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## REVIEW

# Novel functions of platelets in the liver

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**Key words**

platelet, liver, chronic liver disease, cirrhosis, thrombocytopenia, thrombopoietin, liver regeneration, blood transfusion, eltrombopag, adenosine tri-phosphate (ATP), hepatocyte growth factor (HGF), Kupffer cell, liver sinusoidal endothelial cell (LSEC)

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**Abstract**

Platelets contain not only proteins needed for hemostasis but also many growth factors that are required for organ development, tissue regeneration, and repair. Thrombocytopenia, which is frequently observed in patients with chronic liver disease (CLD) and cirrhosis, is due to various causes, such as decreased thrombopoietin production and accelerated platelet destruction caused by hypersplenism; however, the relationship between thrombocytopenia and hepatic pathogenesis and the role of platelets in CLD are poorly understood. Thus, in this paper, the experimental evidence for platelets improving liver fibrosis and accelerating liver regeneration is summarized and addressed based on studies conducted in our laboratory and current progress reports from other investigators. Platelets improve liver fibrosis by inactivating hepatic stellate cells to decrease collagen production. The level of intracellular cAMP is increased by adenosine through its receptors on hepatic stellate cells, thereby resulting in inactivation of these cells. Adenosine is produced by degradation of adenine nucleotides, which are stored in abundance within the dense granules of platelets. The regenerative effect of platelets in the liver consists of three mechanisms: a direct effect on hepatocytes, a cooperative effect with liver sinusoidal endothelial cells, and a collaborative effect with Kupffer cells. Based on these experiments, a clinical trial suggested that the increase in platelets induced by platelet transfusion improved liver function in patients with CLD in a clinical setting. We highlight the current knowledge concerning the role of platelets in CLD and expect to open a novel avenue for application of these clinical therapies to treat liver disease.

**Introduction**

Chronic liver disease (CLD) is a major cause of mortality and morbidity in many countries,<sup>1</sup> and cirrhosis is the end stage of CLD. Cirrhosis carries a poor prognosis and an increased risk of carcinogenesis.<sup>2</sup> Liver fibrosis represents the consequence of a sustained wound-healing response to chronic liver injury induced by a variety of causes, including viral infection, alcohol abuse, autoimmune disorders, drug use, cholestasis, and metabolic diseases.<sup>3</sup> Currently, liver transplantation is still the only curative approach for end-stage cirrhosis, but this process is associated with serious problems, such as graft shortage in living-donor-liver transplantation, surgical complications, organ rejection, and high cost.<sup>3</sup> Although liver fibrosis has traditionally been regarded as an irreversible process, recent reports indicate that even advanced fibrosis may be reversible.<sup>4</sup> Based on these studies, novel treatments have been developed to treat patients with cirrhosis.

Thrombocytopenia is a common complication of CLD and is due to various causes, including decreases in thrombopoietin (TPO) production, increases in platelet destruction with splenomegaly, and an inability of bone marrow to perform hematopoiesis.<sup>5</sup>

Therefore, thrombocytopenia is thought to be intimately related to the pathogenesis of CLD and cirrhosis.

The effect of thrombocytopenia on liver damage and the exact mechanisms that lead to thrombocytopenia in CLD and cirrhosis are still unclear, and further study is required. In our previous studies, we revealed that platelets play a crucial role in promoting liver regeneration.<sup>6-9</sup>

Platelets (anuclear blood cells) are derived from megakaryocytes (MKs), which contain not only proteins needed for hemostasis but also many growth factors that are required for tissue regeneration and repair.<sup>10</sup>

We also reported that platelets have a preventive effect on the progression of liver fibrosis *in vitro* and *in vivo* and that the increase in platelets induced by platelet transfusion can improve the liver function of patients with CLD and cirrhosis in a clinical setting.<sup>11-14</sup>

In addition, it was reported that splenectomy, which is a platelet increment therapy, contributes to improvement of liver function.<sup>15</sup>

However, there are many contradictory reports that platelets have harmful effects on liver fibrosis.<sup>16,17</sup>

The aim of this review is to summarize the clinical and experimental studies that have broadened our understanding of the role of platelets in CLD.

## Platelets

Platelets are derived from MKs. MKs are derived from multipotent hematopoietic stem cells toward MK progenitors. Mature MKs produce platelets by cytoplasmic fragmentation, which occurs through a dynamic and regulated process called proplatelet formation; these proplatelets consist of long pseudopodial elongations that break in the blood flow.<sup>18</sup> Recently, it has been reported that IL-1 $\alpha$  induces thrombopoiesis through MK rupture in response to acute platelet needs.<sup>19</sup>

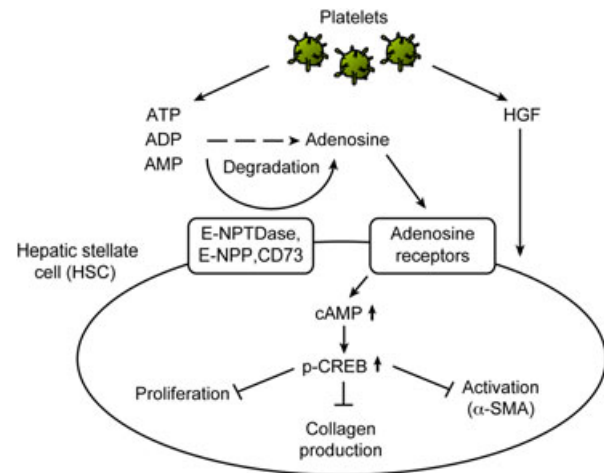
Platelets are discoid and have anucleate structures that contain a large number of secretory granules,<sup>20</sup> alpha granules, dense granules, and lysosomal granules, of which three types of secretory granules are recognized.

