

A molecular view of liver regeneration¹

Uma visão molecular da regeneração hepática

Marissa Rabelo Tarlá², Fernando Silva Ramalho³, Leandra Naira Zambelli Ramalho⁴, Tiago Castro e Silva⁵, Daniel Ferracioli Brandão², Juliana Ferreira², Orlando Castro e Silva⁶, Sérgio Zucoloto⁷

1. Study performed in the Laboratory of Cellular Proliferation of the Department of Pathology of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, (FMRP-USP), Brazil.
2. Fellow PhD degree of the Department of Pathology, (FMRP-USP), Brazil.
3. PhD, Professor of the Department of Surgery and Anatomy, (FMRP-USP), Brazil
4. PhD, Professor of the Department of Pathology, (FMRP-USP), Brazil.
5. Graduate student of the Physical Institute of São Carlos – University of São Paulo (IFSC-USP), Brazil.
6. Full Professor, Head of Division of Gastroenterology of the Department of Surgery and Anatomy, Coordinator of the Liver Transplant Program, (FMRP-USP), Brazil.
7. Full Professor of the Department of Pathology, (FMRP-USP), Brazil.

ABSTRACT

The purpose of this review was to carry out an analysis of the liver regenerative process focusing on the molecular interactions involved in this process. The authors undertook a review of scientific publications with a focus on the liver regeneration. The cellular processes involved in liver regeneration require multiple systematic actions related to cytokines and growth factors. These interactions result in the initiation of mitogenic potential of the hepatocytes. The action of these modulators in the regenerative process require a processing in the extra-cellular matrix. Serines and metal proteins are responsible for the bio availability of cytokines and growth factors so that they can interact as receptors in the cellular membrane generating signaling events for the beginning and end of the liver regenerative process. The exact mechanism of interaction between cells, cytokines and growth factors is not well established yet. A series of ordered events that result in the hepatic tissue regeneration has been described. The better understanding of these interactions should provide a new approach of the treatment for liver diseases, aiming at inducing the regenerative process.

Key words: Liver Regeneration. Cytokines. Grow Factors. Hepatocytes.

RESUMO

O objetivo desta revisão foi desenvolver uma análise do processo regenerativo do fígado, focando as interações moleculares envolvidas neste processo. Os processos celulares envolvidos na regeneração hepática requerem múltiplas ações sistemáticas relacionadas com citocinas e fatores de crescimento. Estas interações resultam na iniciação do potencial mitogênico dos hepatócitos. A ação destes moduladores do processo regenerativo necessita de processamento no meio extra celular. As serinas e metaloproteínas são responsáveis pela biodisponibilização de citocinas e fatores de crescimento, para que então possam interagir com receptores na membrana celular gerando os eventos sinalizadores para o início e o término do processo regenerativo hepático. O exato mecanismo de interação entre células, citocinas e fatores de crescimento não está bem estabelecido. Tem-se descrito uma série de eventos ordenados que resulta na regeneração do tecido hepático. O melhor entendimento destas interações leva a uma nova abordagem de tratamento para doenças hepáticas, objetivando a indução do processo regenerativo.

Descritores: Regeneração Hepática. Citocinas. Fatores de Crescimento. Hepatócitos.

Introduction

It is well-known that the liver is an organ of unequalled regenerative capacity. It is well-established that the hepatocytes are able to reconstruct the liver tissue after the occurrence of damages such as surgical and toxic lesions. The elucidation of the events that make up this restorative capacity becomes crucial for the understanding of the hepatocyte dynamics during the liver regeneration. The regenerative process requires a close interplay between the signals of the extracellular matrix and the intracellular means. A response from the remanescant cells, such as the genes transcription and the new protein codification, must be established early resulting in the beginning of the cellular proliferation. A series of events regulated by cytokines and growth factors. These regulators of the regenerative process require a processing in the extracellular matrix. Metalproteins process cytokines and factors associated with the regeneration, making them available so that they signal with the extracellular membrane, starting and finishing an intracellular event cascade that will result in the reestablishment of the liver tissue.

