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審 査 研 究 科	人間総合科学研究科
学位論文題目	Platelets stimulate liver regeneration in a rat model of partial liver transplantation (部分肝移植のラットモデルにおいて血小板は肝再生を刺激する)
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## 論文の内容の要旨 Abstract of thesis

In this doctoral dissertation, the author described the impact of thrombopoietin-induced perioperative thrombocytosis on graft regeneration. The content is summarized as follows:

### （目的 Purpose）

Liver regeneration is mainly mediated by the proliferation of hepatocytes. Besides, non-parenchymal cells such as KCs (Kupffer cells), LSECs (liver sinusoidal endothelial cells), and hepatic stellate cells contribute to liver growth via their own proliferation and proliferation-stimulatory effects on hepatocytes. Platelets were also previously reported to stimulate liver regeneration in models of hepatectomy, however, how platelets associate with liver regeneration in partial liver transplantation (LT) remains unclear. In this study, the author examined the impact of platelets on thrombopoietin (TPO)-induced thrombocytosis in a rat model of partial LT, mimicking the condition of living donor liver transplantation (LDLT).

### （対象と方法 Materials and Methods）

The impact of thrombopoietin-induced perioperative thrombocytosis on graft regeneration, I/R injury and survival was investigated.

#### **Ex vivo graft preparation:**

A 14-gauge catheter was used to form the cuff for the portal vein (PV), and a 10-gauge catheter was used to form the cuff for the inferior hepatic vena cava (IHVC). For 70% or 80% liver resection, the 6-0 thread used in the donor operation step was ligated to prevent the PV blood from flowing into the median lobe (ML) and left lobe (LL). The

hepatic veins (HVs) of the LL and ML were ligated together at the pedicle levels with a 4-0 silk thread. The resected liver surface was cauterized with bipolar forceps and the graft was stored in the preservation solution at 4°C.

#### **Recipient operation:**

The recipient rat's abdomen was opened by a midline incision. After mobilization of the liver, the BD and vessels were dissected, and the recipient liver was removed. The 30% or 20% liver graft was placed orthotopically. The anastomosis of the superior hepatic vena cava (SHVC) was performed in an end-to-end fashion using a continuous suture with 7-0 polypropylene thread.

#### **Mechanism analysis:**

To analyze the regeneration-related effects, quantitative real time PCR, ELISA, and Western blot analysis were performed.

#### **(結果 Results)**

The author pointed out that following four different mechanisms exist to explain about the involvement of platelets in liver regeneration: (1) the direct effect on hepatocytes; (2) the cooperative effect with LSECs; (3) the collaborative effect with KCs; (4) the transfer of messenger RNA to hepatocytes.

The author found the hepatocyte regeneration-related cytokines, including insulin-like growth factor-1, hepatocyte growth factor, interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), were elevated.

In addition, the author showed the proliferative signaling pathways, Ki67-labeling index, PCNA-labeling index, mitotic index and liver/body weight ratio were increased under thrombocytosis. Of note, the author identified the platelet-induced regeneration was independent of thrombopoietin, as increases in the Ki67-labelling and PCNA-labelling indexes were abolished after reducing platelet counts by anti-platelet serum in rats administered with thrombopoietin.

In case of I/R injury, the author found thrombocytosis did not aggravate oxidative stress, downstream signaling pathways, necrosis or apoptosis in the graft. Moreover, the author demonstrated that the platelet-activated Akt signaling pathways rescued I/R injury, suggesting Akt agonists or inhibitors can be administered to rats in future studies to provide direct evidence.

After KC depletion, the serum levels of AST, ALT, TBIL and regeneration-related cytokines after partial LT were analyzed. The author found AST, ALT and TBIL were significantly higher in the KDTPO group than in the TPO group. Further, the author showed the serum levels of IL-6 and TNF- $\alpha$  decreased significantly after KC depletion, and the tissue levels of IL-6, HGF and IGF-1 decreased significantly after KC depletion, but there was no significant difference in the tissue levels of TNF- $\alpha$ . The platelet levels did not change after KC depletion between the TPO and KDTPO groups after reperfusion. Hence, the author concluded Kupffer cells (KCs) vitally contributed to platelet-derived regeneration.

#### **(考察 Discussion)**

This is the first study to clarify the role of platelets in promoting liver regeneration and survival in partial LT. The author demonstrated that platelet-induced graft regeneration outweighed its associated risk of I/R injury. In the present study, the author showed the platelet levels were increased to twice of normal levels in the TPO group. Under TPO-induced thrombocytosis, significant accumulation of platelets was observed in the liver graft after partial LT with elevated levels of HGF, IGF-1, IL-6 and TNF- $\alpha$ . The author identified that platelets were efficient carriers of proliferation-related growth factors and stimulators of Kupffer cells (KCs), which led to subsequent activation of downstream proliferative transcription cascades. While hepatocyte growth factor (HGF) in the serum might be also derived from other organs, the author confirmed the significant increase of HGF in the TPO group, suggesting that thrombocytosis would play an important role in the secretion of HGF.

Finally, the author proposed further evaluation of the mid- and long-term efficacy and safety of TPO treatment or platelet transfusion that will be necessary before translating it into clinical practice.

## 審査の結果の要旨 Abstract of assessment result

### (批評 General Comments)

The author clearly proved that thrombocytosis stimulated graft regeneration and prolonged survival without aggregating I/R injury after partial LT. It is highly laudable that all detailed analysis were performed using an animal model created by making full use of appropriate and accurate surgical procedure. In addition, it is well described for the careful discussion of the findings that have observed in this study by citing previous papers on various possibilities.

### (最終試験の結果 Assessment)

The final examination committee conducted a meeting as a final examination on June 25, 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

### (結論 Conclusion)

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.