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Review

Research progress in stem cell therapy for Wilson disease

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ABSTRACT

Wilson disease (WD), also known as hepatolenticular degeneration, is an autosomal recessive disorder characterized by disorganized copper metabolism caused by mutations in the *ATP7B* gene. Currently, the main treatment options for WD involve medications such as d-penicillamine, trientine hydrochloride, zinc acetate, and liver transplantation. However, there are challenges that encompass issues of poor compliance, adverse effects, and limited availability of liver sources that persist. Stem cell therapy for WD is currently a promising area of research. Due to the advancement in stem cell directed differentiation technology in vitro and the availability of sufficient stem cell donors, it is expected to be a potential treatment option for the permanent correction of abnormal copper metabolism. This article discusses the research progress of stem cell therapy for WD from various sources, as well as the challenges and future prospects of the clinical application of stem cell therapy for WD.

Contents

Abbreviations: BMSCs, bone marrow mesenchymal stem cells; CiHeps, Chemically induced hepatocytes; Cp, ceruloplasmin; CTR1, copper uptake protein 1; DMPS, sodium dimercaptosulphonate; DMSA, dimercaptosuccinic acid; DPSCs, dental pulp-derived mesenchymal stem cells; ESCs, embryonic stem cells; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HLCs, hepatocyte-like cells; iPSCs, induced pluripotent stem cells; LEC, long-evans cinnamon; LPCs, liver progenitor cells; LT, liver transplantation; MSCs, mesenchymal stem cells; OSM, Oncostatin M; TGN, trans-Golgi network; WD, Wilson disease.

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1. Introduction

Wilson disease, also known as hepatolenticular degeneration, is a rare autosomal recessive inherited disease characterized by a primary dysfunction of copper metabolism [1]. The pathogenic gene *ATP7B* in WD encodes transmembrane copper-transporting ATPase 2, which is predominantly expressed in hepatocytes. Mutations in the *ATP7B* gene result in functional defects or loss of the enzyme, leading to obstacles in ceruloplasmin synthesis and copper excretion in bile [2]. Copper metabolism disorders can cause excessive deposition of copper in liver cells, leading to necrosis of the cells. After cell lysis, copper ions loosely bind to serum albumin and are deposited in large quantities in various organs through the bloodstream, causing liver damage, neurological and psychiatric symptoms, kidney damage, bone and joint disease, and the observation of Kayser-Fleischer rings in the cornea [3–6]. The estimated worldwide frequency of carriers of the *ATP7B* mutation gene is approximately 1 in 90 individuals, considering the prevalence of symptomatic diseases ranging from 1:2600–1:30000 [7]. WD is underestimated, according to a statistical report from the UK, the estimated frequency of individuals carrying two pathogenic *ATP7B* mutant alleles is approximately 1:7000, with potentially up to 2.5% of the general population with heterozygous mutations [8]. The incidence of WD might be underestimated as a result of the intricate nature of its clinical symptoms and the challenges involved in diagnosing it solely through clinical manifestations and physiological or biochemical tests. This may cause misdiagnosis or failure to identify cases. With growing physician awareness and decreasing costs of whole-exome sequencing, there appears to be an increasing trend in the number of diagnosed cases of WD.

The common clinical symptoms of WD are mainly in the following areas. Firstly, liver disease is the first clinical manifestation in 40%–60% of patients with WD, including cirrhosis, chronic hepatitis and acute liver failure. Secondly, among the most prevalent neurological manifestations of WD, tremor, dystonia and parkinsonism are predominant. Thirdly, Kayser-Fleischer rings in the cornea and sunflower cataracts in the eye are pathological manifestations of copper deposition in the eyes of WD patients. Fourthly, skeletal involvement is also a common manifestation of WD, including osteoporosis, chondrocalcinosis, osteoarthritis, and joint pain. Copper buildup in the synovium and cartilage is widely recognized as the primary factor contributing to arthritis in patients with WD. Finally, WD-induced acute copper toxicity results in Coombs-negative hemolytic anemia with varying degrees of rhabdomyolysis and renal tubular damage. In addition to typical clinical symptoms, low serum ceruloplasmin levels, increased urinary copper levels, and abnormal serum biochemical markers such as aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, and bilirubin can also be used as auxiliary diagnostic indicators for WD.

