Journal of Translational Medicine

REVIEW

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Influencing factors and mechanism of hepatocyte regeneration

Xiaoyi Zhang[1,2,3], Shenghao Li[1,2,3], Liyuan Hao[1,2,3], Fukang Jia[4], Fei Yu[1,2,3] and Xiaoyu Hu[1,2*]

Abstract

As a research hotspot in the field of regenerative medicine, hepatocyte regeneration has great potential in the treatment of liver diseases. This paper comprehensively summarizes the diverse sources of hepatocyte regeneration and its complex influencing factors, and deeply discusses the typical mechanism. According to the existing research, we observed that Wnt signaling pathway and Notch signaling pathway can play a synergistic role in the process of hepatocyte regeneration. So we further analyzed the crosstalk between Wnt and Notch signal pathway and the cross mechanism with TGF-β, YAP/TAZ pathway during regeneration. Despite the remarkable progress in the study of liver regeneration at the cellular and molecular levels, the comprehensive understanding of the fine regulation of influencing factors and the interaction between mechanisms still needs to be deepened. This paper aims to systematically analyze the interaction between influencing factors and classical mechanisms of hepatocyte regeneration by integrating multi-group data and advanced bioinformatics methods, so as to provide feasible ideas for the treatment of liver diseases and lay a solid theoretical foundation for the future development of regenerative medicine. It is believed that focusing on the rational development of innovative means such as inducing gene tendentiousness expression and anti-aging therapy, and in-depth analysis of the complex interactive network between hepatocyte regeneration mechanisms are expected to open up a new road for the development of more effective treatment strategies for liver diseases.

Keywords Hepatocytes, Regeneration, Signal channel

Introduction

With the continuous expansion of hepatectomy in clinical application, the regenerative characteristics of liver have become an important research direction in the field of modern medicine, and remarkable progress has been made [1]. Since the first discovery of the phenomenon of volume and mass recovery after liver injury in rodents in 1930s [2], the study of hepatocyte regeneration not only reveals the self-repair mechanism of life in response to injury, but also opens up a new perspective and strategy for the treatment of liver diseases. The development of regenerative medicine has ushered in an unprecedented opportunity in the face of the grim reality of high mortality rate of end-stage liver diseases.

Since the beginning of this century, with the rapid development of biotechnology and medical research, the research on the mechanism of hepatocyte regeneration has made significant breakthroughs in theory at the cellular and molecular levels, and achieved significant progress in clinical application. Especially in recent years, for example, two major discoveries [3–5] about hepatocyte

*Correspondence:
Xiaoyu Hu
18582311048@163.com
[1]Chengdu University of Traditional Chinese Medicine, Chengdu, China
[2]Department of Infectious Diseases, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, No.39, Shierqiao Road, Jinniu District, Chengdu, Sichuan, China
[3]Clinical Medical College of Chengdu, University of Traditional Chinese Medicine, Chengdu, China
[4]Henan University of Traditional Chinese, Zhengzhou, China

regeneration published in Science in 2021 have pushed the research in this field to a new climax [6, 7]. These studies not only deepen our understanding of the process of hepatocyte regeneration, but also inspire more new insights on the regeneration mechanism, such as "the asynchronism between wound closure and hepatocyte proliferation after injury", "the key role of dual-energy transitional hepatic progenitor cells derived from biliary epithelium in liver regeneration" and "whether there is partition bias in liver regeneration" [8–10] and so on.

In addition, the application of high-throughput sequencing technology and other emerging means further reveals the complexity and diversity of hepatocyte regeneration. These technologies not only help us discover many new regeneration-related genes and signal pathways, but also promote the application of nanotechnology [11, 12], organ-like culture [13] and various new models in regeneration research, shortening the distance between laboratory and clinic. In terms of signal pathways, some recent studies have emphasized the key role of classical signal pathways such as Wnt [14], Notch [15], Hedgehog [16] and TGF-β [17] in the process of hepatocyte regeneration and their crosstalk mechanism. These findings not only deepen our understanding of the molecular mechanism of hepatocyte regeneration, but also provide an important basis for developing new strategies for the treatment of liver diseases.

Therefore, the study of hepatocyte regeneration not only has theoretical significance, but also shows great application potential in clinical treatment. By deeply exploring the influencing factors and mechanisms of hepatocyte regeneration, we are expected to provide more effective and safe treatment programs for patients with liver diseases, thus improving their prognosis and quality of life. However, although the research on the mechanism of hepatocyte regeneration is in full swing at present, there are still many challenges in the rational regulation of influencing factors and the combing of classical pathway-related crosstalk.

Source and cell process of regenerated hepatocytes

Researchers have observed that the liver has a certain ability to recover when it undergoes partial resection or faces various damages. Strong curiosity and the urgent need for treatment of liver diseases jointly promote the in-depth study of liver regeneration [18]. Liver regeneration is a process of self-replication and repair in the whole region after liver injury. Although hepatocytes are not always the with the greatest contribution in the process of regeneration [19–21], they are still often regarded as the main contributors to liver regeneration as the liver parenchyma cells with the largest proportion [22].

New hepatocytes from multiple sources

Source of new hepatocytes is the primary consideration of hepatocyte regeneration, which can not only reveal the potential mechanism of liver regeneration, but also provide potential targets for the treatment of liver diseases.

