well culture plates, and the medium was changed to the serum-free medium (2mL/well) and cultured for 2 h. Then, platelets suspended in 0.5% HSA-PBS at 1 x 10^9/mL density were added to each well of the group cocultured with platelets (200μL/well). Nothing was added to each well of the group cultured without platelets. After incubation at 37°C for 0, 6, and 24 h, the supernatant of TMNK-1 was sampled and centrifuged at 1000 x g for 15 min. Then amounts of HGF, IL-6 VEGF and IGF-1 in the supernatant were measured. In the present study, to quantify the amounts of IL-6, VEGF and IGF-1, commercially available Quantikine ELISA kits (R&D Systems, Minneapolis, MN) and the Human specific HGF ELISA kit (Institute of Immunology, Tokyo, Japan), were used.

2.7. Effects of inhibitors on excretion of IL-6 from TMNK-1 with platelets

To determine which platelet component had an effect on the excretion of IL-6 from TMNK-1, we inhibited the excreted soluble factors; i.e., VEGF and PDGF; using neutralizing antibodies or receptor antibodies and evaluated the excretion of IL-6 from TMNK-1. The following antibodies were diluted in each defined medium and used: Anti-human VEGF Antibody MAB293 (R&D Systems), Anti-human IGF-I Antibody AF-291-NA (R&D Systems), PDGFR Tyrosine Kinase Inhibitor IV (Calbiochem, San Diego, CA) and a specific antagonist for S1P2 receptors JTE-013 (Cayman Chemical, Ann Arbor, MI). TMNK-1 cells were precultured under confluent conditions in 24-well culture plates, and the medium was changed to the serum-free medium (1mL/well). Then, various

concentrations of the receptor antibodies PDGFR Tyrosine Kinase Inhibitor IV and JTE-013 were added to the cells and cultured for 2 h. The platelets were then suspended in serum-free medium at 1 x 10^9/mL density and were added to each well (100μL/well). For assays involving the neutralizing antibodies MAB293 and AF-291-NA, the platelets were preincubated with the antibodies for 30 min at 37°C before being added to TMNK-1. After incubation at 37°C for 6 h, the supernatant of TMNK-1 was sampled and centrifuged at 1000 x g for 15 min. Then amounts of IL-6 in the supernatant were measured using Quantikine ELISA kits.

2.8. Effects of S1P on excretion of IL-6 from TMNK-1

To determine whether S1P had an effect on the excretion of IL-6 from TMNK-1, we added S1P to TMNK-1 and evaluated the excretion of IL-6 from TMNK-1. TMNK-1 cells were precultured under confluent conditions in 24-well culture plates, and the medium was changed to the serum-free medium (1mL/well) and cultured for 2 h. Then, various concentrations of S1P (Sigma) were added to the cells. After incubation at 37°C for 6 h, the supernatant of TMNK-1 was sampled and centrifuged at 1000 x g for 15 min. Then amounts of IL-6 in the supernatant were measured using Quantikine ELISA kits.

2.9. Necessity of contact with platelets for excretion of IL-6 from TMNK-1

To investigate the necessity of direct contact between platelets and TMNK-1, TMNK-1 cells and platelets were cocultured using a cell culture insert (pore size; 0.4 μm; Becton Dickinson, Bedford, MA), and excretion of IL-6 from TMNK-1 was measured as described above. TMNK-1 cells were precultured under confluent conditions in 6-well culture plates. The medium was then changed to the serum-free medium (2mL/well) and cell culture

inserts were placed in each well, and cultured for 2 h. Then, platelets suspended in serum-free medium at 1 x 10^9/mL density were added to the lower chamber of each well of the group cocultured with platelets (mixed), or to the upper chamber of each well of the group cocultured with platelets (separated) (200μL/well). Only serum-free medium was added to each well of the group cultured without platelets (200μL/well). After incubation at 37°C for 6 h, the supernatant of TMNK-1 was sampled and centrifuged at 1000 x g for 15 min. Then amounts of IL-6 in the supernatant were measured using Quantikine ELISA kits.