2. Current treatment strategies for WD

WD patients can be reported at any age, but they are mostly observed in the 5 to 35-year-old range, primarily in children and adolescents. The prevalence is comparable between males and females, and significant variations in symptoms exist among different patients [9]. Once diagnosed, WD patients should receive prompt treatment, with the principle being early treatment, lifelong treatment, and lifelong monitoring [10]. Because the organs involved and the degree of damage also vary, the main symptoms manifest as liver and neurological damage. Of these, symptoms of liver disease appear earliest, with some infant WD patients had symptoms at the age of two weeks after the end of breastfeeding, and the neurological disease develops relatively late, usually after the age of 15 [11,12]. During the initial stages of WD treatment, primary therapeutic approaches encompass the administration of copper chelating agents (such as d-penicillamine and trientine hydrochloride) and zinc acetate therapy (Table 1). The pharmacological treatment principle outlined above revolves around inducing a negative copper balance within the body through the administration of specific drugs, chelating copper in the blood and tissues, and promoting urinary excretion of copper, and reducing copper intake in the digestive tract [13,14]. Presymptomatic WD patients who are taking their medications as prescribed have similar mortality rates to the general population [15]. Although drug therapy for WD has been effective in some patients, particularly those with hepatic symptoms, there are limitations to current treatment methods. Approximately 50% of WD patients still experience neurological symptoms during treatment [16]. About 1/3 of WD patients experience adverse drug reactions, with d-penicillamine being associated with more adverse reactions such as nausea, vomiting, skin rash, fever, and even systemic lupus erythematosus [17,18]. The treatment regimens for WD are often complex and cumbersome (3 times daily, 2 h before meals, etc.) with a high number of adverse effects, resulting in poor medication adherence, ultimately leading to a mortality rate for WD patients that is 5–6.1% higher compared to the general population, mainly due to severe liver disease or severe neurological symptoms, with a few patients committing suicide due to disease burden or depression [1,10,19]. Hence, it is imperative to expedite the development of novel therapeutic alternatives that can both relieve neurological symptoms, reduce the number of medications taken, improve patient confidence in adhering to treatment, and also restore ATP7B function.

When WD patients experience late-stage symptoms, such as, acute liver failure or decompensated cirrhosis, liver transplantation (LT) is the only feasible treatment option [20–22]. Given that WD primarily affects the liver and is characterized by impaired copper metabolism in hepatocytes, liver transplantation can be viewed as a corrective measure for the gene deficiency, effectively restoring copper homeostasis and addressing the phenotypic manifestations of the disease. After LT, biliary copper excretion is restored, neurologic and mental illness stabilize or improve, and Kayser-Fleischer rings gradually disappear [23–25]. Nevertheless, LT is not without its inherent limitations, namely the scarcity of liver donors, the heightened frequency of postoperative complications, and the requirement for prolonged administration of immunosuppressive agents. Additionally, paradoxical neurological deterioration may be seen in transplant recipients, and there is still ongoing debate about whether uncontrolled neurological symptoms alone should be considered a sufficient indication for liver transplantation [20].

Given the ability of transplanted hepatocytes to integrate into the liver parenchyma and reinstate crucial functions, such as facilitating copper transfer into bile, it seems that cell-based therapies hold promise as a feasible approach for treating WD [35]. Park et al. conducted intrasplenic injection of healthy hepatocytes into 8-week-old Long-Evans Cinnamon (LEC) rats exhibiting clinical

Table 1
Clinical symptoms and current corresponding treatment strategies.