At present, the sources of new hepatocytes in the process of regeneration are mainly summarized as the proliferation of residual hepatocytes after injury [23], the differentiation of hepatic progenitor cells [24] and those obtained from bile duct cells [25] and other non-parenchymal cells [26–29]. Although the origin of regenerative hepatocytes is different, the emergence of regenerative hepatocytes faces the steps of gene replication, transcription and translation. The new study also found that liver cells also have the ability to dedifferentiate into hepatocyte-like cells and transdifferentiate into biliary epithelial cells, which broke the previous thinking that mature liver cells did not have the ability to differentiate, and this circular model further benefited liver regeneration. In fact, Rodrigo-Torres D and others also tried to trace the bile duct epithelial cells expressing Hnf1β in mice with injury model through the genetic pedigree tracing technique mediated by mouse Cre/loxP, and finally confirmed that oval cells can differentiate into bile duct cells under certain conditions [30], which is a great discovery of the origin of bile duct cells. However, like other related experiments of conventional mouse liver injury models, the exact evidence that oval cells differentiate into hepatocytes has not been found at last [31, 32]. Actually, in 2021, Professor Hao Zhu and his team used CreER knock-in mouse model to mark the sub-groups of hepatic lobules and verified that the cells in area 2 between the periportal vein and the pericentral vein are the important sources of regenerative hepatocytes. At the same time, Professor Bin Zhou's team reached the conclusion that the hepatocytes in area 2 contribute the most to liver regeneration under steady-state conditions through proliferation ProTracer [33]. More and more evidences show that hepatocytes in different regions will express different regeneration abilities and choose different regeneration pathways (hypertrophy or hyperplasia, etc.) when injured. This is a major breakthrough in the research history of hepatocyte regeneration [6, 7].

Although there are many achievements in exploring the source of regenerated hepatocytes at present, it is still an unsolved mystery which cells can directly or participate in the differentiation into hepatocytes and which type of hepatocytes plays a leading role.

Regeneration process of hepatocytes

Regardless of the specific source of hepatic progenitor cells, the process of hepatocyte regeneration can be roughly divided into three stages: start, proliferation and termination. In the initial stage of hepatocyte

regeneration, cells are stimulated by external or internal synthetic mitogens (such as growth factors, hormones, etc.), thus entering G1 phase from the static G0 phase, preparing for the subsequent DNA replication. In this process, the mRNA expression of proto-oncogenes c-Jun and c-Fos increased significantly [34], and these genes play an important role in the regulation of cell proliferation. Subsequently, the key molecular mechanisms in G1 phase include Cyclin D/Cdk4/6 [35] and Cyclin E/Cdk [36] gradually participating and being activated. These complexes promote the cell cycle process by phosphorylating downstream substrates, so that cells can pass through the restriction point (R point) smoothly and enter the DNA synthesis stage (S stage). In S phase, cells complete DNA replication and provide genetic material for subsequent cell division. Then, the cell cycle continues, passing through the G2/M checkpoint and entering the G2 phase, an important stage of prophase. In this cycle, cells will further synthesize RNA and protein, especially protein and enzymes related to cell division, so as to make full preparations for the upcoming mitosis. Finally, under the action of various regulatory factors (such as Foxm1b transcription factor [37], etc.), it entered the mitotic phase (M phase) and successfully completed cell division. Through the precise coordination of these molecular mechanisms and regulatory steps, hepatocytes can regenerate efficiently and restore damaged liver tissue.

The formation process of new hepatocytes can be briefly summarized by cell division cycle, but the specific molecular mechanism behind it is extremely complex and has not been fully clarified yet.

Research circles generally believe that the efficiency and quality of hepatocyte regeneration are influenced by a series of complex and intertwined factors, which jointly determine the final results such as the speed of hepatocyte regeneration and the degree of functional recovery. Usually, these factors include the dynamic changes of cell microenvironment, the types and severity of injury, the participation of immune system, and the fine regulation of endogenous growth factors and signal pathways. Hence, it is not only the key to understand the mechanism of hepatocyte regeneration, but also of great clinical significance to improve the therapeutic effect of liver diseases and promote liver health.

Analysis of influencing factors of hepatocyte regeneration

As a unique feature of life, genes play a key role in liver regeneration. Considering the influence of physiological and pathological conditions on regeneration [38], the influencing factors of hepatocyte regeneration process are briefly summarized as endogenous factors, exogenous factors and disease state.

Endogenous factors

With the deepening of research, researchers have focused on the root cause of hepatocyte regeneration at the genetic level, and gradually realized the role of polyploid hepatocytes in liver regeneration. T Matsumoto's team administered rAAV700-Ttr-Cre to 27-week-old and 3-week-old mice, and found that the frequency of diploid hepatocytes in the liver of old mice was similar to that of young mice [39]. It shows that polyploidy may be an adaptive mechanism for hepatocytes to cope with aging and regeneration pressure. By increasing the number of genomes, hepatocytes may gain stronger metabolism and regeneration ability. The latest research results reveal that even in the models of hepatectomy and compensatory regeneration after acute injury, diploid hepatocytes also show significant ability to accelerate liver regeneration [40]. These findings reveal that the difference of genome structure or chromosome number is related to the regeneration ability of hepatocytes, and future research can explore the potential of reasonably inducing the expression of polyploid hepatocytes in promoting the repair of liver tissue injury.