Each granule contains secretory substances, such as platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), serotonin, adenosine diphosphate (ADP), adenosine tri-phosphate (ATP), epidermal growth factor (EGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), and sphingosine 1-phosphate (S1P).<sup>20</sup> Platelets are activated by various types of stimulation and release active substances from their granules.<sup>20–22</sup>

Opposite effects have been reported: the positive ones include processes such as hemostasis,<sup>22</sup> wound healing,<sup>23</sup> and tissue regeneration,<sup>24</sup> whereas the negative effects of platelet degranulation include inflammation,<sup>25</sup> malignancy<sup>26</sup> and immune response.<sup>27</sup> It has been reported that platelets accumulate in the liver under certain pathological conditions, such as ischemia/reperfusion,<sup>28,29</sup> cirrhosis, cholestasis,<sup>16</sup> viral hepatitis,<sup>30</sup> and the residual liver after hepatectomy.<sup>6</sup>

## Platelets and liver fibrosis

Currently, liver fibrosis is known to be part of a dynamic process of continuous extracellular matrix (ECM) remodeling in the setting of chronic liver injury; this process leads to excessive accumulation of several extracellular proteins, proteoglycans, and carbohydrates.<sup>3</sup> Among the cellular populations in the liver, hepatic stellate cells (HSCs) have been reported to have the most involvement in liver fibrosis through production of large amounts of ECM and secretion of TGF- $\beta$ , which appears to be a key mediator of liver fibrogenesis.<sup>3</sup> In the normal liver, HSCs reside in the space of Disse, and their primary function is storage of vitamin A and other retinoids.<sup>31</sup> In addition, HSCs are now well established as the key cellular element that is involved in the development of liver fibrosis.<sup>31</sup> In response to liver injury, HSCs undergo morphological and functional transdifferentiation, converting from vitamin A-storing star-like cells into contractile myofibroblastic cells; this process is called activation.<sup>32</sup> Ikeda *et al.* reported that human platelets contribute to suppression of both HSC activation and type I collagen production via a cyclic AMP signaling pathway *in vitro* (Fig. 1).<sup>33</sup> The level of intracellular cAMP is increased by adenosine through its receptors on HSCs. Large amounts of adenosine around HSCs are produced by degradation of adenine nucleotides, such as ADP and ATP, which are stored



**Figure 1** Scheme showing the functions of platelets in the suppression of liver fibrosis. The signs “ $\uparrow$ ” and “ $\downarrow$ ” in the figure indicate increases and decreases, respectively. Platelets come in contact with HSCs and release adenine nucleotides, such as ADP and ATP. These adenine nucleotides subsequently lead to production of adenosine through degradation by HSCs, which inhibits the activation of HSCs. Platelets also contribute to the expression of HGF in the liver. Quiescent HSCs inactivated by adenosine or HGF reduce the production of TGF- $\beta$  and ECM.

in abundance within the dense granules of platelets.<sup>33</sup> Quiescent HSCs that are inactivated by adenosine may have a decreased ability to produce TGF- $\beta$  and secrete ECM. In addition, platelet-derived HGF plays a critical role in suppression of type I collagen gene expression in cultured HSCs.<sup>34</sup> HGF has also been reported to attenuate liver fibrosis through suppression of HSC activation and hepatic TGF- $\beta$  expression.<sup>35</sup> These findings indicate that platelets can play a crucial role in suppression of liver fibrogenesis via inhibition of HSC activation. Because human platelets contain a smaller amount of HGF than rodent platelets, it is unclear whether the mechanisms observed in rodents are applicable to humans.<sup>36</sup>

Thrombopoietin is the most important growth factor in the regulation of MK development and platelet production.<sup>37</sup> Several promising novel agents that stimulate TPO receptors and increase platelet levels, such as eltrombopag and romiplostim, are currently in development for the treatment of thrombocytopenia in patients with CLD and cirrhosis.<sup>38,39</sup> The ability to increase platelet levels could significantly reduce the need for platelet transfusions and facilitate the use of interferon-based antiviral therapy and other treatments in patients with liver disease.<sup>39</sup> Recently, it was reported that the increase in platelets induced by TPO administration could improve liver fibrosis, even in subjects with CLD and cirrhosis, in experimental studies.<sup>11,13</sup> Dimethylnitrosamine was administered three times a week for three weeks to induce liver fibrosis in rats. Five days after administering TPO intravenously, 70% hepatectomy was performed, and the liver fibrosis was compared 24 h after the hepatectomy. The increase in platelets inhibited the activation of HSCs and reduced the fibrotic area of the cirrhotic liver; these effects were diminished by administration of antiplatelet serum.<sup>11</sup> Carbon tetrachloride (CCl<sub>4</sub>) was

administered twice a week for 8 weeks to induce liver fibrosis in mice. TPO was administered intraperitoneally once a week from 5 to 8 weeks during the experiment. <sup>13</sup>By administering TPO, liver fibrosis was decreased. <sup>13</sup> Although the precise mechanisms that relate the increase in platelets and the liver anti-fibrotic effect are still unclear, one reason may be that platelets enhanced the expression of HGF by approximately 14%, <sup>11</sup> whereas the matrix metalloproteinase 9 (MMP9) was enhanced by approximately three times, thereby stimulating fibrolysis and decreasing pro-fibrotic growth factor TGF- $\beta$ . <sup>13</sup> MMPs such as MMP-8, MMP-9, and MMP-13 possess the ability to degrade the extracellular matrix via the breakdown of collagen type I. <sup>40</sup> MMP-9 may indirectly contribute to fibrolysis by accelerating HSC apoptosis. <sup>41</sup> In the murine bile duct ligation model, thrombocytopenia exacerbates liver fibrosis and platelets have an anti-fibrotic role by suppressing type I collagen expression via the HGF–Met signaling pathway. <sup>34</sup> Recently, Takahashi *et al.* reported that transfused human platelets improved liver fibrosis in severe combined immune deficiency (SCID) mice induced by CCL4. <sup>42</sup> An increase in murine HGF and a decrease in TGF- $\beta$  were observed in the liver. <sup>42</sup> Although the precise mechanism that relates the increase in platelets and liver fibrolysis remain unclear, one reason is that platelets enhanced the expression of HGF without an increase in the expression of pro-fibrotic growth factors derived from platelets, such as TGF- $\beta$  and PDGF, in the cirrhotic liver in rodent models. <sup>11,35</sup>

