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Molecular mechanisms of platelet-mediated liver regeneration after partial hepatectomy

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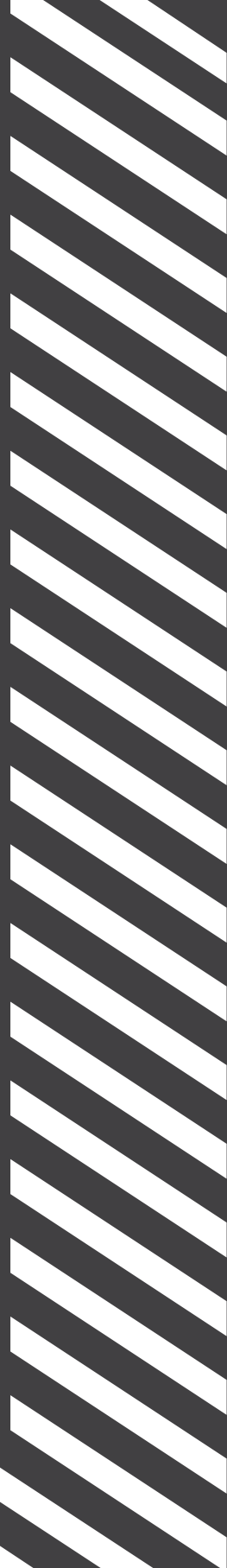
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**INTRODUCTION
AND OUTLINE OF
THIS THESIS**

Liver regeneration

The liver has a unique regenerative capacity. The ultimate regenerative response of the liver occurs after a partial liver resection. Up to 70% of liver tissue can be safely removed in patients that require a liver resection for removal of a liver tumor (1,2). Also, healthy individuals can donate part of their liver for transplantation purposes, resulting in partial livers in both donor and recipient. Following partial liver resection, the liver eventually regenerates to its original size with substantial regeneration in humans already after one week, while regeneration is virtually complete after three months. In rodents, liver regeneration is even faster, with complete regeneration in mice after ~5 days.

Liver regenerative responses also occur when liver tissue is damaged by for example toxins or viruses, or by ischemia/reperfusion injury. Diseases associated with damage to liver tissue are either chronic or acute. Chronic liver disease, for example causes formation of fibrous tissue replacing healthy liver tissue leading to fibrosis and eventually cirrhosis. Acute liver failure, for example caused by intoxication with acetaminophen, results in rapid necrosis by a mechanism involving sterile inflammation. The most severe cases lead to necrosis of virtually the entire liver.

In the context of liver transplantation, livers also suffer from acute hepatocellular injury as a consequence of combined warm and cold ischemia and the subsequent reperfusion injury. Livers that have suffered substantial damage in the process of transplantation can also fully recover. Animal experiments as well as biopsy studies in humans have shown that moderate fibrosis can resolve when the initiating trigger is removed or when successful treatment is given (3,4). The liver can thus regenerate not only following physical removal of liver tissue but also following damage from an acute or chronic insult. Although the liver can regenerate and sustain vital functions, it sometimes fails to regenerate and fails to maintain the metabolic demand of the body. This condition is associated with high mortality and morbidity rate in patients. In those patients little therapeutic options are available and patients die from liver insufficiency. A liver transplantation is the only lifesaving procedure. Therefore, strategies for preventing liver damage and accelerating liver regeneration after a partial liver resection or liver transplantation are of great interest.

Platelets

Plates are anucleated, discoid cellular fragments derived from the cytoplasm of megakaryocytes in the bone marrow. They are the smallest of the many types of cells in circulating blood, averaging only from 2 to 5 micrometer in diameter and 0.5 micrometer in thickness. In contrast, the number of platelets in the circulation is enormous. A normal human platelet count in healthy individuals ranges from 150.000 to 450.000 platelets per microliter of blood. Per day megakaryocytes release approximately 10^{11} platelets into the bloodstream and the average life span of circulating platelets is between 7 and 10 days. Although platelets lack a nucleus, and they are per definition no cells, they contain a

numerous amounts of intracellular organelles, which are also present in cells. Beside platelets specific cytoplasmic compartments, alpha and dense granules, platelets contain mitochondria to maintain their energy balance and also an endoplasmic reticulum and ribosomes. Because of their anatomy platelets are often considered simple, noncomplex "cells" with their primary responsibilities to stop bleeding. Platelet functions in primary hemostasis have been investigated extensively in the last decades. Circulating platelets are recruited to the site of injury, where they become a major component of the developing thrombus. However platelets are more than bleeding stoppers. Platelets are multifunctional "cells" that are involved in a variety of biologic and pathologic processes, including host defense, angiogenesis, wound healing, inflammation, and also liver regeneration (5-8).

Platelet-mediated liver regeneration

Liver regeneration after partial liver resection is a complex and well-organized process, which involves the participation of all liver cells, immune cells and also platelets. It is well-known that in experimental animal models in which platelets were depleted or functionally impaired, liver regeneration is substantially delayed after a partial liver resection (9,10). In a clinical study, our research group showed that a low platelet count immediately after partial liver resection is an independent predictor of delayed postoperative liver function recovery following liver surgery, suggesting that platelets stimulate liver regeneration also in humans (11). Nevertheless the molecular mechanisms behind platelet-mediated stimulation of liver regeneration are largely unexplored. It seems that platelets use various mechanisms to perform these extra-hemostatic functions.