Clinical Diseases	Specific symptoms	Current treatment strategies	References
Hepatopathy	Cirrhosis, chronic hepatitis acute liver failure	D-penicillamine, zinc acetate, DMPS, DMSA liver transplantation	[26–28]
Neuropsychology	Mild dystonia, Tremor, muscle rigidity, movement disorders, dysarthria, severe dystonia	DMPS, DMSA (first choice), zinc acetate, D-penicillamine (use cautiously or not)	[29–31]
Ophthalmic diseases	Kayser-Fleischer ring, sunflower cataract	D-penicillamine, DMPS, DMSA, zinc acetate	[1]
Osteoarticular diseases	Osteoporosis, chondrocalcinosis, osteoarthritis, joint pain	D-penicillamine, DMPS, DMSA, zinc acetate	[32]
Other systemic diseases	Coombs-negative haemolytic anaemia, rhabdomyolysis	Hemodialysis, plasmapheresis	[33,34]

symptoms of WD due to mutant *ATP7B* [36]. After 16 weeks post-transplantation, all transplanted rats exhibited restored biliary copper excretion and reduced hepatic copper concentration, causing improved 6-month survival rates in LEC rats. The results were further confirmed by Sauer et al. through repeated transplantation, they found that multiple hepatocyte transplantations led to better outcomes without requiring preconditioning [37]. Based on current data, it is estimated that restoring normal copper metabolism requires the transplantation of approximately 40% of healthy hepatocytes. However, the limited supply of primary human hepatocytes (PHHs) poses a barrier, thereby limiting the feasibility of PHHs therapy as a potential treatment option for WD [38]. Stem cells have the advantages of self-renewal and differentiation into various cell types, including hepatocytes. With the ongoing advancement of in vitro-directed induced differentiation techniques, stem cell-derived hepatocytes have the potential to serve as a stable source of cells.

3. Stem cell therapy

Recent scientific research has showcased the immense potential of various types of stem cells in the field of regenerative medicine, such as mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs) and other stem cells. Through in vitro induction technology, stem cells can be programmed to differentiate into hepatocytes [39–41], providing a continuous source of cells for WD patients or obtaining "customized" cells through gene modification technology. Moreover, the use of patient-derived MSCs or iPSCs to circumvent immune rejection reactions [42]. Establishing a large-scale bank of adult stem cells to screen for high-matching cell lines or using CRISPR-Cas9 technology to artificially construct low-immunogenic pluripotent stem cell lines are also a feasible approach for reducing alloimmune antigenicity [43]. These approaches have brought stem cell therapy a step closer to clinical practice, and numerous research has demonstrated that hepatocytes derived from multiple sources of stem cells can effectively replace damaged hepatocytes and function effectively [44]. Additionally, these treatments utilizing them for WD have been put into practice in both clinical and preclinical settings (Fig. 1).

3.1. Potential mechanisms of stem cell therapy for WD

Copper is a vital cellular element that functions as both an electron donor and acceptor, actively engaging in diverse reactions and serving as a crucial cofactor in numerous enzymes. Copper obtained from the diet is absorbed in the stomach and duodenum, subsequently transported to the liver via the portal vein [45]. Hepatocytes and other cells take up copper via the high-affinity copper uptake protein 1 (CTR1), where divalent copper ions are reduced to monovalent copper ions on cell membrane, which are then taken up by CTR1. The copper transport protein ATOX1 (ATOX1) plays a crucial role in delivering copper to the copper-transporting ATPase 2 (ATP7B). In hepatocytes, ATP7B has two functions: firstly, in the trans-Golgi network (TGN), it facilitates the transfer of six copper ions to apoceruloplasmin, leading to the formation of ceruloplasmin (Cp), which is subsequently released into the bloodstream. Secondly, in the cytoplasm, ATP7B is