## Platelets and liver regeneration

Liver regeneration is performed by proliferation of hepatocytes, biliary epithelial cells, liver sinusoidal endothelial cells (LSECs), Kupffer cells, and hepatic stellate cells, all of which proliferate to rebuild the lost hepatic tissue. <sup>43</sup> Intercellular interactions via many growth factors and cytokines, including HGF, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), transforming growth factor- $\alpha$  (TGF- $\alpha$ ), and EGF, play important roles. Each growth factor and cytokine leads to subsequent activation of downstream transcription cascades, which is associated with the transitions of quiescent hepatocytes into the cell cycle and progression beyond the restriction point in the G1 phase of the cycle. <sup>43</sup> Several transcription factors are known to have functions in this process: nuclear factor-kappa B (NF- $\kappa$ B), activator protein 1 (Ap-1), CCAAT/enhancer binding protein-b (C/EBPb), extracellular signal-regulated kinase 1/2 (ERK 1/2), <sup>44</sup> signal transducer and activator of transcription 3 (STAT3), and phosphatidylinositol-3-kinase (PI3 K)/Akt. <sup>45</sup> The current study focused on the TNF- $\alpha$ /NF- $\kappa$ B, IL-6/STAT3, and PI3 K/Akt pathways as the three major cascades in which platelets exert their effects during the process of liver regeneration.

The TNF- $\alpha$ /NF- $\kappa$ B pathway is activated within 30 min after hepatectomy, and the activation usually lasts 4–5 h. NF- $\kappa$ B is found in almost every cell, including hepatocytes and non-parenchymal cells. It is usually inactivated by Inhibitor of NF- $\kappa$ B (I $\kappa$ B), which binds to the p65 subunit. <sup>46</sup> After NF- $\kappa$ B is stimulated by TNF- $\alpha$  and activated by removal of I $\kappa$ B from the p65 subunit, it migrates to the cell nucleus and binds to the promoter of cyclin-D1, which regulates the G0/G1-to-S-phase transition. <sup>47</sup> STAT3 is activated 1–2 h after hepatectomy and lasts 4–6 h. STAT3 is phosphorylated after being stimulated by IL-6 and

translocates to the nucleus, where it promotes the expression of cyclin-D1 and p21 to control the progression of the cell cycle. <sup>48</sup> Haga *et al.* reported that hepatocytic mitosis was significantly suppressed in STAT3-knockout mice after partial hepatectomy. <sup>49</sup> The PI3 K/Akt pathway was activated shortly after hepatectomy. The pathway is stimulated by HGF, IL-6, TNF- $\alpha$ , TGF- $\alpha$ , or many other signaling molecules. <sup>50</sup> C-met is one of the tyrosine kinase receptors on the surface of hepatocytes that binds with HGF, and HGF/c-met signaling activates PI3 K, which recruits Akt to the site of membranes and, subsequently, phosphorylates Akt. <sup>51</sup> Phosphorylation of Akt results in activation of glycogen synthase kinase (GSK) 3b, thus resulting in accumulation of  $\beta$ -catenin and cyclin-D1 in the nucleus, and induces DNA synthesis and cellular mitosis of hepatocytes. <sup>52</sup>

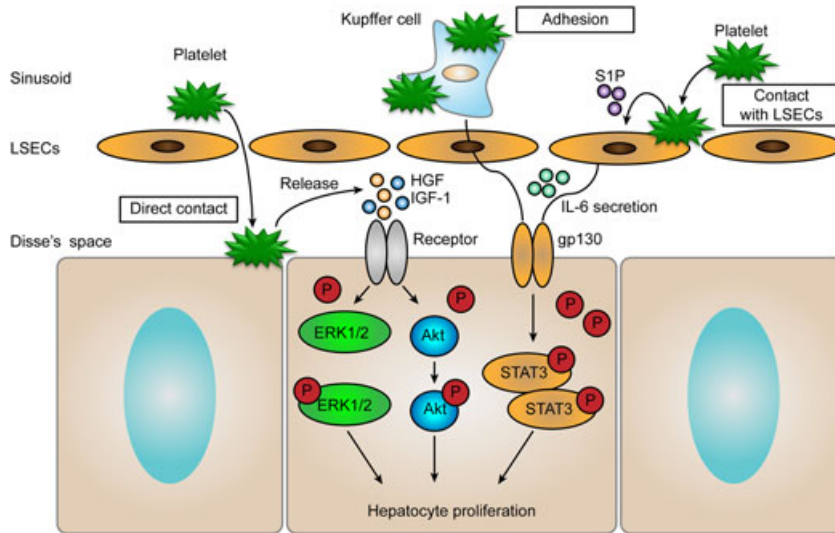
However, it has never been directly demonstrated that release of growth factors from platelets is responsible for platelet-mediated stimulation of liver regeneration *in vivo*. <sup>53,54</sup>

## Mechanisms for the promotive effect of platelets in liver regeneration

**The direct effect of platelets.** Platelets accumulate in the thrombocytotic liver within 5 min after hepatectomy, and a two-fold increase in platelets is observed in the liver in comparison with pre-hepatectomy levels. <sup>55</sup> Electron microscopy has revealed that platelets translocate from the sinusoidal space into the space of Disse and have direct contact with hepatocytes. <sup>6,7</sup> Endo *et al.* <sup>56</sup> reported a similar phenomenon, in which platelets accumulate in the liver and a large number platelets are detected in the space of Disse in response to the administration of lipopolysaccharide (LPS), interleukin-1, and TNF- $\alpha$ . Intravital microscopy reveals that although a few platelets flow rapidly in the liver sinusoids before partial hepatectomy, a significant number of platelets accumulate in the liver sinusoids, and the platelets flow slowly with rolling and adhering after hepatectomy. <sup>8</sup> These results suggest that platelets accumulate in the liver within a few minutes after hepatectomy and provide signals for rapid hepatocyte proliferation through direct contact with hepatocytes. A co-culturing chamber system was used to clarify the role of the direct contact of platelets with hepatocytes. Platelets and hepatocytes are separated by a permeable membrane. <sup>10</sup> Direct contact between platelets and hepatocytes triggers the release of soluble mediators from platelets, such as HGF, IGF-1, and VEGF, which leads to hepatocyte proliferation (Fig. 2).