2.10. Effects of the supernatants of TMNK-1 on hepatocyte proliferation

The effect of the supernatant of TMNK-1 on hepatocyte proliferation was evaluated using the BrdU assay kit (Roche Diagnostics GmbH, Penzberg, Germany). The supernatant of TMNK-1 cultured with or without platelets was sampled as mentioned in Materials and methods 2.6 (cultured for 24 h). Primary hepatocytes (3 x 10^3 in 100μL Williams' E medium supplemented with 10% FBS) were seeded in 96-well plates. After incubation at 37°C for 24 h, the medium was changed to the serum-free Williams' E medium (100μL/well) and cultured for 2 h. Then, the supernatant of TMNK-1 was added to each well (100μL/well). After incubation at 37°C for 24 and 48 h, BrdU uptake was measured according to the manufacture's instruction.

2.11. Western blot analysis of transcriptional factor in primary hepatocytes

The supernatant of TMNK-1 cultured with platelets was sampled as mentioned in Materials and methods 2.6 (cultured for 24 h). Primary hepatocytes were precultured in 6-well culture plates, and the medium was changed to the serum-free Williams' E medium (2mL/well) and cultured for 2 h. Then, the supernatant of TMNK-1 was added

to each well (105μL/well). Hepatocytes were harvested at 5, 10, 15, 30 and 60 min after the addition of the supernatant. For Western blot analysis, hepatocyte lysates were subjected to 10% SDS-PAGE and transferred to a PVDF membrane. The following antibodies were used as primary antibodies: STAT3, phospho-STAT3, Akt, phospho-Akt, ERK1/2, GSK3β, phospho-GSK3β and phospho-ERK1/2.

2.12. Effects of IL-6 in the supernatants of TMNK-1 on hepatocyte proliferation

To investigate the effect of IL-6 in the supernatant of TMNK-1 on hepatocyte proliferation, we inhibited effect of IL-6 using neutralizing antibody MAB206 (R&D Systems) and BrdU uptake was measured. The supernatant of TMNK-1 cocultured with platelets was preincubated with or without MAB206 for 30 min at 37°C before being added to hepatocytes. We used cryopreserved hepatocytes (viability 83%) and all other methods were the same as Materials and methods 2.10. The final concentration of MAB206 was 10μg/ml.

2.13. Statistical analysis

All data are expressed as the mean ± standard deviation of samples. Statistical analyses were carried out with Mann-Whitney U-test and one-way analysis of variance (ANOVA), and significant data were examined by the Bonferroni-Dunn multiple comparisons post hoc test. In all cases, a p value < 0.05 was considered significant.

3. Results

3.1. Effects of platelets on TMNK-1 proliferation

Proliferation of TMNK-1 in the group cocultured with platelets (1 x 10^7/mL) was significantly higher than in the group cultured without platelets after 48 h incubation (Fig. 1A).

3.2. Signal transduction of TMNK-1

The phosphorylation of Akt, GSK3β and ERK1/2 were analyzed in TMNK-1 cells stimulated by platelets for up to 180 min (Fig. 1B). The Akt and ERK1/2 pathways were activated within 30 min after the addition of platelets; the STAT3 pathway was not activated (data not shown).

3.3. Assay of HGF, IL-6, VEGF and IGF-1 in the supernatant of cultured TMNK-1

Concentrations of IL-6 and VEGF were significantly increased in the supernatant of the group cocultured with platelets compared to the supernatant of the group cultured without platelets at 6 and 24 h (IL-6) or 24 h (VEGF) after the addition of platelets (Fig. 2). HGF was not detected in either supernatant of groups cultured with or without platelets (data not shown).

3.4. Effects of inhibitors on excretion of IL-6 from TMNK-1 with platelets

Concentration of IL-6 was significantly decreased in the group administered with JTE-013 compared to those without JTE-013 after the addition of platelets and antibodies. There was no significant difference in the concentration of IL-6 in groups with and without administration of other antibodies (Fig. 3).

3.5. Effects of S1P on excretion of IL-6 from TMNK-1

Concentration of IL-6 was significantly increased in the group administered with S1P compared to those without S1P (Fig. 4).

3.6. Necessity of contact with platelets for excretion of IL-6 from TMNK-1

In the group in which the platelets and TMNK-1 were cultured separately, no significant increase of IL-6 was observed (Fig. 5).

3.7. Effects of the supernatants of TMNK-1 on hepatocyte proliferation

BrdU uptake of the hepatocytes in the group administered with the supernatant cocultured with platelets was significantly higher than in the group administered with the supernatant cultured without platelets (Fig. 6A).