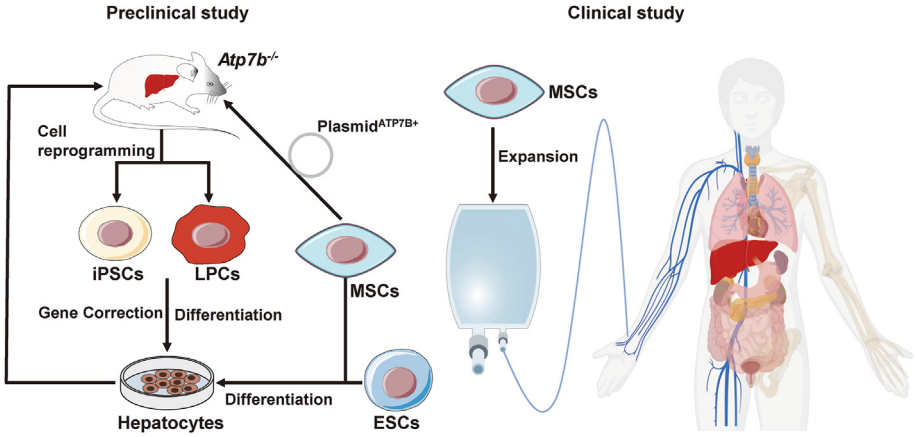

Fig. 1. Schematic representation of different sources of stem cells for the treatment of WD.

responsible for transferring excess copper ions into vesicles, which are ultimately excreted into bile through the apical membrane [46]. Finally, copper is excreted in the feces via the gastrointestinal tract. Therefore, in the liver, the ATP7B mainly achieves copper balance by excreting copper through two channels: the bile duct and the bloodstream.

In WD, mutations in the *ATP7B* gene cause aberrant or absent ATP7B protein functionality, resulting in copper metabolism disorders and the development of pathological states. Therefore, copper gradually accumulates in the liver, exceeding its capacity. Excessive quantities of non-ceruloplasmin-bound copper enter the systemic circulation and are deposited and accumulate in various organs, triggering extrahepatic copper toxicity. Copper accumulates in the brain, cornea, skeletal and red blood cells, causing the various clinical manifestations of WD (Fig. 2).

Patient-derived stem cells were genetically modified to correct the *ATP7B* gene and are differentiated into hepatocytes in vitro to rebuild the copper excretion system of the liver after transplantation [35]. Alternatively, allogeneic adult stem cells or pluripotent stem cells with low immunogenicity and high versatility can be selected and prepared for this purpose. In addition, the treatment of WD mainly relies on the function of ATP7B, thereby high-expression *ATP7B* hepatocytes can be obtained through gene modification in vitro, which means that combined cell and gene therapy is the optimal solution. The mechanism of stem cell therapy for WD is to restore hepatic copper excretion capacity by enabling healthy hepatocytes to perform ATP7B functions, as well as mitigating copper-induced oxidative stress and alleviating liver injury through paracrine secretion (Fig. 3).

Considering that the liver is the largest parenchymal organ in the human body, treatment of WD requires the transplantation of a large number of stem cell-derived hepatocytes and the ability to homing these cells to integrate with the liver parenchyma and restore the hepatobiliary capacity for copper excretion. However, only a portion of the transplanted cells enter the liver, and most of the cells are retained in the hepatic sinusoids. Research indicates that over 70% of transplanted cells are cleared within 48 h [47]. Therefore, there is a need to improve delivery methods or develop therapeutic strategies to inhibit cell loss.