The mechanism that underlies the direct effect of platelets can be explained. Platelets accumulate in the liver immediately after hepatectomy, translocate from the liver sinusoids to the space of Disse and release growth factors, such as HGF, IGF-1, and VEGF, through direct contact with hepatocytes. Growth factors stimulate the initiation of hepatocyte mitosis, which eventually promotes liver regeneration. Human platelet does not include most of the HGF; <sup>36</sup> thus, IGF-1 is considered to be the most important mediator for liver regeneration in human platelets. Recently, it has been reported that RNA transfer mechanism as an additional contributor to platelet-mediated liver regeneration. <sup>57</sup>

**The collaborative effect with Kupffer cells.** Kupffer cells play a role in the liver as macrophages that act against bacteria, endotoxins, and microbial debris derived from the



**Figure 2 Liver regeneration promoted by platelets. Platelets accumulate in the liver immediately after hepatectomy.** Platelets translocate from the sinusoidal space to the space of Disse and release growth factors, such as IGF-1 and HGF, through direct contact with hepatocytes, which, subsequently, induces initiation of hepatocyte mitosis. Direct contact between platelets and LSECs triggers the release of S1P from platelets, which leads to excretion of IL-6 from LSECs. IL-6 from LSECs promotes proliferation of hepatocytes, and interaction of Kupffer cells (KCs) with platelets activates KCs after hepatectomy. Activated KCs release TNF $\alpha$  and IL-6. IGF-1 and HGF activates Akt and ERK1/2 in the hepatocytes. IL-6 stimulates STAT3 activation. These signal transduction molecules proliferate hepatocytes.

gastrointestinal tract. Kupffer cells produce important cytokines that have a stimulatory influence on hepatocyte proliferation after hepatectomy.<sup>58</sup> One of the most important events following hepatectomy is an increase in the plasma levels of TNF- $\alpha$ . An experiment using an antibody against TNF- $\alpha$  demonstrated a significant reduction of hepatocyte proliferation,<sup>59</sup> and mice lacking the TNF- $\alpha$  receptor exhibit severe impairment in liver regeneration after hepatectomy.<sup>60</sup> Activation of the TNF- $\alpha$  receptor increases the hepatic expression of the NF- $\kappa$ B in both hepatocytes and non-parenchymal cells and is followed by production and release of IL-6 from Kupffer cells.<sup>43</sup> Kupffer cells are considered to be the most important source of both TNF- $\alpha$  and IL-6. Kupffer cell-depleted mice fail to exhibit increases in TNF- $\alpha$  and IL-6 to levels that are equivalent to those in mice with Kupffer cells after hepatectomy.<sup>61</sup> The interrelationship between platelets and Kupffer cells has been well studied using ischemia/reperfusion models. A triangular interaction among platelets, Kupffer cells, and leukocytes has been demonstrated as the major mechanism of injury.<sup>62</sup> Rats depleted of Kupffer cells were subjected to ischemia/reperfusion, which suppressed platelet adherence in sinusoids, and, as a consequence, there was attenuation of sinusoidal perfusion failure and endothelial damage.<sup>29</sup> Tamura *et al.*<sup>63</sup> reported that platelets adhered to Kupffer cells at the early period of ischemia/reperfusion and that the platelets that adhered Kupffer cells were involved in apoptosis of hepatocytes and the mechanism of hepatic ischemia/reperfusion injury. Nakamura *et al.* described different characteristics for platelets and Kupffer cells. The authors reported that direct cellular interactions between platelets and Kupffer cells play an important role in platelet migration to the space of Disse after administration of LPS.<sup>64</sup> It is therefore likely that platelets are activated by direct contact with Kupffer cells, and Kupffer cells are stimulated by direct contact with platelets. The collaborative effect of platelets with Kupffer cells on liver regeneration is thought to occur after hepatectomy, when activated Kupffer cells induce accumulation and activation of platelets in the liver, and the functions of Kupffer cells are enhanced by the accumulated platelets. Liver regeneration is promoted by the direct effect of

growth factors released from platelets and by the paracrine effect of Kupffer cells, which is enhanced by the platelets (Fig. 2).