3.8. Signal transduction of primary hepatocytes by cultured with supernatant of TMNK-1 activated by platelets

The Akt pathway was activated within 5 min, the ERK1/2 pathway within 10 min, and the STAT3 pathway within 5 min after the addition of the supernatant of TMNK-1 activated by platelets (Fig. 6B).

3.9. Effects of IL-6 in the supernatants of TMNK-1 on hepatocyte proliferation

BrdU uptake of the hepatocytes was significantly decreased in the group administered with anti-IL-6 antibody MAB206 compared to those without MAB206 (Fig. 6C).

4. Discussion

Platelets have an important role in a various stress mechanisms of the liver and we previously reported that platelets promote hepatocyte proliferation [12, 13, 16-23]. Recently, Lesurtel et al. reported that platelet-derived serotonin mediates liver regeneration in vivo [27]. However, there are no prior studies regarding the relationship between human platelets, LSEC and hepatocytes in liver regeneration. In this study, we focused on the role of LSECs and hepatocytes in liver regeneration in addition to platelets. We used human platelets and human primary hepatocytes and we used not primary LSECs but immortalised cell line TMNK-1 as LSECs. Using cocultured LSECs with human platelets, it was revealed that platelets proliferate LSECs and induce the excretion of IL-6, VEGF and IGF-1 from LSECs through activating Akt and ERK1/2 signals. The excretion of IL-6 from LSECs requires direct contact between platelets and LSECs. The S1P of platelets are responsible for producing IL-6 in LSEC. IL-6 from LSECs proliferate hepatocytes by activating STAT3. The results in this study indicate that platelets induced hepatocyte proliferation through LSEC activation.

The PI3/Akt pathway is activated by growth factors and is known as a survival signaling pathway [28]. GSK3β is located downstream of Akt and plays a critical role in liver regeneration by regulating cell size [29]. It was reported that Akt of rat LSEC was phosphorylated after the addition of VEGF [30] or PDGF [31]. The ERK1/2 pathway is also activated by growth factors and involved in growth and differentiation [32]. There are no reports indicating that any growth factors activate the ERK1/2 pathway of LSEC. It was reported that the ERK1/2 of human umbilical vein endothelial cells was phosphorylated after the addition of VEGF [33]. In this study, the Akt and ERK1/2 pathways of TMNK-1 were activated after the addition of platelets and the proliferation of TMNK-1

was induced. This effect may be caused by some growth factors or S1P. Platelets contain VEGF, PDGF and S1P; therefore, they are likely involved in the activation of the Akt and ERK1/2 pathways of TMNK-1.

IL-6 is known to play a crucial role in parenchymal hepatocyte proliferation by activating STAT3 [34]. In this study, concentrations of IL-6 in the supernatant of TMNK-1 were significantly increased in the groups with platelets compared to the groups without platelets. The effects that activate the STAT3 pathways of hepatocyte as well as Akt and ERK1/2 and promote DNA synthesis were presumably due to IL-6 in the supernatants of TMNK-1. VEGF does not have a strong effect on hepatocyte proliferation [35]. Therefore, it is likely that IL-6, excreted from SEC, mainly contributed to hepatocyte proliferation rather than VEGF.

S1P belongs to lipid mediators and has been revealed to regulate diverse biological processes [24]. S1P is excreted in a large amount from activated platelets [36]. It was reported that S1P promotes DNA synthesis of LSECs and protects LSECs from ethanol-induced apoptosis through endothelial NO synthase activation in rat [30]. It was also reported that S1P causes the excretion of IL-6 from other cells, such as human airway smooth muscle cells [37] and immature human dendritic cells [38]. When platelets and TMNK-1 were separated, no significant increment of IL-6 was observed. The concentration of IL-6 was significantly decreased in the supernatant of TMNK-1 cocultured with platelets after the addition of S1P inhibitor. On the other hand, the concentration of IL-6 was significantly increased after the addition of S1P alone. These results indicate that direct contact between platelets and LSECs triggered the excretion of S1P from the platelets, S1P derived from platelets induced the excretion of IL-6 from LSECs (Fig. 7). Also, the DNA synthesis of hepatocytes was then significantly promoted by stimulating IL-6 excretion from LSECs. Platelets would contribute to hepatocyte proliferation with activation of LSECs through S1P and IL-6.