Problems in the process of gene replication, transcription and translation will cause abnormal gene expression to affect hepatocyte regeneration, but the influence of significant characteristics such as gender and age on this link is rarely discussed. In the model of liver fibrosis induced by CCl4 injection, it was found that male mice showed more fibrosis areas and higher expression of αSMA protein than female mice at 6 weeks [41]. In another experiment, the recovery rate of liver after 2/3 hepatectomy was compared. It was found that the mRNA of B-myc in the liver of female mice increased more significantly than that of male mice, but the regeneration rate was lower [42]. It seems that gender may indeed affect gene expression and can have an impact on liver regeneration [43]. Although there is no strong evidence to use sex hormones to help hepatocyte regeneration to treat liver diseases, it provides a new idea for the treatment of diseases through micro-regulation of gene expression.

Aging, similar to gender, can also have a significant impact on liver regeneration [44–46]. The aging process is often accompanied by the down-regulation of many genes such as GSK3β and Dnmt3b44. At the same time, aging can also lead to the silent expression of some cyclins, which makes the methylation ability of many tissues lose [47], thus affecting gene expression and ultimately reducing their ability to cope with external damage. Genereb et al.' s research shows that fetal liver-derived mesenchymal stem cells (fMSCs) have significantly stronger proliferation ability than adult bone marrow-derived mesenchymal stem cells (aMSCs) [48], which suggests that young cells have stronger

proliferation ability than aging cells. Studies have found that aging is related to Notch [49] and Wnt [50] mechanisms, and it has been applied in the treatment of diseases such as Alzheimer's disease and senile osteoporosis. Just as drugs that selectively induce apoptosis of aging cells have become the focus of research in recent years, anti-aging may become a key breakthrough in the field of disease treatment in the future. At present, the discussion of aging problems mostly stays at the level of animal experiments, so it is necessary to build a human aging model in the future.

Nowadays, existing treatments have promoted the regeneration by transferring RNA to hepatocytes through platelet transfusion [51, 52], which makes us realize that further analysis of endogenous factors affecting hepatocyte regeneration is expected to open up a new way for hepatocyte regeneration and liver injury repair.

Exogenous factors

Normally, the liver does not exhibit regenerative ability, and its regenerative potential can be activated after external stimulation. Thus, some external conditions will also affect the regeneration of hepatocytes. As early as 17 years ago, Moriyama M's team found that the irregular regeneration of liver cells in patients with hepatitis C and cirrhosis treated with interferon was effectively improved, and the prevalence of hepatocellular carcinoma in patients with hepatitis C was also reduced [53]. Even though direct antiviral drugs (DAAs) have been used to treat hepatitis C, this conclusion still has important clinical value. In addition, Li L et al. also studied 8-week-old male mice through a 9-week CDE diet test, and then found that the differentiation process of activated offspring cells into hepatocytes would be inhibited after tamoxifen treatment, thus guessing that hormone receptor modulators have inhibitory effects on hepatocyte regeneration [54].

In fact, besides drugs, cytokines have also been found to have certain effects on hepatocyte regeneration. After Al-Ghamdi TH et al. gave exogenous IL-6 to rats undergoing 70% hepatectomy, they not only induced significant proliferation of hepatocytes, improved the quality of frozen liver, but also improved biochemical indexes, reduced apoptosis and enhanced the regeneration rate of hepatocytes [55]. In addition, the use of IL-1 receptor antagonists can also promote hepatocyte regeneration in alcoholic hepatitis mice [56].

Based on the effects of drugs and cytokines on hepatocyte regeneration, we speculate that physical factors such as radiation and temperature can also affect hepatocyte regeneration. Sure enough, the experiment shows that the morphological changes caused by liver radiotherapy (including SBRT) can be observed in the later radiological examination [57]. By testing the liver/segment volume and radiation dose of liver stereotactic radiotherapy (SBRT), the staged dose threshold of hepatocyte regeneration was found, and it was finally verified that retaining some liver segments or giving smaller radiation dose was helpful to promote hepatocyte regeneration [58]. Although it has been confirmed that radiation and other physical factors have an effect on hepatocyte regeneration, the mechanism of radiation and other physical measures on hepatocyte regeneration is not completely clear at present, and further research is needed.

Briefly, in the treatment of liver diseases, the regulation of exogenous factors on hepatocyte regeneration can not be ignored.

Disease state

In the treatment of liver diseases such as liver cancer, partial hepatectomy or chemotherapy can activate the regeneration ability of liver cells. It can be seen that the disease state is also an important aspect affecting hepatocyte regeneration. Studies have shown that there are significant differences in the process and effect of hepatocyte regeneration under different disease States.

Taking liver fibrosis/cirrhosis as an example, Hung KC et al. found that the liver regeneration rate of patients with liver fibrosis showed unique changes after liver zoning, portal vein ligation and staged hepatectomy through careful grouping experiments. In the first week, the liver regeneration rate of hepatic fibrosis group was the fastest, in contrast, the liver volume of non-hepatic fibrosis group reached the highest level in the second week [59]. We suspect that this difference may be caused by the limitation of hepatocyte regeneration space caused by fibrous tissue hyperplasia in pathological state, but the specific reasons still need further exploration in related experiments.

