

Liver regeneration in traditional Chinese medicine: advances and challenges

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Abstract – Liver diseases pose a serious problem for national health care system all over the world. Liver regeneration has profound impacts on the occurrence and development of various liver diseases, and it remains an extensively studied topic. Although current knowledge has suggested two major mechanisms for liver regeneration, including compensatory hyperplasia of hepatocytes and stem or progenitor cell-mediated regeneration, the complexity of this physiopathological process determines that its effective regulation cannot be achieved by single-target or single-component approaches. Alternatively, using traditional Chinese medicine (TCM) to regulate liver regeneration is an important strategy for prevention and treatment of liver disorder and the related diseases. From the perspectives of TCM, liver regeneration can be caused by the disrupted balance between hepatic damage and regenerative capacity, and the “marrow”-based approaches have important therapeutic implications for liver regeneration. These two points have been massively supported by a number of basic studies and clinical observations during recent decades. TCM has the advantages of overall dynamic fine-tuning and early adjustment, and has exhibited enormous therapeutic benefits for various liver diseases. Here, we review the recent advances in the understanding of liver regeneration in TCM system in the hope of facilitating the application of TCM for liver diseases via regulation of liver regeneration.

Keywords: Liver regeneration, traditional Chinese medicine, hepatocyte, liver injury, imbalance, marrow

1 Introduction

It has been one of the directions that academic community strives to prevent and treat liver diseases from the perspective of internal mechanism of human body, and more attention has been gained in recent years. The mechanism of liver regeneration has long-termly been an important host factor for the development of liver diseases. After nearly a century of efforts, especially with the rapid development of stem cell-centered regenerative medicine, mechanistic understanding of liver regeneration progresses rapidly [1]. However, studies with high level evidence-based measures and methods for regulating liver regeneration are still relatively limited.

It has currently been known that the regulation of liver regeneration is not only the partial function of the liver, but also the overall comprehensive effect of dynamic changes of human body. Therefore, regulation of liver regeneration must be based on the overall dynamic regulation. The treatment using one-sided therapeutic method will fail to meet the needs of various aspects and ever-changing regulation of liver regeneration, and the curative effect must be limited [2,3]. Due to the functional characteristics of multi-target, multi-level and multi-system of the overall dynamic fine-tuning adjustment, traditional Chinese medicine (TCM) has gradually been a research hotspot, especially given that it can meet the needs of regulating liver regeneration in multi-aspects, complexity and fickleness [4]. Here, we present a brief review of the general cellular and molecular mechanisms of liver regeneration followed by detailed discussion on the

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recognition of liver regeneration in TCM theoretical system and its therapeutic implications.

2 General mechanism of liver regeneration

Current paradigm generally describes two mechanisms of liver regeneration: hepatocyte compensatory hyperplasia, and stem or progenitor cell-driven regeneration [5]. It is acknowledged that hepatic parenchymal turnover is maintained solely through hepatocyte division controlled by some pivotal signaling pathways, including Wnt/ β -catenin and Hippo/Yap [6,7]. This spatiotemporal regulation contributes to metabolic zonation and determines hepatocyte function [8]. Rodent models such as the classical partial hepatectomy in rats demonstrate that the liver size is restored through hepatocyte hypertrophy and hyperplasia in the remaining lobes. This is a highly regulated process dependent on changes in blood flow, sinusoidal endothelium, immune cells, hepatic stellate cells, and a host of growth factors and paracrine signals [9,10]. This regenerative potential is both highly efficient and of almost infinite capacity.

For the alternative regeneration mechanism, blood-borne cells of the bone marrow origin also play a role, in addition to the resident liver cells [11–13]. Hepatic stellate cells (HSCs) are also an important element of the liver regeneration machinery being part of liver stem cell niche, supporting regeneration at early stages by producing growth factors and inducing regeneration arrest after restoration of normal organ mass [14]. Notably, recent studies have concentrated on determining the contribution of expansion of the putative hepatic progenitor cell population resident in the bile ductules. It appears that when the ability of hepatocytes to proliferate is inhibited or overwhelmed, the liver maintains the ability to regenerate via mobilization of a population of hepatic progenitor cells [15,16]. For example, in the zebrafish models, where hepatocytes are ablated or prevented from entering the cell cycle, hepatic progenitor cells are activated, proliferate and can restore the hepatocyte mass ensuring survival of the organism [17]. However, much debate has surrounded the true significance of this hepatic progenitor cell pool, awaiting further elucidation.