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Figure legends

Fig. 1

(A) Effects of platelets on TMNK-1 proliferation. The effect of platelets on TMNK-1 proliferation was evaluated counting TMNK-1 cells cocultured with platelets for 24 and 48 h. Data are expressed as mean ± SD. n = 4 in each group. *P < 0.05 versus the platelet (-) group. (B) Signal transduction of TMNK-1. To investigate the mechanism of platelet-induced TMNK-1 proliferation, TMNK-1 cells were harvested at 0, 30, 60, 90, 120 and 180 min after the addition of platelets, and activation of the Akt, ERK1/2, and STAT3 pathways was examined by

Western blotting.

Fig. 2

Assay of HGF, IL-6, VEGF and IGF-1 in the supernatant of cultured TMNK-1. The amounts of HGF, IL-6, VEGF and IGF-1 in the supernatant of TMNK-1 were measured at 0, 6, and 24 h after the addition of platelets. Data are expressed as mean ± SD. n = 6 in each group. *P < 0.05 versus the platelet (-) group. IL-6, interleukin-6; VEGF, vascular endothelial growth factor; IGF-1, insulin like growth factor 1.

Fig. 3

Effects of inhibitors on excretion of IL-6 from TMNK-1 with platelets. To determine which platelet component had an effect on excretion of IL-6 from TMNK-1, we inhibited the excreted soluble factors using neutralizing antibodies or receptor antibodies and evaluated excretion of IL-6 from TMNK-1. Anti-human VEGF Antibody MAB293, Anti-human IGF-I Antibody AF-291-NA, PDGFR Tyrosine Kinase Inhibitor IV and a specific antagonist for S1P2 receptors JTE-013 were added to TMNK-1 or platelets. TMNK-1 cells were then cocultured with platelets for 6 h, and the amounts of IL-6 in the supernatant of TMNK-1 were measured. Data are expressed as mean ± SD. n = 4 in each group. *P < 0.05 versus the inhibitor (-) group. IL-6, interleukin-6.

Fig. 4

Effects of S1P on excretion of IL-6 from TMNK-1. To determine whether S1P had an effect on excretion of IL-6 from TMNK-1, TMNK-1 cells were cultured with S1P for 6 h, and the amounts of IL-6 in the supernatant of

TMNK-1 were measured. Data are expressed as mean ± SD. n = 4 in each group. *P < 0.05 versus the S1P (-) group. IL-6, interleukin-6; S1P, sphingosine 1-phosphate.

Fig. 5

Necessity of contact with platelets for excretion of IL-6 from TMNK-1. To investigate the necessity of direct contact between platelets and TMNK-1, TMNK-1 and platelets were cocultured using a cell culture insert for 6 h, and excretion of IL-6 from TMNK-1 was measured. Data are expressed as mean ± SD. n = 4 in each group. *P < 0.05 versus the platelet (+) separated group. IL-6, interleukin-6.

Fig. 6

(A) Effects of the supernatants of TMNK-1 on hepatocyte proliferation. Primary hepatocytes were cultured with the supernatants of TMNK-1, and after 24 and 48 h incubation, BrdU uptake was measured. Data are expressed as mean ± SD. n = 8 in each group. *P < 0.05 versus the platelet (-) supernatant group. (B) Signal transduction of primary hepatocytes cultured with the supernatant of TMNK-1 activated by platelets. To investigate the mechanism of effects of the supernatants of TMNK-1 on hepatocyte proliferation, hepatocytes were harvested at 0, 5, 10, 15, 30 and 60 min after the addition of the supernatants of TMNK-1 with platelets, and activation of the Akt, ERK1/2 and STAT3 pathways was examined by Western blotting. (C) Effects of IL-6 in the supernatants of TMNK-1 on hepatocyte proliferation. Primary hepatocytes were cultured with the supernatant of TMNK-1, which was preincubated with or without anti-IL-6 antibody MAB206, and after 24 h incubation, BrdU uptake was measured. Data are expressed as mean ± SD. n = 4 in each group. *P < 0.05 versus the MAB206 (-) supernatant

group. IL-6, interleukin-6.

Fig. 7

Scheme for liver regeneration promoted by LSECs and platelets. It was reported that platelets promote hepatocyte proliferation. Our results indicate that direct contact between platelets and LSECs triggered the excretion of S1P from the platelets, which then caused the excretion of IL-6 from LSECs. IL-6 from LSECs promoted hepatocyte proliferation. IGF-1, insulin-like growth factor 1; HGF, hepatocyte growth factor; LSEC, liver sinusoidal endothelial cell; S1P, sphingosine 1-phosphate; IL-6, interleukin-6.