In addition to liver fibrosis/cirrhosis, other types of liver diseases will also affect the process of hepatocyte regeneration. For example, alcohol exposure caused by long-term drinking will cause large-scale inflammation and sporadic bleeding in the liver, and then interfere with the transmission of microRNA signals, affect DNA synthesis, weaken the proliferation ability of mature hepatocytes and ultimately inhibit liver regeneration [60]. Viral infection is also an important factor affecting hepatocyte regeneration. In the experiment of transgenic mice, Quétier I team found that hepatitis B virus HBx protein can delay liver regeneration by inducing IL-6 overexpression. Also related to viral hepatitis, Barthel SR team found that HBV infection can reduce the sensitivity of liver cells to insulin, which also shows the delay of liver regeneration [61]. Studies have shown that the use of Wnt signal modulators in the treatment of nonalcoholic fatty liver disease can affect the regeneration of hepatocytes and promote the repair of damage [62]. The DLL4-Notch signal also

plays an important role in the treatment of acute liver failure [63].

At this point, we guess that other diseases besides liver disease itself may indirectly affect the process of hepatocyte regeneration. Perhaps in the state of diabetes, the abnormal metabolism of hepatocytes caused by hyperglycemia will also affect their regenerative ability? Or in cardiovascular diseases such as cardiogenic shock or congestive heart failure, the reduction of liver blood flow limits the oxygen supply for regeneration of hepatocytes and weakens their regeneration ability? Because similar systematic research is limited, we can't draw a definite conclusion for the time being. We guess that different diseases may also regulate hepatocyte regeneration through signals such as Wnt and Notch, which can be further verified by interested scholars in the future.

After in-depth analysis of the influencing factors of hepatocyte regeneration, it is not difficult to find that many factors are intertwined and jointly affect the complex process of hepatocyte regeneration. In order to understand the mechanism of hepatocyte regeneration more comprehensively, it is necessary to put these influencing factors into a unified framework for investigation in the future, so as to develop more effective treatment strategies and explore the treatment ideas of liver diseases. Next, we will discuss the related mechanisms of hepatocyte regeneration in detail, and try to reveal the specific role of these mechanisms in the process of hepatocyte regeneration. Through in-depth understanding of these mechanisms, we can better explain the phenomenon of hepatocyte regeneration and provide new ideas and methods for the treatment of liver diseases.

Mechanism of hepatocyte regeneration

Hepatocyte regeneration is a complex process regulated by multiple factors. Although scientists have made remarkable achievements in the field of hepatocyte regeneration for many years, the specific mechanism of liver regeneration still needs to be further explored. In recent years, remarkable progress has been made in the study of molecular signaling pathways in hepatocyte regeneration. However, due to different experimental conditions, animal models and research methods, the conclusions drawn by different research teams are different. Although the understanding of the mechanism becomes more and more complicated with the deepening of research, the related research still focuses on a few classic signaling pathways. Based on the comprehensive analysis of the existing literature, compared with other signal transduction pathways, Wnt and Notch signal pathways show a more solid research foundation. The new study found that Notch and Wnt/β-catenin signal transduction also coordinated the transformation from biliary epithelial cells to transitional hepatic progenitor cells and from transitional hepatic progenitor cells to hepatocytes, respectively [9]. In a word, these two kinds of signal transduction not only have complex interactions with various signal pathways, but also have significant clinical transformation potential.

Wnt signaling pathway mechanism

Wnt pathway is widely recognized in the process of liver regeneration [64, 65]. Wnt pathway can be divided into two types according to whether it depends on β-catenin or not, which play different roles in hepatocyte regeneration. Studies have shown that activation of Wnt signal can promote the regeneration of liver cells under certain conditions, but abnormal activation of Wnt signal can promote the progress of liver tumors [14, 66, 67] In the classical Wnt/β-catenin signaling pathway, Wnt ligands interact with Frizzled receptors and LRP5/6, and then combine with the destructive complexes formed by GSK-3β, Axin2 and APC, so that β-catenin accumulation further triggers the transcription of Wnt target genes [68]. In the non-classical Wnt/Ca2 + pathway, the activation of Frizzled receptors can promote the generation of Ca2 + and further activate Ca conduction [69]. In another nonclassical Wnt/PCP pathway, binding to Wnt ligand leads to phosphorylation of Dishevelled and binding to DAAM, thus activating Rac1, profilin and RhoA. Then Rac1 activates JNK and then activates c-Jun to enter the nucleus [70]; And RhoA activates ROCK to further activate MRLC [71].

Wnt signaling plays a dual role in liver regeneration and repair. For example, Wnt/β-catenin signaling in the classical pathway can promote liver regeneration through lysosomal degradation of APC protein, whereas downregulation of Wnt/β-catenin signaling will damage liver regeneration [72]. Overall, it is clear that Wnt signal activation plays a key role in liver regeneration, metabolic grouping, liver diseases and liver cancer [73]. With the in-depth understanding of Wnt mechanism, pathway inhibitors such as Dickkopf-1 [74] will bring new therapeutic hope to patients (Table 1).