3 Understanding of liver regeneration from perspectives of TCM

3.1 Imbalance between liver injury and regeneration

Having experienced the research hotspots, including anti-virus, anti-liver fibrosis, anti-hepatoma and immune-regulation for the treatment of liver diseases, a new research hotspot of regulating liver regeneration by TCM has been emerged. Many past studies have paid attention to studying the effects of TCM on liver damage or liver regeneration and to elucidating the underlying mechanisms [18]. With the deepening of research, the biological nature of “deficiency syndrome” has been demonstrated to

be the imbalance between pathological damage and tissue regeneration. In recent years, the regulatory mechanisms for TCM coordination of liver damage and liver regeneration for treatment of liver diseases have gradually been acknowledged [19]. Through understanding the nature of “hepatic deficiency syndrome” from the perspective of TCM vitality theory, the progression of liver disease syndrome can be roughly divided into several development trends or outcome patterns as follows. (1) The damaged liver structure can be repaired by induced regeneration in a short and mild liver injury. The liver damage and liver regeneration are only temporarily imbalanced, and the equilibrium state is quickly restored. In this case, liver physiological function is not significantly affected, and the liver disease-related deficiency syndrome is not formed, and the disease syndrome tends to recover. Most self-healing liver diseases follow such trend or outcome pattern. (2) The liver damage is too severe and/or too urgent, which is beyond the repair capacity of liver regeneration. The patients are too late and/or insufficient to regenerate the heavy liver damage, in which the liver damage and liver regeneration are seriously imbalanced, and the patients will be dead because of acute failure of liver function. Patients with acute liver failure who are too late for emergent treatment follow this trend or outcome pattern. (3) Although liver injury is not urgent or heavy, it will be repeated or persistent, eventually leading to disorder of liver regeneration and repair. Under such a condition, the liver damage and regeneration will be imbalanced, affecting liver tissue remodeling and functional recovery, and forming liver disease-related “deficiency syndrome”. The progressive and incurable chronic liver disease syndromes follow this trend or outcome pattern. Taken together, it can be concluded that one of the basic rules for prevention and treatment of liver disease syndrome is to regulate the imbalance between liver damage and liver regeneration, and that is, to reduce liver damage concomitant with promoting the normal repair mechanism of liver regeneration. Under normal circumstances, reducing liver damage is conducive to liver regeneration and repair, and regulating liver regeneration and repair is also beneficial for alleviating liver damage. Both aspects are mutually beneficial for and complement to each other, which is a virtuous circulation. Thus, the correction of the imbalance between liver damage and regeneration will be the most efficient, and the treatment effect will be the best.

The therapeutic practice of TCM or integrated Chinese and Western medicine for hepatic failure has demonstrated the scientific and advanced nature of reducing the mortality of liver failure, alleviating complications, and improving the quality of life by regulating the imbalance between liver damage and liver regeneration [20–27]. TCM believes that the damp, heat, and epidemic pathogenic factors are the main cause of hepatic failure. The pathological changes caused by damp, heat and epidemic pathogenic factors are mainly inflammatory injuries and necrosis. The Chinese medicines for clearing heat and promoting diuresis and detoxification exhibit the