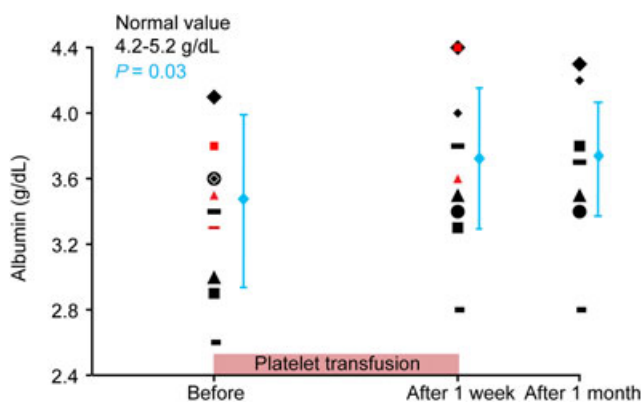
**The cooperative effect with liver sinusoidal endothelial cells.** Sinusoidal cells account for 70% of LSECs. The construction of a thin and continuous layer of sinusoidal endothelium forms a structural barrier that separates the hepatic parenchyma from blood constituents that pass through the liver.<sup>65</sup> LSECs enable contact between circulating blood and hepatocytes and help exchange various soluble macromolecules and nanoparticles, such as lipoproteins.<sup>66</sup> LSECs are involved in liver regeneration and produce immune-regulatory and pro-inflammatory cytokines, including HGF, IL-1, IL-6, and interferons. The elevation of the IL-6 concentration after hepatectomy is an important component of the early signaling pathways in liver regeneration, and IL-6 activates the acute phase of protein synthesis by hepatocytes as a part of the overall inflammatory response.<sup>67</sup> The plasma IL-6 concentration increases after hepatectomy from 6 h, with a peak by 24 h.<sup>68</sup> IL-6 binds to the receptor on hepatocytes, which leads to phosphorylation of STAT3 monomers. The relationship between platelets and LSECs is well documented in ischemia/reperfusion models.<sup>69</sup> In contrast, no studies have focused on the relationship between platelets and LSECs in liver regeneration. The role of platelets in liver regeneration in relation to LSECs was evaluated using co-culturing chamber systems.<sup>9</sup> These studies clarified that platelets increase the proliferation of LSECs and induce IL-6 release from LSECs, and IL-6 derived from LSECs accelerates DNA synthesis in hepatocytes. Direct contact between platelets and LSECs was required for IL-6 excretion. In addition, the high level of sphingosine-1-phosphate (S1P) in platelets plays an important role in IL-6 secretion.<sup>9</sup> S1P is a lipid mediator that regulates diverse biological processes, including proliferation, migration, and cytoskeletal reorganization.<sup>70</sup> S1P is excreted in large amounts from activated platelets and interacts with endothelial cells under the conditions of critical platelet-endothelial interactions, such as thrombosis, angiogenesis, and atherosclerosis.<sup>70</sup>

The cooperative effect of platelets with LSECs on liver regeneration occurs when direct contact between platelets and LSECs induces S1P release from platelets, which subsequently induces excretion of IL-6 from LSECs. IL-6 derived from LSECs promotes hepatocyte proliferation through the STAT3 pathway (Fig. 2).

**Effect of platelet transfusion on chronic liver disease and cirrhosis.** Platelet transfusion is an established therapy for thrombocytopenia, with well-known benefits and complications. The results suggest that both endogenous and exogenous platelets may play a role in the improvement of liver function, although whether platelet transfusion is appropriate for CLD patients with thrombocytopenia is still unclear, even in the published guidelines for platelet transfusion.<sup>71</sup> Based on these animal experiments, clinical trials were performed. Maruyama *et al.* recently reported on a clinical trial to investigate whether platelet transfusion improves liver function in patients with CLD and cirrhosis (Child-Pugh class A or B) who all presented with thrombocytopenia (platelet counts between 50 000 and 100 000/ $\mu$ L). The subjects received 10 units of platelet concentrate once per week for 12 weeks. One and three months after the last transfusion, significant improvements in serum albumin were observed (Fig. 3). Serum cholinesterase improved for 9 months after the last transfusion. Serum hyaluronic acid, which represents liver fibrosis, exhibited a tendency to improve after the last transfusion. This clinical trial was a non-controlled, non-randomized study, in which only 6 patients were eventually analyzed.<sup>14</sup>

### Limitations of platelet increment therapy for CLD and cirrhosis

Platelet increment therapies, such as administration of a TPO receptor agonist, splenectomy, and platelet transfusion, have been reported to have adverse effects, although they have ameliorating effects for CLD and cirrhosis.<sup>39,72,73</sup> Portal vein thrombosis has been observed among patients who received eltrombopag or splenectomy.<sup>39</sup> In addition, operative complications of splenectomy include hemorrhage,



**Figure 3** Liver function improvements after platelet transfusion. The serum albumin level at 1 month ( $P = 0.03$ ) after the last transfusion was significantly greater than that before the platelet transfusion. The data are expressed as the means  $\pm$  standard deviations. \* $P < 0.05$  compared with the levels before the platelet transfusion.

infection, and injury to the pancreatic tail.<sup>72</sup> In platelet transfusion, platelets are frequently activated and may induce inflammatory reactions and unexpected side-effects, including febrile non-hemolytic reactions and acute lung injury.<sup>73</sup> There are some reports regarding the detrimental effects of platelets on CLD and cirrhosis. Zaldivar *et al.* provided evidence that chemokine (C-X-C motif) ligand 4 (CXCL4), which is known as a platelet-derived factor, modulated liver fibrosis in animal models of chronic liver injury *in vivo*, although direct release of CXCL4 by platelets was not demonstrated.<sup>16</sup> In addition, the proliferation and chemotactic migration of HSCs was significantly enhanced by CXCL4 without synthesis of collagen and expression of TGF- $\beta$  *in vitro*.<sup>16</sup> As one of the side-effects of platelet increment therapy, promotion of liver carcinogenesis should be considered because of the high risk of HCC in patients with CLD and cirrhosis.<sup>3</sup> Carr *et al.* reported that patients with thrombocytosis (platelet levels  $> 400 \times 10^9/L$ ) had larger HCC sizes and better liver function than patients with platelet levels in the normal range.<sup>74</sup> Sitia *et al.* demonstrated that antiplatelet drugs reduced the development of HCC in a mouse model of chronic hepatitis B virus infection, although this approach was not effective in the carbon tetrachloride-induced cirrhosis model.<sup>75</sup> These findings indicate that platelets might have a promotive effect on liver carcinogenesis. However, it has been reported that TPO itself had no proliferative effect on HCC in *in vitro* and *in vivo* experiments.<sup>76</sup> Currently, two TPO-R agonists, eltrombopag and romiplostim, have been approved for treatment of chronic immune thrombocytopenic purpura. Eltrombopag is a small-molecule, non-peptide TPO-R agonist, whereas romiplostim is a peptide TPO-R agonist that is composed of an IgG Fc fragment.<sup>77</sup> The efficacy of eltrombopag in hepatitis C virus-infected patients with thrombocytopenia before the initiation of pegylated interferon and ribavirin therapy has previously been reported.<sup>38</sup> In contrast to TPO, eltrombopag does not activate the PI3K/Akt pathway.<sup>78</sup> Another report demonstrated that eltrombopag has no proliferative effect in myelodysplastic syndromes and AML patients but rather inhibits the proliferation of the leukemia cell line.<sup>79</sup> Thus, eltrombopag would be secure in HCC, and use of this type of TPO-R agonist can be anticipated as a novel treatment for liver disease.