Notch signal path mechanism

In addition, as the downstream effector of Hippo transducer YAP, Notch signaling pathway is also very important in hepatocyte regeneration [104]. Notch protein is transported to endoplasmic reticulum in the classical Notch pathway, where it is glycosylated [105]. Subsequently, the glycosylated Notch single-stranded precursor will be transported to Golgi apparatus, where it will be cleaved by protease into two fragments: NECD(Notch extracellular domain) and TMD (transmembrane domain) [106]. Then, with the participation of Ca2 + ions, mature Notch receptors are formed, and the binding with ligands is completed on the cell surface. After that, Notch

Table 1 Wnt path research node

Literature	Time		Result
[75]	1982.11	Int1	Int1 gene was cloned from mouse breast cancer cells, and it was found that Int1 could promote the development of cancer.
[76]	1987.08	Dint-1	Dint-1, a homologue of int-1, was isolated from Drosophila melanogaster by cross hybridization. And Dint-1/wingless plays an important role as a signal of intercellular communication.
[77]	1989.06	catenin	The catenin α, β and γ are named.
[78]	1991.11	β- catenin	β -catenin was found in all the embryonic stages of Xenopus laevis, and was related to C- cadherin.
[79]	1996.05	GSK3β	GSK3β can bind to β -catenin and Drosophila adenomatous polyposis coli gene (APC).
[80]	1996.08	TCF-1/Lef-1	TCF-1/Lef-1 can bind to β -catenin and can bind to target DNA as a complex.
[81]	1997.02	Frizzled	The curled protein was identified as Wnt receptor and the function of Dishevelled was discussed.
[82]	1998.06	GBP	GBP (a parent Xgsk-3 binding protein) inhibits the phosphorylation of Xgsk-3 in vivo and is an inhibitor of GSK-3.
[83]	1998.07	Dsh	Dsh activates JNK pathway; Wnt/Frizzled signaling pathway uses multiple intracellular signal cascades.
[84]	1998.1	TCF	CBP inhibits TCF.
[85]	1999.03	Axin	Axin protein family is very important in Wnt/Wingless signal of Drosophila.
[86]	2000.09	LRP6	LRP6 plays a wide role in the transduction of several Wnt signals in mammals.
[87]	2002.05	Wnt/Ca2+	Wnt/Ca2 + pathway activates NF-AT and mediates ventral signals of Xenopus laevis embryos.
[88]	2002.04	Notch	Wnt and Notch cascade
[89]	2002.04	Hedgehog	Shaggy/GSK3 is also a negative regulator in Hedgehog (Hh) pathway.
[90]	2005.09	c-Jun、 TCF4	Phosphorylation-dependent interaction between c-Jun and TCF4 regulates intestinal tumorigenesis by integrating JNK and APC/β-catenin.
[91]	2007.05	WTX	WTX promotes ubiquitination and degradation of β-catenin, thus antagonizing Wnt/β- catenin signal transduction.
[92]	2009.12	Wnt	The axial mode of Wnt signal transduction is earlier than the evolution of bilaterally symmetrical animals.
[93]	2012.12	TAZ	A large part of Wnt transcription reaction is mediated by TAZ.
[94]	2013.01	YAP	YAP inhibits Wnt signal by limiting DVL nuclear translocation during regenerative growth.
[95]	2014.07	YAP/TAZ	The release of YAP/TAZ in the complex contributes to Wnt/β-catenin signal transduction.
[96]	2015.06	DVL	As a WNT signaling pathway, DVL promotes complex assembly and downstream signal transduction, and also serves as a linker to recruit ZNRF3 and RNF43 to WNT co-receptors to promote their degradation and down-regulation.
[97]	2020.12	Lgr5	Wnt/β-catenin signaling realizes bile duct regeneration by regulating the expression of Lgr5 gene in ductal response cells (DRCs) located near peribiliary glands.
[72]	2021.02	TMEM9	Transmembrane protein 9 (TMEM9) is a Wnt signal amplifier, and Tmem9 knockout will damage liver regeneration.
[98]	2022.02	Wnt2	C-kit + SECs promotes liver partition and regeneration through Wnt2, and is regulated by Notch signal transduction.
[99]	2022.03	TCF1 、LEF1	TCF1 and its homologue LEF1 have historically been called effective transcription factors downstream of wnt signaling pathway, which are essential for early T cell development.
[100]	2023.04	Wnt/β-catenin	Wnt/β-catenin pathway is activated in the early stage of LR, and the conditional loss of β-catenin liver cells will delay LR.
[101]	2023.10	Wnt/β-catenin	Activation of Wnt/β-catenin signal transduction is the main mitogenic clue of adult primary human hepatocytes.
[102]	2023.12	Wnt	Cell maturation is mediated by the activation of NF-κ B and the inhibition of Wnt signal respectively.
[103]	2024.05	Cachd1	Cachd1 can bind to both Lrp6 and Wnt co-receptors of Frizzled family.

receptor will face the cleavage of ADAM enzyme at a new site, and then release extracellular fragments to produce instantaneous intermediate peptides composed of TMD and NICD(Notch intracellular domain) [107]. Then, presenilin-dependent γ secretase cleaves the intermediate peptide at the third site. With the completion of cleavage, NICD will be released into the nucleus and interact with CSL protein to regulate gene expression [108]. The nonclassical Notch pathway is independent of the classical ligand-receptor interaction and the regulatory mechanism of CSL/NICD complex in the nucleus. For example, mTOR-Rictor can interact with NICD, thus activating AKT/PKB and regulating cell survival [109].