Regulating Factors in Hepatocyte Division

Liver regeneration is an event that involves multiple cellular processes and a complex interaction with cytokines and growth factors. Related factors that start the regeneration and make the regenerated liver functional, is a well-elucidated process with animal models after partial hepatectomy. More specifically, the liver regeneration may be divided into three phases: (1) Initiation; (2) Proliferation; and (3) Inhibition. The mitogenic response divides into two phases, first due to cytokines that stimulate the G0-G1 transition, and then the progression until phase S. In rats, after hepatectomy, hepatocytes go into G1 around 4 hours after the procedure and progress to G1-S with DNA synthesis peak within 24 hours¹. After hepatectomy, non-parenchymatous liver cells and remanescant hepatocytes increase the production of tumoral necrosis factor (TNF)- α , linfotoxine (LT)- β and interleukins (IL)-1 and 6. (TNF)- α bonds to the soluble receptor TNFR1, resulting in the translocation of the nuclear factor, Kappa-B (NF-kB). The transcription factor NF-kB controls the expression of the cytokine-codifying genes, regulate the cellular cycle and is an antagonist of the apoptosis in the liver regenerative process. NF-kB consists of different proteins with distinct biological activities. An activity form of NF-kB is found retained in the cytoplasm, and its activation occurs a few minutes after the HP process and lags for 1 to 2 hours². (TNF)- α also induces the expression of IL-6, which is a signal for the transduction and activation of (STAT)³ transcription, possibly attaching to C/Erb β protein in the liver remanescant cells³. STAT³ is important in the inhibition of hepatocyte apoptosis by inhibition of the caspasis 3 and 8 activation, in addition to improving the survival rate of hepatocytes through the inhibition of TGF β mediated by Fas activation⁴. TNFR1 or IL-6 deficiency diminishes the liver regeneration in mice³. In *tnf 0/0 mice*, there is no significant blockage of regeneration, with the chance of initialization compensating factor occurrence⁵ As a result of

the activation of these transcription factors (NF-kB, STAT3 and C/EBP β), the hepatocytes program the response of primary growth (immediate genes, activation of AP-1 and pro-oncogenesis: c-Myc, c-fos, c-Has, c-met, c-Erb². Nitric oxide takes part in these events through the generation of nitric oxide sintetase (NOS-2) that prevents the pro-apoptotic activation mediated by TNF α (via caspase 3), and protects the hepatocytes against cellular death mediated by procaspases S-nitrosilantes⁶. In a second phase, the hepatocyte growth factor (HGF) and its receptor MET are considered the central stimulation for the G1-S progression in the remanescant hepatocytes⁷. Hepatocytes are sensibilized by TNF α , IL-6 and nitric oxide (NO) in order to respond to stimulations of growth factors. HGF, a potent mitogenic agent, is a peptide secreted by non-parenchymatous cells after hepatectomy and induces early genes in the regeneration². It has been shown that HGF may be able to induce the differentiation of stem cells of the bone marrow in hepatocyte⁸. The c-met deficiency, receptor HGF, results in the necrosis generation of hepatocytes and esteatosis⁹. Other mitogens involved in the proliferative phase include the transforming growth factor (TGF α) and the epidermal growth factor (EGF). The expression of TGF α is induced in the hepatocytes around 2-3 hours after PH and suggests that it stimulates the proliferation of both hepatocytes and endothelial cells¹⁰. TGF α and EGF signal to the cell through a receptor of tirosine kinase in common, a receptor EGFR. Endothelial cells can also be induced by the growth factor of the vascular endothelium (VEGF), angipoetinas 1 and 2 and growth factor of fibroblast (FGF)¹¹. The growth factor similar to insulin (IGF) is an auxiliary element that bonds to receptor IGFP-1 that is induced in hepatocytes by the stimulation of IL-6. The amino acids sequence RGD in IGFBP-1 can bonds to the fibronectine-integrine $\alpha 5 \beta 1$ receptor, affecting the migration and cellular adhesion. The IGFBP-1 deficiency results in defects in the DNA synthesis, diminishing of the expression of proteins that regulate the cellular cycle and the liver necrosis after hepatectomy, indicating a possible mitogenic role in the liver regeneration¹². The substance of hepatic stimulation (HSS), a peptidic growth factor that is specific in the liver, is related to the protection and proliferation of hepatocytes through the repairing of DNA synthesis and self-fosforilation of the residues of tiroxine of EGFR receptor¹³. In the inhibition phase, the signals for the termination of growth or the stop signals (stop signals) are responsible for the decrease of DNA synthesis. The most known of these is the TGF β , secreted by hepatocytes and plaquetes¹⁴. In mice, the TGF β increased expression results in the absence of regeneration and development of hepatocarcinoma¹⁵. As a member of the superfamily TGF, it sgnals through TGF α receptor, antagonizes with TGF- α , a growth factor that competes for the same TGF α receptors. The control between these signals occurs by the earlier secretion of TGF α and later secretion of TGF β ¹⁶. Another mitosis inhibitor is IL-1 that expresses itself in the two phases of the regeneration, first as a proliferative stimulation and in the terminal phase of this process. The end of the hepatocyte regenerative process is not well defined, such as the initial and proliferative phases, possibly this latter initiates through the stimulation of hepatocyte growth factors¹⁷