3.2. Stem cell sources for WD treatment

3.2.1. Mesenchymal stem cells

MSCs are isolated from various tissues, including umbilical cord blood, endometrial tissue, bone marrow and dental pulp [48]. MSCs have several advantages, firstly, they exhibit the capability of self-renewal and multi-lineage differentiation, and various induction methods have been developed to differentiate MSCs towards hepatocytes [49]. Secondly, due to their low immunogenicity, MSCs are extensively utilized in allogeneic transplantation for diverse diseases [50]. Thirdly, MSCs induce peripheral tolerance, migrate to injured tissues, and contribute to inhibiting pro-inflammatory cytokine release and enhancing cell survival [51]. Therefore, MSCs can reach the liver via various administration routes, including intravenous, intraperitoneal, intrahepatic, splenic, and portal vein injections [52]. Fourthly, the secreted factors generated by MSCs significantly contribute to tissue regeneration, supporting graft and nutritional functions (autocrine and paracrine). Mounting evidence from numerous studies has demonstrated the therapeutic potential of MSCs in the treatment of liver damage [53]. Extensive research has revealed that co-culturing hepatocytes with MSCs facilitates hepatocyte growth. Similarly, transplantation of MSCs into the liver has been shown to enhance hepatocyte growth and expedite liver function restoration in cases of hepatic damage [54].

Presently, MSC transplantation research for liver diseases has primarily concentrated on cirrhosis treatment, yielding favorable therapeutic outcomes [55,56], and the application of MSCs in WD treatment has been primarily examined through limited animal experiments. Previous evidence has shown that human bone marrow mesenchymal stem cells (BMSCs) can integrate into the livers of rats and mice, differentiating into functional human hepatocytes [57,58], indicating their potential to use in the treatment of WD. Both BMSCs by tail vein injection and intrasplenic injection corrected copper metabolism and restored liver function in early toxic milk mice, and the therapeutic effect was superior to that of late injections [59,60]. Vanessa et al. used retroviruses to construct BMSCs overexpressing *ATP7B*, this cell line had stronger resistance to copper than HepG2 cells, and could serve as an innovative approach for cell transplantation in WD treatment [61].

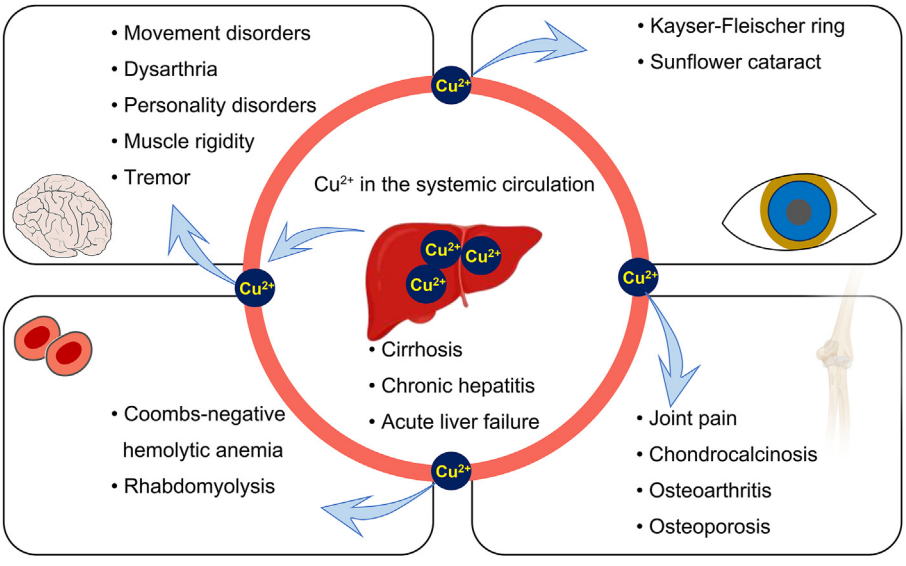

Fig. 2. Copper toxicity in the pathogenesis of WD.