Studies have shown that in the rat model after partial hepatectomy (PHx), inhibition of Notch activity leads to significant impairment of hepatocyte proliferation and regeneration [110], while the expression intensity of hepatocyte growth factor (HGF), epithelial growth factor (EGF), vascular growth factor (VEGF) and Notch signal in serum will increase significantly after hepatectomy.

This suggests that the activation of Notch is very important for hepatocyte regeneration, and future research can further explore how to promote hepatocyte regeneration by regulating Notch pathway (Table 2).

Interaction and regulation between molecular signal pathways

Wnt and Notch pathway cross talk with each other, and Notch can complex with β-catenin to promote the degradation of β-catenin Notch can also increase the expression of Frizzled receptor and the transcription of TCF1 [140]. TCF/LEF can also regulate the expression

Table 2 Notch path research node

Literature	Time		Result
[111]	1917.12	Notch locus	Notch locus was determined by the mutant strain of Drosophila melanogaster with notched wings
[112]	1917.09	Notch	Notch gene found in mutant Drosophila melanogaster
[113]	1983.04	Notch	Notch was isolated by molecular cloning.
[114]	1985.12	Nucleotide sequence	The nucleotide sequence was published.
[115]	1988.10	LIN12	Notch homologue LIN12 was found in Caenorhabditis elegans.
[116]	1989.08	GLP1	Lin-12/Notch repeats correspond to glp-1.
[117]	1990.05	Delta	Interaction between gene products of Notch and Delta loci.
[118]	1992.12	Notch2	A new Notch gene Notch2 was encoded by cDNA cloning, which proved that Notch1 and Notch2 were equally related to Notch in Drosophila.
[119]	1993.01	Delta2	Delta-2, Another Member of Excitatory Amino Acid Receptor Superfamily
[120]	1994.09	Notch3	The adjacent RAGE and HOX12 genes were completely sequenced, and the corresponding gene of human int-3 was partially sequenced and classified as Notch homologue. And the homologue was named Notch3.
[121]	1996.07	Notch4	The cDNA clone corresponding to the whole coding potential of int-3 proto-oncogene was isolated. And named the gene Notch4.
[122]	1996.11	Jagged-2	Jagged-2 was isolated.
[123]	1997.05	delta3	Identified from human cerebral cortex by library screening and PCR, and named as delta1 and delta3 subtypes.
[124]	2000.05	LAG-3	LAG-3 is an effective transcriptional activator in yeast.
[125]	2003.11	Jagged-1	Overexpression of Jagged-1 induces allogenic antigen-specific human regulatory T cells.
[126]	2003.04	Gycosylation	The difference of glycosylation degree is an important mechanism to regulate Notch signal.
[127]	2006.06	Stem cell amplification	The expansion of stem cells in vitro and in vivo are two core goals of regenerative medicine, which can be achieved by Notch ligands through ways that are crucial to development and cancer.
[128]	2007.07	Delta4	Delta-like ligand 4(DLL4), a ligand of Notch receptor, is usually induced by VEGF and is a negative feedback regulator, which can inhibit vascular germination and branching.
[129]	2012.09	HCC	Notch signal can be activated in human HCC samples.
[130]	2016.05	Notch signal	The results of Notch signal transduction vary greatly with the signal dose and cell environment.
[131]	2017.07	DLL4	DLL4 activates NF-κ β signal transduction to enhance the secretion of vascular endothelial factor (VEGF) and tumor metastasis.
[132]	2019.06	DLL3	DLL3 inhibits cell growth by inducing apoptosis.
[133]	2020.10	M1 macrophages.	Capsaicin can alleviate liver fibrosis by inactivating Notch signal transduction and further inhibiting the secretion of TNF α by M1 macrophages.
[134]	2021.02	Notch	Notch signaling involves many aspects of cancer biology, including angiogenesis, tumor immunity and maintenance of cancer stem cell-like cells.
[135]	2021.07	IGF1R	Notch signaling increased the abundance of insulin-like growth factor 1 receptor (IGF1R) in BEC, and inhibited the differentiation of BEC into hepatocytes.
[15]	2022.03	Sirt1	Sirt1 inhibition accelerates liver regeneration by eliminating Notch-driven aging.
[136]	2022.10	YAP/TAZ-TEAD	The coiled helix coiled helix domain 2cchd2 is up-regulated by YAP/TAZ-TEAD in NASH liver of nonalcoholic steatohepatitis, thus promoting liver fibrosis by activating Notch pathway and enhancing osteopontin production.
[137]	2023.02	MCP-1	The infiltration of liver monocyte-derived macrophages (MoMF) decreased, and liver fibrosis decreased. Activation of Notch in hepatocytes promotes the increase of the dependence of monocyte chemoattractant protein − 1 (MCP-1) on MoMF infiltration and fibrosis in liver.
[138]	2023.08	Jagged1/notch	Monocyte-derived macrophages (MoMFs) can activate the Jagged1/notch 2 axis in the early stage of injury, prevent further damage of hepatocytes, and play a key role in repairing necrotic lesions.
[139]	2024.06	Notch1	Notch1 may be a target for regulating the level of thrombopoietin in liver.