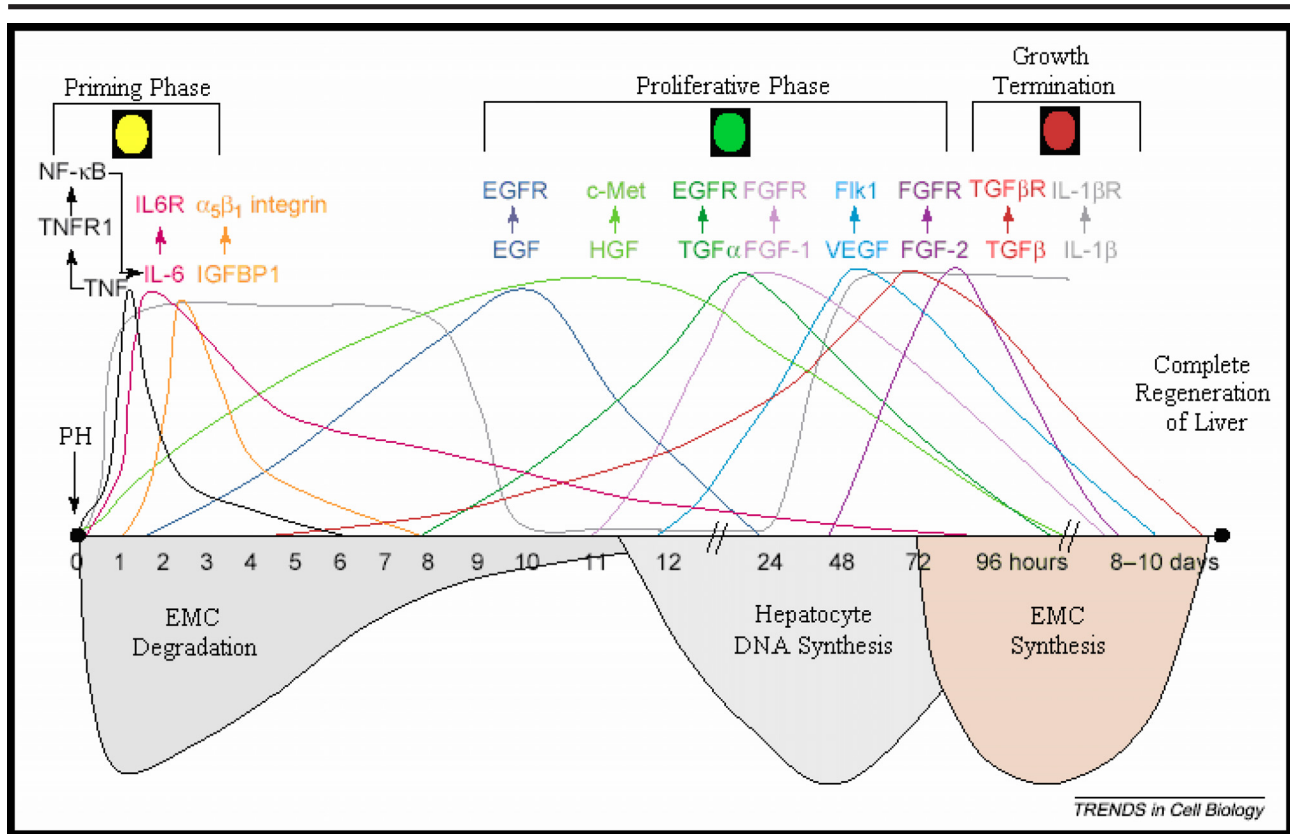


FIGURE 1 - Scheme for the start and stop of the liver regeneration (11).

Proteolytic Modulation: Extra Cellular Matrix

The liver is made up by 60% of parenchymatous cells (hepatocytes) and non-parenchymatous cells (Kupffer's cells, colangiocytes, epithelial, endothelial and oval cells). Basically, the liver parenchyme structure is organized into hexagonal lobes that contain a central vein surrounded by six portal spaces (Figure 2). The portal space consists of a vein, a hepatic artery and a biliar duct that drain spaces between the hepatocytes and is surrounded by endothelial cells termed sinusoides. The Kupffer's cells are found in the sinusoides and the stellat cells are located in the perisinusoidal spaces (Figure 3). The liver extra cellular matrix (MEC) works as a foundation for the cells and occupy less than 3% of the liver area. Collagen is the main protein of the extra cellular matrix, with collagen type I, III and V located in the portal area and central vein, and collagen type IV together with laminine and entactino-nidogen making up the basal membrane ¹⁸. Other molecules include glicoproteins (laminine, fibronectine, tenascine, nidogen