dual effects of anti-inflammation and regulation of liver regeneration. Such medicines used most commonly include rheum officinale and its compound preparations, capillary artemisia and its compound preparations, liquorice and its modern preparations (glycyrrhizic acid glycosides, diammonium glycyrrhizinate oral and intravenous preparations), etc. Animal studies and clinical trials have confirmed that rheum officinale can alleviate hepatocyte damages induced by inflammatory factors such as TNF- α , IL-1 and IL-6 due to enteral endotoxemia, promoting liver regeneration [28,29]. The water-soluble active ingredients of capillary artemisia can reduce the activities of ALT and AST, and increase the activity of superoxide dismutase to produce anti-oxidative effects in animal models of liver injury. IL-6 and TNF- α are essential regulatory factors for initiating liver regeneration, but if overexpressed, they become inflammatory mediators leading to liver damage. Capillary artemisia can inhibit the overexpression of inflammatory mediators such as IL-6 and TNF- α in liver tissues, so as to produce anti-inflammatory and liver protective effects. On the other hand, capillary artemisia can also promote liver protein synthesis and liver regeneration, and accelerate the repair of necrotic liver tissues by increasing ribonucleic acids and hepatic glycogen [30]. Capillary mixture can reduce the expression of TNF- α , promote the expression of hepatocyte growth factor, and inhibit the apoptosis of hepatocytes in model rats with fulminant hepatic failure [31]. The ethanolic extracts of capillary artemisia have direct and significant protective effects on FFA-caused hepatotoxicity, and the underlying mechanism may be related to inhibition of excessive activation of nuclear factor- κ B [32,33]. The effective constituents glycyrrhizic acid and glycyrrhetinic acid in licorice can alleviate liver degeneration and necrosis caused by drug-induced liver damage in animals, exerting significant anti-inflammatory and hepatoprotective effects via regulating the multi-stages of inflammatory progression [34]. Furthermore, liver sinusoidal endothelial cells play a very important role in the occurrence and development of liver injury. Shen et al. found that isoliquiritigenin and diamine glycyrrhizate have significant protective effects against CoCl₂-induced hypoxia injuries in SK-HEP-1 cells, and meanwhile significantly improved the NO content and NOS activity concomitant with a decrease in reactive oxygen content [35]. Studies have shown that the expression of NF- κ B in patients with severe hepatitis and liver failure is significantly lower than that in the patients with normal or mild liver injury [36]. Specific enhancement of NF- κ B expression in hepatocytes can reduce liver damage and promote hepatocyte regeneration and repair [37]. In addition, Qiu et al. found that the compound glycyrrhizin can reduce the severity of acute liver injury in mice by regulating NF- κ B pathway [38].

3.2 Liver regeneration via “marrow”

“Marrow engenders liver” is an important academic thought of *Nei Jing* [39,40]. Recent studies have revealed that the biological nature of “marrow” is the stem cells and

related tissue microenvironment [41]. “Marrow engenders liver” includes at least two mechanisms for liver repair and regeneration: direct transformation of “marrow” into liver, and regulatory transformation of “marrow” into liver. Through the central link of the “marrow”, TCM can make the pathophysiological changes of “marrow” conform to and meet the needs of occurrence and development of regenerative repair, so as to maintain or promote the physiological state of “liver generation via marrow”, and to prevent or improve the pathological state of hypofunction of “liver generation via marrow”. In short, “engendering marrow” is to make marrow engendered, which is to maintain or promote the normal physiological state of “marrow” [42]. Therefore, comprehensive use of the following two strategies shall be adopted: on the one hand, to maintain or promote the normal physiological state of hepatic stem cells and their tissue microenvironment; on the other hand, to prevent or improve the abnormal pathological state of hepatic stem cells and their tissue microenvironment. The two strategies complement to each other.