### Summary and future work

This review discussed the current evidence for platelets improving liver fibrosis and promoting liver regeneration. There is significant evidence that platelets play a role in improving fibrosis. ATP and ADP inside platelets are degraded by HSCs, and adenosine is incorporated into the HSCs. Cyclic AMP is increased by adenosine, and HSCs become inactivated by cyclic AMP. There are three different mechanisms of liver regeneration induced by platelets: a direct effect on hepatocytes; cooperation with Kupffer cells; and a cooperative effect with LSECs. Therefore, platelet therapy, that is, platelet transfusion and TPO receptor agonist administration, could open a new avenue to develop novel strategies for treatment of liver diseases for which there is currently no effective treatment except transplantation.

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## References

- Pinzani M, Marra F. Cytokine receptors and signaling in hepatic stellate cells. *Semin. Liver Dis.* 2001; **21** (3): 397–16.
- Montalto G, Cervello M, Giannitrapani L, Dantona F, Terranova A, Castagnetta LAM. Epidemiology, Risk Factors, and Natural History of Hepatocellular Carcinoma. *Ann. N. Y. Acad. Sci.* 2006; **963** (1): 13–20.
- Battaller R, Brenner DA. Liver fibrosis. *J. Clin. Invest.* American Society for Clinical Investigation; 2005; **115** (2): 209–18.
- Iwamoto T, Terai S, Hisanaga T *et al.* Bone-marrow-derived cells cultured in serum-free medium reduce liver fibrosis and improve liver function in carbon-tetrachloride-treated cirrhotic mice. *Cell Tissue Res.* 2013; **351** (3): 487–95.
- Kajihara M, Okazaki Y, Kato S *et al.* Evaluation of platelet kinetics in patients with liver cirrhosis: similarity to idiopathic thrombocytopenic purpura. *J. Gastroenterol. Hepatol.* 2007; **22** (1): 112–8.
- Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R. Platelets Promote Liver Regeneration in Early Period after Hepatectomy in Mice. *World J. Surg.* 2007; **31** (4): 808–16.
- Murata S, Matsuo R, Ikeda O *et al.* Platelets promote liver regeneration under conditions of Kupffer cell depletion after hepatectomy in mice. *World J. Surg.* 2008; **32** (6): 1088–96.
- Matsuo R, Nakano Y, Ohkohchi N. Platelet administration via the portal vein promotes liver regeneration in rats after 70% hepatectomy. *Ann. Surg.* 2011; **253** (4): 759–63.
- Kawasaki T, Murata S, Takahashi K *et al.* Activation of human liver sinusoidal endothelial cell by human platelets induces hepatocyte proliferation. *J. Hepatol.* 2010; **53** (4): 648–54.
- Matsuo R, Ohkohchi N, Murata S *et al.* Platelets Strongly Induce Hepatocyte Proliferation with IGF-1 and HGF In Vitro. *J. Surg. Res.* 2008; **145** (2): 279–86.
- Murata S, Hashimoto I, Nakano Y, Myronovych A, Watanabe M, Ohkohchi N. Single administration of thrombopoietin prevents progression of liver fibrosis and promotes liver regeneration after partial hepatectomy in cirrhotic rats. *Ann. Surg.* 2008; **248** (5): 821–8.
- Ikeda N, Murata S, Maruyama T *et al.* Platelet-derived adenosine 5'-triphosphate suppresses activation of human hepatic stellate cell: In vitro study. *Hepatol Res.* 2012; **42** (1): 91–102.
- Watanabe M, Murata S, Hashimoto I *et al.* Platelets contribute to the reduction of liver fibrosis in mice. *J. Gastroenterol. Hepatol.* 2009; **24** (1): 78–89.
- Maruyama T, Murata S, Takahashi K *et al.* Platelet transfusion improves liver function in patients with chronic liver disease and cirrhosis. *Tohoku J. Exp. Med.* 2013; **229** (3): 213–20.
- Murata K, Ito K, Yoneda K, Shiraki K, Sakurai H, Ito M. Splenectomy improves liver function in patients with liver cirrhosis. *Hepatogastroenterology* 2008; **55** (85): 1407–11.
- Zaldivar MM, Pauels K, von Hundelshausen P *et al.* CXC chemokine ligand 4 (Cxcl4) is a platelet-derived mediator of experimental liver fibrosis. *Hepatology* 2010; **51** (4): 1345–53.
- Lisman T. Platelets and fibrin in progression of liver disease: friends or foes? *J. Thromb. Haemost.* 2015; **13** (1): 54–6.
