Molecular mechanisms of platelet-mediated liver regeneration after partial hepatectomy
Kirschbaum, Marc

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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 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 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 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 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 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 treatment is given (3,4).

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 been 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
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 pancreatoco-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 6 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.
References