of DLL1Notch receptor ligand. In addition to conventional Wnt and Notch, its family members can also produce crosstalk and play a role. For example, Notch1 can also be combined with β-catenin and Lamp1 to promote lysosomal degradation of β-catenin. Notch2 and Wnt4 can participate in positive feedback regulation together [141]. In addition, β-catenin, as the main member of Wnt pathway, can enhance the transcription of Hes1, inhibit the degradation of Notch1 and NICD, and upregulate the transcription of JAG1Notch ligand [142]. As an important member of Wnt signal, GSK-3β can not only phosphorylate NICD, stabilize Notch and positively regulate Notch signal transmission, but also affect the interaction between SUFU and Gli transcription factor by phosphorylating SUFU, thus increasing the activity of Gli transcription factor in Hedgehog signal transduction [143]. Besides Notch pathway, TGF-β also has crosstalk with Wnt signal. Eger et al. once found that the deletion of E-cadherin can increase the signal transduction of LEF/TCF-β-catenin by inducing EMT (epithelial mesenchymal transition) of fully polarized mouse mammary epithelial cells, and can in turn cooperate with autocrine TGF-β signal transduction to maintain the undifferentiated mesenchymal phenotype, which proves that TGF-β and Wnt signal transduction play a synergistic role in EMT. Moreover, the overlapping of related pathways also plays a role in regeneration: Chen Z team found that blocking TGF-β signaling can delay proliferation, impair progenitor cell response and scar repair, and promote HSC transformation. At the same time, this process is related to Wnt/β-catenin [144]. In addition, it has long been proved that the effect of Wnt signaling on stem cells is regulated by combining with other signaling pathways, including Notch, Sonic hedgehog and TGF-β signaling. Besides, YAP/TAZ and Wnt, as the effectors of the Hippo pathway, also cross each other in signal transduction [145]. In Wnt pathway, YAP/TAZ can inhibit Dishevelled and β-catenin, and make the combined β-catenin more stable, thus reducing Wnt signal conduction and inhibiting regeneration. In fact, apart from working with TAZ, YAP can also work with SHP2 to enhance the activity of β-catenin in the nucleus. It can be seen that with the discovery of β-catenin, GSK-3β, TCF and TAZ in multichannel conduction, it means a deeper understanding of the complex crosstalk relationship between multiple channels.

In fact, besides Wnt and Notch pathways, there are many related signal pathways, such as Hippo [146, 147], hedgehog [148], NF-κB [149, 150], TGF-β [151], Jak-Stat [152], etc., which participate in the regulation of hepatocyte regeneration, and all of them interfere with each other [153]. Although the crosstalk relationship of multiple regeneration-related channels is gradually revealed, it is still only the tip of the iceberg of its complex relationship. In the future, the development of targeted signal transduction inhibitors for such signaling pathways will be a new direction of disease treatment in the future. The new achievements of mechanism research will lay a solid experimental and theoretical foundation for discovering new strategies and therapeutic targets for hepatocyte regeneration (Fig. 1).

Application of high-throughput sequencing technique in the study of hepatocyte regeneration

In recent years, with the demand of clinical and laboratory research, high-throughput sequencing has been used frequently. Through this method, the possibility of regeneration of damaged liver tissue can be indirectly predicted by genes, and it will move from laboratory to clinic [154].

Single cell RNA sequencing (scRNA-seq), as an effective method to explore cell structure and identity, has been widely used in clinic [155]. Because of its limitations in the detection of frozen liver samples, Richter ML team developed a new method of single-core snRNA-seq2. Based on the effective cleavage of nuclear membrane, they successfully realized the unbiased characterization of the main cell types in the liver by combining transcriptomics and high-efficiency low-volume reaction technology for separating single-core from frozen liver. Finally, it is proved that polyploid hepatocytes have proliferation characteristics and contribute to liver regeneration [156]. In addition, some researches have used the advantage that ChIP-seq can be used to explore the whole genome information and applied it to clinical research [157]. Because ChIP-seq is mostly used to detect binding sites, it has become another detection method to find the potential binding sites of other regulatory proteins other than the target gene by sequencing the open chromatin region with ATAC [158]. Some teams went further and drew a high-definition spatio-temporal map of mouse liver regeneration by combining the new means of Stereo-seq with single cell transcriptomics [159], which is another breakthrough in sequencing technology.

In addition to high-throughput sequencing technology, remarkable progress has been made in gene editing, organ-like culture model and the application of stem cell transplantation in the field of hepatocyte regeneration. These new technologies and methods not only provide new tools for in-depth study of the molecular mechanism of hepatocyte regeneration, but also bring new ideas for clinical treatment. Continue to explore the molecular mechanism of hepatocyte regeneration, pay attention to the crosstalk between signal pathways, and realize the precise regulation of influencing factors and the in-depth analysis of crosstalk mechanism. This will help us better understand the complex process of hepatocyte