- Chang Y, Bluteau D, Debili N, Vainchenker W. From hematopoietic stem cells to platelets. *J. Thromb. Haemost.* 2007; **5** (s1): 318–27.
- Nishimura S, Nagasaki M, Kunishima S *et al.* IL-1 $\alpha$  induces thrombopoiesis through megakaryocyte rupture in response to acute platelet needs. *J. Cell Biol.* 2015; **209** (3): 453–66.
- Suzuki H, Yamazaki H, Tanoue K. Immunocytochemical aspects of platelet membrane glycoproteins and adhesive proteins during activation. *Prog. Histochem. Cytochem.* 1996; **30** (1): 1–106.
- Murata S, Maruyama T, Nowatari T, Takahashi K, Ohkohchi N. Signal transduction of platelet-induced liver regeneration and decrease of liver fibrosis. *Int. J. Mol. Sci.* 2014; **15** (4): 5412–25.
- Holmsen H. Physiological functions of platelets. *Ann. Med.* 1989; **21** (1): 23–30.
- Yamaguchi R, Terashima H, Yoneyama S, Tadano S, Ohkohchi N. Effects of platelet-rich plasma on intestinal anastomotic healing in rats: PRP concentration is a key factor. *J. Surg. Res.* 2012; **173** (2): 258–66.
- Radice F, Yáñez R, Gutiérrez V, Rosales J, Pinedo M, Coda S. Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. *Arthroscopy* 2010; **26** (1): 50–7.
- McNicol A, Israels SJ. Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovasc. Hematol. Disord. Drug Targets* 2008; **8** (2): 99–117.
- Nash GF, Turner LF, Scully MF, Kakkar AK. Platelets and cancer. *Lancet Oncol.* 2002; **3** (7): 425–30.
- Sowa JM, Crist SA, Ratliff TL, Elzey BD. Platelet influence on T- and B-cell responses. *Arch. Immunol. Ther. Exp. (Warsz.)* 2009; **57** (4): 235–41.
- Pak S, Kondo T, Nakano Y *et al.* Platelet adhesion in the sinusoid caused hepatic injury by neutrophils after hepatic ischemia reperfusion. *Platelets* 2010; **21** (4): 282–8.
- Nakano Y, Kondo T, Matsuo R *et al.* Platelet dynamics in the early phase of postischemic liver in vivo. *J. Surg. Res.* 2008; **149** (2): 192–8.
- Lang PA, Contaldo C, Georgiev P *et al.* Aggravation of viral hepatitis by platelet-derived serotonin. *Nat. Med.* 2008; **14** (7): 756–61.
- Flier JS, Underhill LH, Friedman SL. The Cellular Basis of Hepatic Fibrosis--Mechanisms and Treatment Strategies. *N. Engl. J. Med.* 1993; **328** (25): 1828–35.
- Friedman SL. Mechanisms of Hepatic Fibrogenesis. *Gastroenterology* 2008; **134** (6): 1655–69.
- Ikeda N, Murata S, Maruyama T *et al.* Platelet-derived adenosine 5'-triphosphate suppresses activation of human hepatic stellate cell: In vitro study. *Hepatol Res* 2011; **42** (1): 91–102.
- Kodama T, Takehara T, Hikita H *et al.* Thrombocytopenia Exacerbates Cholestasis-Induced Liver Fibrosis in Mice. *Gastroenterology* 2010; **138** (7): 2487–7.
- Xia JL, Dai C, Michalopoulos GK, Liu Y. Hepatocyte Growth Factor Attenuates Liver Fibrosis Induced by Bile Duct Ligation. *Am. J. Pathol.* 2006; **168** (5): 1500–12.
- Nakamura T, Nishizawa T, Hagiya M, *et al.* Molecular cloning and expression of human hepatocyte growth factor. *Nature* 1989; **342** (6248): 440–3.
- Wolber EM, Jelkmann W. Thrombopoietin: the novel hepatic hormone. *News Physiol. Sci.* 2002; **17**: 6–10.
- McHutchison JG, Dusheiko G, Shiffman ML *et al.* Eltrombopag for Thrombocytopenia in Patients with Cirrhosis Associated with Hepatitis C. *N. Engl. J. Med.* 2007; **357** (22): 2227–36.
- Afdhal NH, Giannini EG, Tayyab G *et al.* Eltrombopag before procedures in patients with cirrhosis and thrombocytopenia. *N. Engl. J. Med.* 2012; **367** (8): 716–24.
- Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin. Liver Dis.* 2001; **21** (3): 373–84.
- Hemmann S, Graf J, Roderfeld M, Roeb E. Expression of MMPs and TIMPs in liver fibrosis - a systematic review with special emphasis on anti-fibrotic strategies. *J. Hepatol.* 2007; **46** (5): 955–75.
- Takahashi K, Murata S, Fukunaga K, Ohkohchi N. Human platelets inhibit liver fibrosis in severe combined immunodeficiency mice. *World J. Gastroenterol.* 2013; **19** (32): 5250–60.
- Malik R, Selden C, Hodgson H. The role of non-parenchymal cells in liver growth. *Semin. Cell Dev. Biol.* 2002; **13** (6): 425–31.