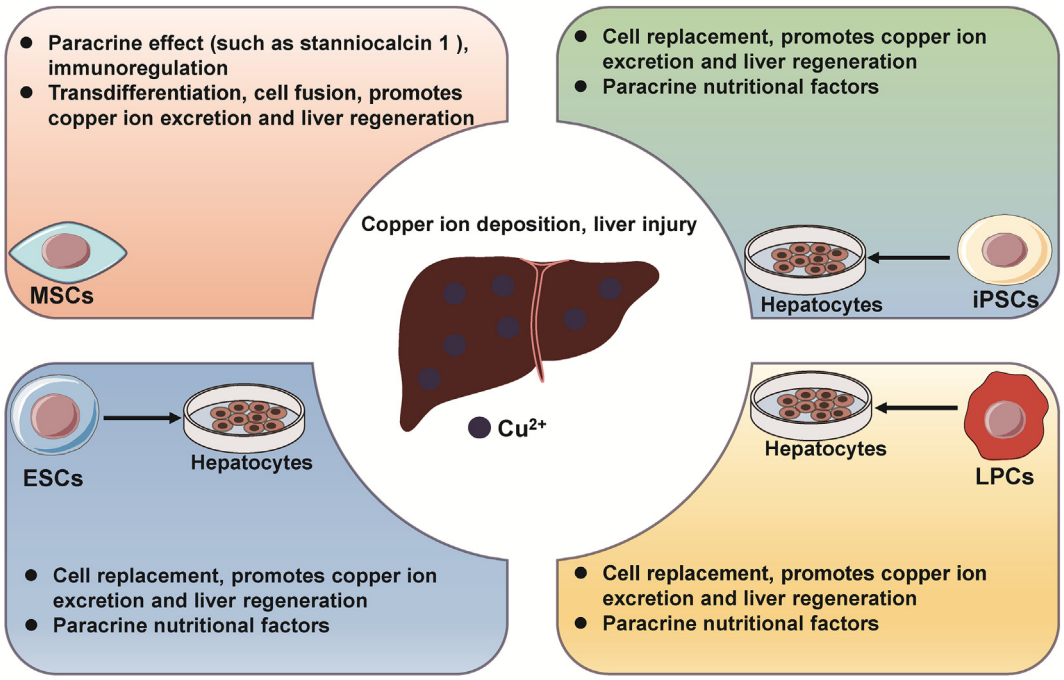

Fig. 3. Potential mechanisms of stem cell therapy for WD.

Subsequently, Chen et al. utilized this method to construct *ATP7B*-transduced BMSCs(BMSCs^{ATP7B}). Transplantation of BMSCs^{ATP7B} into LEC rats via the portal vein reduced copper load and rescued 8-week-old LEC rats [62]. As the LEC rats did not exhibit symptoms of WD during treatment, it was a preventative measure rather than evidence of disease improvement. Fujiyoshi et al. induced differentiation of dental pulp-derived mesenchymal stem cells (DPSCs) into hepatocyte-like cells (DPSC-Heps), and then transplanted them into LEC rats via the spleen. Compared to the simple transplantation of DPSCs, DPSC-Heps substantially extended the lifespan of fulminant LEC rats and exhibited superior therapeutic effects in repairing liver dysfunction and tissue damage [63]. Subsequent investigations revealed that DPSC-Heps exhibited the ability to mitigate copper-induced oxidative stress in fulminant LEC rats through ATP7B-independent secretion of stanniocalcin 1, likely attributable to the paracrine activity of DPSC-Heps.

In 2014, a clinical study was conducted at the Gulhane Military Medical Academy in Turkey to treat WD-induced cirrhosis with BMSCs via peripheral intravenous injection [64]. Improvement in end-stage liver disease model scores was observed in eight patients, and serum albumin levels showed a significant increase in the third month. Consecutive liver biopsy examinations indicated that BMSCs did not reach the liver in sufficient quantities, and the observed improvement in the patients' condition may be attributed to the immunomodulatory effects of BMSCs. Subsequently, Zhang et al. expanded WD patients' autologous BMSCs in vitro and infused them back to the patients through a peripheral vein, and showed that the combination of BMSCs and D-penicillamine showed a significant improvement in WD-induced liver fibrosis [65]. Although there have been few cases of adverse reactions following BMSC therapy, collecting autologous BMSCs can exacerbate the patient's pain. In addition, this study only evaluated the efficacy of BMSCs in treating liver fibrosis and did not assess changes in serum copper and ceruloplasmin levels. Therefore, it remains unclear whether BMSCs are effective in removing copper from WD patients.