and SPARC) and proteinoglicans (heparam, dermatam, condritine sulphate, perlacam, hialuronic acid and decorim). The MEC continuous network around the cells make it possible to store and transmit signals through bonds and liberations of growth factors, hormones, enzymes and cytokines. The process of restoration of the hepatic mass involves a series of proteolytic mechanisms located in MEC, which allows a rapid, but controlled response through the sending of signals for the initiation and stop of the regenerative process. Among these extra cellular

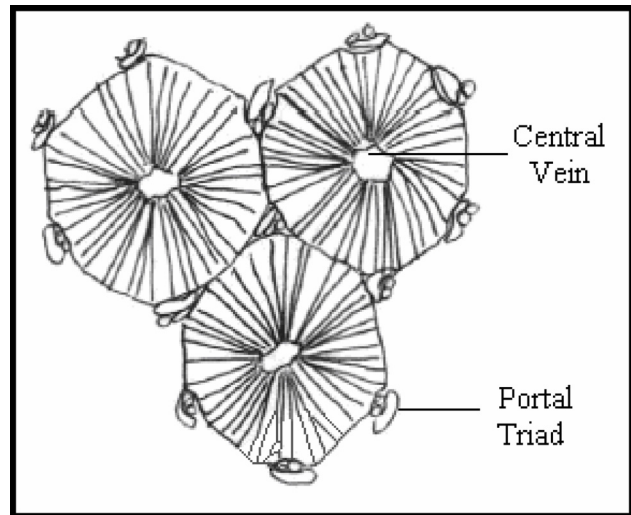


FIGURE 2 - Portal spaces

factors, proteinases, serines and metalproteins (MMPs) exist as a complex proteolytic cascade that requires a specific mechanism of activation or as latent enzymes, inhibited by specific inhibitory proteases (TIMPs) and a 2 microglobulines ¹⁹. The proteolytic process is a mechanism to modify pre-existent signals without the need of a new synthesis. The proteolytic cleavage that occurs in the pericellular interface (MEC) may function both as an activator or inhibitor of the cellular signaling processes. It may acts directly or modifying the bonding