- 44 Factor VM, Seo D, Ishikawa T *et al.* Loss of c-Met disrupts gene expression program required for G2/M progression during liver regeneration in mice. Ng IOL, editor. *PLoS One*. 2010; **5** (9): e12739.
- 45 Jackson LN, Larson SD, Silva SR *et al.* PI3K/Akt activation is critical for early hepatic regeneration after partial hepatectomy. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2008; **294** (6): G1401–10.
- 46 Solt LA, May MJ. The I $\kappa$ B kinase complex: master regulator of NF- $\kappa$ B signaling. *Immunol. Res.* 2008; **42** (1-3): 3–18.
- 47 Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M. NF- $\kappa$ B function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol. Cell Biol.* 1999; **19** (4): 2690–8.
- 48 Terui K, Ozaki M. The role of STAT3 in liver regeneration. *Drugs Today* 2005; **41** (7): 461–9.
- 49 Haga S, Ozaki M, Inoue H *et al.* The survival pathways phosphatidylinositol-3 kinase (PI3-K)/phosphoinositide-dependent protein kinase 1 (PDK1)/Akt modulate liver regeneration through hepatocyte size rather than proliferation. *Hepatology*. 2009; **49** (1): 204–14.
- 50 Tulasne D, Foveau B. The shadow of death on the MET tyrosine kinase receptor. *Cell Death Differ.* 2008; **15** (3): 427–34.
- 51 Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat. Rev.* 2004; **30** (2): 193–204.
- 52 Gotoh J, Obata M, Yoshie M, Kasai S, Ogawa K. Cyclin D1 over-expression correlates with beta-catenin activation, but not with H-ras mutations, and phosphorylation of Akt, GSK3 beta and ERK1/2 in mouse hepatic carcinogenesis. *Carcinogenesis*. 2003; **24** (3): 435–42.
- 53 Meyer J, Lejmi E, Fontana P, Morel P, Gonelle-Gispert C, Bühler L. A focus on the role of platelets in liver regeneration: Do platelet-endothelial cell interactions initiate the regenerative process? *J. Hepatol.* 2015; **63** (5): 1263–71.
- 54 Lisman T, Kirschbaum M, Porte RJ. The role of platelets in liver regeneration - What don't we know? *J. Hepatol.* 2015; **63** (6): 1537–8.
- 55 Myronovych A, Murata S, Chiba M *et al.* Role of platelets on liver regeneration after 90% hepatectomy in mice. *J. Hepatol.* 2008; **49** (3): 363–72.
- 56 Endo Y, Nakamura M. The effect of lipopolysaccharide, interleukin-1 and tumour necrosis factor on the hepatic accumulation of 5-hydroxytryptamine and platelets in the mouse. *Br. J. Pharmacol.* 1992; **105** (3): 613–9.
- 57 Kirschbaum M, Karimian G, Adelmeijer J, Giepmans BN, Porte RJ, Lisman T. *Blood*. 2015; **126** (6): 798–806.
- 58 Meijer C, Wiezer MJ, Diehl AM *et al.* Kupffer cell depletion by CI2MDP-liposomes alters hepatic cytokine expression and delays liver regeneration after partial hepatectomy. *Liver*. 2000; **20** (1): 66–77.
- 59 Akerman P, Cote P, Yang SQ *et al.* Antibodies to tumor necrosis factor- $\alpha$  inhibit liver regeneration after partial hepatectomy. *Am. J. Physiol.* 1992; **263** (4 Pt 1): G579–85.
- 60 Yamada Y, Webber EM, Kirillova I, Peschon JJ, Fausto N. Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. *Hepatology*. 1998; **28** (4): 959–70.
- 61 Abshagen K, Eipel C, Kalf J, Menger MD, Vollmar B. Loss of NF- $\kappa$ B activation in Kupffer cell-depleted mice impairs liver regeneration after partial hepatectomy. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2007; **292** (6): G1570–7.
- 62 Sindram D, Porte RJ, Hoffman MR, Bentley RC, Clavien PA. Synergism between platelets and leukocytes in inducing endothelial cell apoptosis in the cold ischemic rat liver: a Kupffer cell-mediated injury. *FASEB J.* 2001; **15** (7): 1230–2.
- 63 Tamura T, Kondo T, Pak S *et al.* Interaction between Kupffer cells and platelets in the early period of hepatic ischemia-reperfusion injury—an in vivo study. *J. Surg. Res.* 2012; **178** (1): 443–51.
- 64 Nakamura M, Shibazaki M, Nitta Y, Endo Y. Translocation of platelets into Disse spaces and their entry into hepatocytes in response to lipopolysaccharides, interleukin-1 and tumour necrosis factor: the role of Kupffer cells. *J. Hepatol.* 1998; **28** (6): 991–9.
- 65 Hisakura K, Murata S, Takahashi K *et al.* Platelets prevent acute hepatitis induced by anti-fas antibody. *J. Gastroenterol. Hepatol.* 2011; **26** (2): 348–55.
- 66 Braet F, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. *Comp Hepatol. BioMed Central* 2002; **1** (1): 1.
- 67 Gaudie J, Richards C, Baumann H. IL6 and the acute phase reaction. *Res. Immunol.* 1992; **143** (7): 755–9.
- 68 Badia JM, Ayton LC, Evans TJ *et al.* Systemic cytokine response to hepatic resections under total vascular exclusion. *Eur. J. Surg.* 1998; **164** (3): 185–90.
- 69 Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA, Piña E, Geller DA. Factors in the pathophysiology of the liver ischemia-reperfusion injury. *J. Surg. Res.* 2008; **147** (1): 153–9.
- 70 Yatomi Y, Ohmori T, Rile G *et al.* Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells. *Blood*. 2000; **96** (10): 3431–8.
- 71 Afdhal N, McHutchison J, Brown R *et al.* Thrombocytopenia associated with chronic liver disease. *J. Hepatol.* 2008; **48** (6): 1000–7.
- 72 Kojouri K, Vesely SK, Terrell DR, George JN. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. *Blood*. 2004; **104** (9): 2623–34.
- 73 Khan SY, Kelher MR, Heal JM *et al.* Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood*. 2006; **108** (7): 2455–62.
- 74 Carr BI, Guerra V. Thrombocytosis and hepatocellular carcinoma. *Dig. Dis. Sci.* 2013; **58** (6): 1790–6.
- 75 Sitia G, Aiolfi R, Di Lucia P *et al.* Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. *Proc. Natl. Acad. Sci. U. S. A.* 2012; **109** (32): E2165–72.
- 76 Nozaki R, Murata S, Nowatari T *et al.* Effects of thrombopoietin on growth of hepatocellular carcinoma: Is thrombopoietin therapy for liver disease safe or not? *Hepatol Res.* 2012; **43** (6): 610–20.
- 77 Kuter DJ. New thrombopoietic growth factors. *Blood*. 2007; **109** (11): 4607–16.
- 78 Erhardt JA, Erickson-Miller CL, Aivado M, Abboud M, Pillarisetti K, Toomey JR. Comparative analyses of the small molecule thrombopoietin receptor agonist eltrombopag and thrombopoietin on in vitro platelet function. *Exp. Hematol.* 2009; **37** (9): 1030–7.
- 79 Erickson-Miller CL, Kirchner J, Aivado M, May R, Payne P, Chadderton A. Reduced proliferation of non-megakaryocytic acute myelogenous leukemia and other leukemia and lymphoma cell lines in response to eltrombopag. *Leuk. Res.* 2010; **34** (9): 1224–31.