3.2.2. *Induced pluripotent stem cells*

Shinya Yamanaka in 2007 [66], his team successfully reprogrammed mouse skin fibroblasts into iPSCs by overexpressing four transcription factors (*Oct4, Sox2, Klf4, c-Myc*) using a retroviral system. iPSCs possess the same distinct characteristics as ESCs, namely their pluripotency and ability to undergo indefinite expansion in vitro. Several induction protocols have been used to differentiate iPSCs into hepatocyte-like cells (HLCs) that resemble PHHs and function as PHHs [67–69]. Previous studies have demonstrated that human iPSCs-derived multistage hepatic cells have the potential to repopulate liver tissue in a mouse model of liver cirrhosis [70]. More importantly, they can solve histocompatibility issues and bypass ethical challenges, minimizing immune rejection in allogeneic transplantation. HLA homozygous iPSC lines and their derived cells have been successfully established in both Japan and the UK, facilitating the development of banks dedicated to this purpose. These iPSCs can match more than 90% of the local population [71,72]. Building upon this, HLA-haplotype banking of iPSCs will improve the universal availability of iPSCs-based cell therapy and promote industrialization and commercialization, thus bringing iPSCs closer to clinical applications. Furthermore, a low immunogenicity universal iPSC line can be established through gene editing techniques. When HLA class I and II genes are knocked out and CD47 is overexpressed, human iPSCs exhibit reduced immunogenicity [73]. Therefore, iPSCs have emerged as a highly encouraging cell source for cell therapy.

Apart from their capacity to differentiate into HLCs, iPSCs can also undergo neuronal differentiation, offering a cellular model to investigate the potential impact of specific *ATP7B* mutations on neurological impairments in individuals afflicted with WD [74]. Though iPSCs-based autologous cell therapy and gene editing technology, the patient's autologous iPSCs are obtained, repaired in vitro for mutations in the patient's iPSCs genome, and differentiated into disease-relevant cell types before being transplanted back into the patient [75]. Rui et al. provided conceptual validation

data for the therapeutic use of gene-corrected iPSCs in treating WD [76]. They established iPSCs from WD patients and used CRISPR/Cas9 and ssODNs to correct the R778L mutation in ATP7B, and further differentiated the genetically repaired iPSCs into hepatocytes. Gene correction reversed the R778L phenotype, restored subcellular localization of ATP7B in hepatocytes, and restored copper exportation capacity in copper overload tests. The repaired iPSCs-derived hepatocytes were injected into immunodeficient WD mice (*Atp7b*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$, ARG mice) via splenic injection, resulting in significant amelioration of WD liver disease manifestations, improved liver function, and reduced liver fibrosis. This can be attributed to a decrease in hepatic copper accumulation, ultimately resulting in a reduction of hepatocyte toxicity induced by copper. However, the safety of using gene-corrected hepatocytes in clinical applications still requires further investigation.