Recently, some studies have found that the increasingly severe liver cirrhosis causes hematopoietic dysfunction and loss of hematopoietic stem cells, leading to dysfunction of blood and immunity and reduced regeneration potential [43]. Thus, the idea that restoring bone marrow function may provide new treatment option for cirrhosis has been proposed. Based on the “marrow”-centered therapeutic targets, TCM has established the treatment principle of “reinforcing kidney, engendering marrow, and generating liver”, which effectively guides the prevention and treatment of liver diseases and the related disorders. During the past 20 yr, a number of studies with in-depth experiments and clinical research around this treatment principle have been carried out [44–55]. The results show that this treatment principle is able to delay, prevent, or even reverse the occurrence and development of chronic liver disease, liver failure, cirrhosis, and liver cancer, and relatively high level evidence-based medical evidence has been obtained. The therapeutic mechanisms of Zuogui Pill, Diwu Yanggan Capsule, Kangdu Ruanjian Capsule and Jianghuang Capsule, which reflect the treatment principle of “reinforcing kidney, engendering marrow, and generating liver”, involve multiple pathways and links such as hypothalamic-pituitary-hepatic axis, neuro-endocrine-immuno-network of regulation of liver regeneration, transformation of bone marrow stem cells into liver cells, and liver tissue microenvironment, etc. [44–55]. By using cross-sex bone marrow transplantation model, multiple liver injury animal models, MSG-rat-liver regeneration model, bone marrow stem cell and hepatocyte co-culture technology, gene chip technology, protein mass spectrometry, multiple key proteins and their interaction mechanisms involved in “reinforcing kidney, engendering marrow, and generating liver” affecting the transformation of bone marrow stem cells into hepatocytes have been uncovered [44–55]. Multiple signal pathways related to this principle and to the

regulation of liver regeneration have been found, such as Wnt, mitogen-activated protein kinase, TGF- β , Jak-STAT, Toll-like receptors, etc. [44–55].

4 Therapeutic regulation of liver regeneration by TCM

Because of the extremely complex and verified nature of the process and mechanism of regeneration and repair, in order to maintain or promote the regeneration of the stem cell microenvironment, it is necessary to use the mode of action of overall dynamic fine-tuning early adjustment to comprehensively regulate with multiple targets and multiple pathways. Obviously, single-target chemical medicines cannot meet such requirements. Therefore, there is currently no single-target chemical medicine that can regulate liver regenerative repair effectively. TCM has the comprehensive adjustment advantages of complex composition and overall dynamic fine-tuning features, which fully meet the needs of regulating regeneration and restoration. TCM utilizes the natural healing ability of viscera tissue to regenerate and repair the damage of viscera tissue, and rebuild and restore the structure and function of visceral tissue, which possesses the characteristics of being natural, making the best use of circumstances, dynamic changes, reversing the disease, overall adjustment, high security, and being affirmative in maintaining or improving the regenerative repaired stem cell microenvironment [56]. It has been known that IL-6 and TNF- α are key hepatic inflammation-inducing factors and their overexpression induces hepatic inflammatory damage, which is positively correlated with the severity of liver disease [57]. It has been found that TCM is able to alleviate inflammatory damage in the liver by inhibiting the overexpression of IL-6 and TNF- α [58–60].

Proliferation and activation of HSCs are thought to be the key steps of liver fibrosis, and the attention of treatment has always been focused on how to inhibit the proliferation and activation of HSCs [61]. However, as the research progresses, it is recognized that HSCs also play an important role in liver regeneration as a component of stem cells [62]. In recent years, it has been confirmed that HSCs have the potential of multi-directional differentiation, which can be differentiated into hepatocytes and vascular endothelial cells under certain conditions, and can directly participate in cell regeneration of liver injury repair [63]. Considering the impact on HSCs from the perspective of regulating liver regeneration, it is not necessary to blindly emphasize the inhibition of HSC proliferation and activation, but more attention shall be focused on how to regulate the differentiation direction of HSCs, and to promote the coordinated development of liver regeneration. TGF- β 1, epithelial-mesenchymal transition, hedgehog signaling pathways are important mechanisms affecting HSC proliferation and differentiation [64]. Recent studies found that the TCM compound Diwu Yanggan Capsule is able to inhibit the overexpression of TGF- β 1, inhibit the overactivation of

hedgehog signaling pathway, prevent the occurrence and development of hepatic fibrosis, and promote liver regeneration and repair by inhibiting the EMT process of HSCs and/or promoting its MET process [65–67].