Platelets store a variety of growth factors in their alpha granules, including platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and tissue growth factor (TGF)- β (12,13). Upon platelet activation granules become excreted and growth factors are released. It has been demonstrated *in vitro* that hepatocytes show a mitogenic response to various growth factors stored in platelets (14). It seems plausible that local release of platelet stored growth factors after liver resection is partly responsible for platelet-mediated liver regeneration. Nevertheless it has to be proven *in vivo*, that platelet growth factors are actually responsible. Another potential player in platelet-mediated liver regeneration seems to be serotonin (9). Platelets carry serotonin in blood, which is not only a neurotransmitter but also a hormone with various extraneuronal functions. Serotonin exhibits a vast repertoire of actions including cell proliferation and differentiation. It is a potent mitogenic factor and is involved in the remodeling of tissue (15,16). Several studies have demonstrated that serotonin receptors in the liver are upregulated after liver resection in mice and that treatment with serotonin receptor antagonists inhibits liver regeneration (9,17). However, clinical studies with patients undergoing partial hepatectomy show opposed results regarding the role of serotonin in the stimulation of liver regeneration (18,19).

Beside the release of growth factors from platelet granules, the study of cell-cell interactions

between platelets and various liver cells are of great interest for researchers. It seems that direct interaction between platelets and liver cells is crucial for platelet-mediated stimulation of liver regeneration as demonstrated by an impaired platelet proliferative capacity when platelet-hepatocyte binding was blocked *in vitro* (12). Furthermore, Murata et al. demonstrated *in vitro* that platelet binding to liver endothelial cells (LSEC) and liver specific macrophages (Kupffer cells) is important for the release of pro-inflammatory cytokines in the liver. Those cytokines are crucial for the onset of liver regeneration in the early phase after partial liver resection (20,21)

Platelet RNAs

Although platelets are anucleated, they contain miRNAs and mRNAs and it has been demonstrated that platelets contain the necessary molecular machinery to conduct translation (22,23). Historically, platelet RNA was recognized merely in platelet research and it has long been considered that the cytoplasmic platelet RNAs are residual transcripts of their forming cell, the megakaryocyte. Nowadays several studies challenge this assumption and support a more fluid role for platelet RNA in platelet function and disease development. Platelets can actively translate RNA to protein. In response to various physiologic stimuli, platelets are able to synthesize biologically relevant proteins *de novo* that are regulated at translational RNA level and does not require a nucleus (24). In addition to the capacity to synthesize proteins *de novo*, several independent research groups have demonstrated in the last years that platelets have the ability to transfer their endogenous cytoplasmic miRNAs and mRNAs to recipient cells (25-27). Previously RNA transfer between exosomes/ microvesicles and several recipient cells has been investigated and mentioned as novel mechanism of genetic exchange between cells (28-30). Moreover, it has been demonstrated that microvesicles derived from human liver stem cells were taken up by hepatocytes, resulting in transfer of mRNA (31). The transferred mRNA may result in accelerated hepatocyte proliferation and induced apoptosis resistance. Regarding this result, it seems conceivable that also platelet RNAs are involved in the stimulation of hepatocyte proliferation.

Aim of this thesis

The aim of this thesis is to investigate the molecular mechanism of platelet-mediated liver regeneration after partial hepatectomy. Better insight in the mechanisms of platelet-mediated liver regeneration is an essential step in the development of novel therapies that can be applied in patients with liver failure and insufficient liver regeneration. Until now, in those patients, a liver transplantation is the only lifesaving option.

Chapter 2 is a letter in response to an article published in the Journal of Hepatology, which summarizes current knowledge on the role of blood platelets in liver regeneration and the role of platelet RNA. In **Chapter 3** we investigated platelet-mediated stimulation of hepatocyte proliferation *in vitro* as a model for liver regeneration and gained novel insights into the role of platelet RNA in platelet-mediated liver regeneration. The study of the mechanism of platelet recruitment into the liver parenchyma after partial liver resection is the topic in **Chapter 4**. We test our hypothesis that platelet recruitment in the early phase after liver resection is essential for the regenerative process. **Chapter 5** investigates growth factor levels in blood plasma and in platelets of patients undergoing hemihepatectomy or a pancreatico-duodenectomy (PPPD). In **Chapter 6** we focus on liver regeneration in mice with non-alcoholic fatty liver disease (NAFLD) and the effect of partial liver resection on the progression of NAFLD. In **Chapter 7**, we present an “intermezzo” in this thesis. Recombinant factor VIIa (rFVIIa) has been recently shown to prevent spontaneous bleeding in inhibitor-complicated hemophilia when administered once daily. We propose in this study that redistribution of rFVIIa to the bone marrow compartment and uptake by megakaryocytes which results in production of platelets containing rFVIIa. Finally, in **Chapter 8**, all results are summarized and discussed, followed by a view on the future perspectives of platelet-mediated liver regeneration research and their therapeutic applications.

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