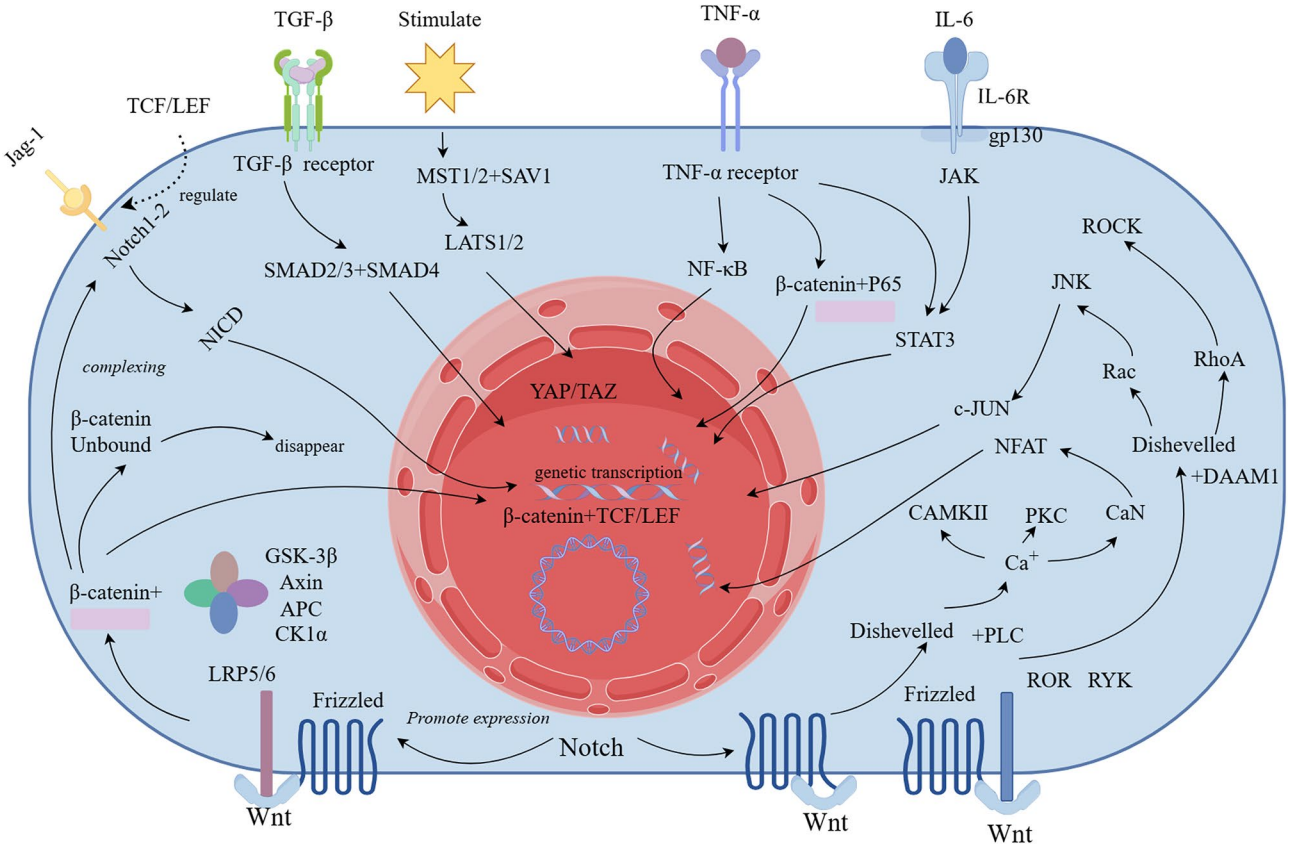

Fig. 1 Hepatocyte regeneration pathway (By Figdraw ID: YPSIUf17cd)

regeneration and lay the foundation for disease treatment by using gene expression regulation mechanism.

Conclusion

To sum up, hepatocyte regeneration is a complex dynamic process, which is regulated by multiple factors such as genes, drugs, cytokines and disease states, and involves many signal pathways such as Wnt, Notch, Hippo, hedgehog and cytokines such as HGF and EGF [160]. Through the combing of this paper, we have a deeper understanding of the influencing factors and molecular mechanisms of hepatocyte regeneration. Under normal circumstances, the compensatory ability of the liver can achieve the balance between damaged and regenerated hepatocytes. However, persistent viral infection (such as hepatotropic virus and non-hepatotropic virus) and metabolic damage (such as long-term alcoholism, high-fat diet and drug intake) will destroy the structure and function of the liver [161]. These stress signals not only weaken the ability of liver regeneration, but also may promote the occurrence and development of hepatocellular carcinoma. In order to cope with acute or chronic liver failure and end-stage liver disease, the current research focuses on advanced technologies such as normal temperature machine perfusion and nano-materials, aiming at providing temporary life support for patients and promoting liver regeneration through alternative therapies such as stem cells, organs and bioartificial liver [162] However, when applying these therapies, it is necessary to carefully evaluate their potential risks, especially the risks brought by gene integration and the use of animal-derived factors, so as to prevent the cancer risk from increasing [163, 164]. It is believed that with the deepening of research, more breakthroughs will be made in the field of hepatocyte regeneration, bringing better treatment options to patients.

Author' contributions

XZ put forward the research and collected the original documents, and was the main contributor to writing the manuscript. SL, LH, FJ, FY put forward constructive amendments to the article and provided some technical support. XH puts forward revision suggestions and coordinates the communication between the author team and the journal editorial department. All the authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare that they have no competing interests.

Received: 23 October 2024 / Accepted: 20 February 2025
Published online: 30 April 2025

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