Current studies have confirmed that the oval cells are one kind of important hepatic stem cells for liver regeneration and repair [68,69]. When hepatocyte proliferation is inhibited or hepatocyte regeneration is insufficient to meet the regenerative repair, oval cell proliferation becomes an important way for liver regeneration and repair. However, oval cells are also progenitor cells of liver cancer, and their long-term excessive hyper-proliferation may promote the occurrence and development of liver cancer [70]. Studies have found that the TCM compound preparation Diwu Yanggan Capsule is able to promote the proliferation and differentiation of bone marrow stem cells and intrahepatic oval cells in 2-AAF/PH model rats at the early and middle stages, which is conducive to liver regeneration and repair [71]. It is also able to inhibit the excessive proliferation and abnormal differentiation of liver oval cells at the middle and late stages, which is conducive to prevent and treat hepatocellular carcinogenesis. Disruption of Wnt/ β -catenin pathway by Diwu Yanggan Capsule may be the molecular mechanism of inhibiting excessive proliferation and abnormal differentiation of oval cells [71]. Regulating the expression of multiple liver regeneration-related cytokines (TNF- α , IL-1, vascular endothelial growth factor, and interferon- γ) in 2-AAF/PH rat models makes them more inclined to the normal levels, thereby inhibiting the excessive proliferation and abnormal differentiation of oval cells and preventing the occurrence and development of precancerous lesions by improving the liver regeneration microenvironment [71]. Moreover, the Solt-Farber two-step method can be adopted to replicate the liver cancer model in rats, in which the liver regeneration microenvironment contains inhibited hepatocyte regeneration and hyperproliferative oval cells. In this model, the pathogenesis of transforming bone marrow stem cells into liver cancer stem cells exists in liver cancer, and Diwu Yanggan Capsule inhibits the occurrence and development of liver cancer. Its mechanism may be to inhibit the transformation of bone marrow stem cells into liver cancer stem cells, promote the regeneration and repair of hepatocytes, inhibit the excessive proliferation of oval cells, regulate the imbalance of EMT/MET (inhibition of EMT and promote MET), affect the expression of proteins in JAK/STAT and Ras/Raf/Mek/Erk signaling pathways, and improve the liver regeneration microenvironment [72–74].

5 Conclusions and challenges

The rapid progress of liver regeneration research, the lack of specific technologies and methods for regulating liver regeneration, and the effective functional characteristics and advantages that the TCM has to regulate liver

regeneration, have collectively brought great opportunities for the basic and clinical researches of preventing and curing liver diseases and related diseases by regulating liver regeneration. Many efforts have been made to improve the ability and level of TCM or integrated Chinese and Western medicine to prevent and cure liver diseases and related diseases. However, faced with the more complex interaction mechanism between the components of TCM and the complex mechanism of liver regeneration, and with the insufficiency of clinical standard system for clinical application, insurmountable challenges for basic and clinical application research of regulating liver regeneration with TCM are proposed.

Normal and abnormal liver regeneration are difficult to be distinguished clinically. It is difficult to establish an index system suitable for clinical application to objectively evaluate the normal or abnormal liver regeneration, which is a key scientific problem that hinders the clinical application of TCM to regulate liver regeneration. TCM regulates liver regeneration mainly through inartificial or natural methods to adjust the internal mechanism of liver regeneration and to normalize liver regeneration. The main mechanism of action is to transform the pathological imbalance mechanism into the normal equilibrium mechanism, so the criterion for therapeutic effect evaluation is “expectation of balance”. The complex composition in TCM, the use of natural therapy, the overall dynamic fine-tuning and early adjustment, and the characteristics of individualized diagnosis and treatment exactly meet the complex and ever-changing needs of liver regeneration regulation, which is of realistic and potential advantages. Furthermore, there are needs to investigate how the physical liver regeneration and pathological liver regeneration are regulated by TCM, given that many Chinese herbs medicines have food function and are commonly used in diets of healthy people. In short, the continuous improvement and development of various systems biology technologies can provide ways and hope to solve the above-mentioned complex problems. The combination of evidence-based medical evidence and the real-world big data can also promote clinical research and application of TCM for regulating liver regeneration.

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Conflict of interests

The authors declare that there is no financial or other relationship that might lead to a conflict of the present article. All authors have reviewed the final version of the manuscript and approved it for publication.

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