proteins. Some important signaling pathways in the regeneration process have been clarified, recent studies have used mice deficient of tissue inhibitors of MMPs, type TIMP 1 or 3²⁰. The proteolytic regulation of the signalization through TNF α is well elucidated and it is known that the TNF α -converting enzyme, TACE/ADAM 17 can be inhibited by TIMP-3, increasing the TNF α action. TACE/ADAM 17 covers TNFR1/2 on the cellular surface, blocking the TNF α action²¹.

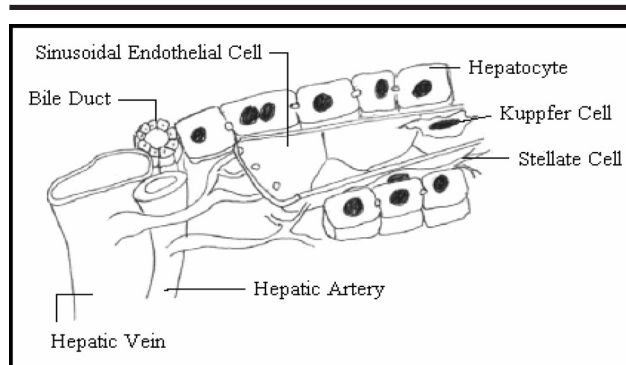


FIGURE 3 - Perisinusoidal spaces

Another important element in the regenerative process, HGF, is secreted in the form of a pro-enzyme, Pro-HGF. This must be metabolized in MEC by MMPs, among them UPA (activator of plasminogen type uroquinase)²². EGF and TNF α act through receptors of the ERBB family. EGF is synthesized as a pro-HB-EGF, has a bonding heparinoid, HB, and requires proteolytic cleavage in MEC so that the fosforilation tyrosine quinase of EGF receptor occurs²³. TNF α exists in the form of a transmembranic precursor that is discharged from the surface by proteolysis action. TACE/ADAM 17 participates in this process, being crucial for the TNF α activation²⁴. VEGF is composed of isoforms with heparinoid domains and HSPGs (proteinoglicans / heparam sulphates). MMP-9 and plasmin are capable to increase free VEGF levels, promoting induced angiogenesis during the regeneration process²⁵. FGF acts through a tirosine quinase receptor, the FGFR1-4. This receptor is of perlacam type, FGF activates these receptors through perlacam bonding elements, that limit the FGF diffusion, protecting it from the proteolytic action. MMP1 and 3 act by the liberation of FGF from the perlacam complex inhibiting the action of this element²⁶. In studies with hematopoietic stem cells, it has been shown that quimioquine SDF-1, a potent chemiotatic secreted by damaged hepatocytes and its receptor, the CXCR4, participates in the process of mobilization of stem cells of the bone marrow and the migration of these cells to the damaged liver tissue²⁷. In fibrosis and hepatic cirrhosis, there is the association with the increase of TIMPs expression and reduction of the expression of MMPs²⁸. The processes of chronic hepatitis are associated with metalproteinases²⁹. The extra cellular proteolysis may show an important role in the development of hepatocarcinomas. The identification and definition of the metalproteinase function in the liver regenerative process must be a new strategy for the development of new clinical treatments of hepatic diseases.

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Correspondence:

Orlando de Castro e Silva Jr.
 Rua Campos Salles, 809 - 9º andar. CEP: 14015-110
 Centro, Ribeirão Preto - SP - Brazil.
 Email: orlando@fmrp.usp.br

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