3.2.3. Embryonic stem cells

ESCs are pluripotent cells obtained from the inner cell mass of early-stage embryos, have self-renewal and infinite proliferation properties and the capacity to differentiate into all cell types derived from the three primary germ layers. Previous reports have indicated the use of various induction approaches to cultivate ESC-derived HLCs in vitro [77–79]. Cai et al. introduced a novel three-stage approach to efficiently differentiate human embryonic stem cells (hESCs) into hepatocytes using serum-free culture medium. These differentiated cells demonstrated functional similarities to mature hepatocytes, such as albumin secretion and glycogen storage [80]. Siller et al. pioneered a growth factor-independent strategy employing small chemical molecules to convert human pluripotent stem cells (hPSCs) to the hepatic phenotype, which effectively induces hPSCs to HLCs [69]. Currently, animal experiments have shown the promising potential of ESCs in the treatment of liver diseases. In a carbon tetrachloride-treated mouse model, Woo et al. injected hESCs-derived hepatocytes into mice via intrasplenic injection. The administered cells facilitated the restoration of injured liver tissue in mice through both cell replacement and the secretion of trophic factors that enhance the endogenous liver regeneration process [81]. Tolosa et al. developed an effective protocol for differentiating ESCs into bipotent liver progenitor cells (LPCs) [82]. When transplanted into mice with acetaminophen-induced acute liver failure, these LPCs exhibited successful repopulation, restored over 10% of the liver tissue and impaired liver function without inducing tumors. LPCs has bi-directional differentiation potential and may differentiate into hepatocytes and cholangiocytes by means of the host liver microenvironment, thereby replenishing damaged liver tissue. Although these studies have validated the effectiveness and safety of ESCs transplantation in animal models, there is currently a lack of preclinical and clinical research on hepatocyte therapy derived from hESCs for the treatment of WD.

3.2.4. Liver progenitor cells

The liver is endowed with an exceptional ability to regenerate itself, allowing it to maintain normal physiological functions even after the removal of 70–80% of its tissue. Furthermore, it gradually achieves restoration of its preoperative weight within a span of approximately six weeks [83]. When there is a significant loss of hepatocytes during severe liver disease or when hepatocyte replication is inhibited due to chronic liver disease or cellular senescence, liver regeneration mediated by LPCs becomes the main mechanism of liver compensation, also known as pathological regeneration [84]. LPCs not only express both stem cell markers but also simultaneously express markers for bile duct cells and hepatocytes, and are therefore recognized as a bipotent stem/progenitor cell population [85]. Wang et al. discovered LPCs that generate functional hepatocytes, and performed lineage tracing in mice to identify a population of LPCs that exhibit the remarkable capability to undergo proliferation and self-renewal in close proximity to the hepatic lobular veins [86]. Therefore, transplantation of LPCs is also an effective treatment option for WD. Transplanted LPCs integrate into the host liver and differentiate into liver cells and bile duct cells in the liver microenvironment, thereby reconstructing the hepatobiliary copper transport system.

Wang et al. successfully utilized small molecules to reprogram hepatocytes isolated from *Atp7b* $^{-/-}$ mice, resulting in the generation of abundant LPCs that exhibited remarkable in vitro proliferative and hepatic differentiation potential [87]. Functional hepatocytes (LPC- *Atp7b* -Heps) were generated through lentiviral-mediated mini *Atp7b* gene transfection and subsequent redifferentiation of LPC- *Atp7b* cells. LPC- *Atp7b* -Heps transplantation into *Atp7b* $^{-/-}$ mice attenuated liver copper accumulation as well as inflammation and fibrosis, achieving comparable levels to normal primary hepatocytes 4 months post-transplantation. This study successfully established an autologous reprogrammed hepatocyte system for in vitro *Atp7b* gene therapy. In a mouse model of WD, transplantation of LPC- *Atp7b* -Heps showed promising therapeutic effects on copper homeostasis. Hence, in the context of treating WD, a combination of gene therapy and stem cell therapy seems to be the best option.

4. Current difficulties and solutions of stem cell therapy for WD

Currently, numerous studies have demonstrated that BMSCs infusion for WD is safe, the low immunogenicity of BMSCs has been fully confirmed, and the therapeutic efficacy of BMSCs or their derivatives has been demonstrated in animal experiments in WD models. However, several obstacles persist, hindering the broad application of BMSCs in clinical therapy for WD. Firstly, in clinical situations, the symptoms in patients with WD often present initially as liver cirrhosis, at which time structurally compromised MSCs may become trapped in the pulmonary capillary bed or fail to properly home in the cirrhotic liver. Therefore, MSCs transplantation for these patients may be suboptimal. Secondly, infusing cells via the portal vein can potentially cause sinusoidal occlusion and portal hypertension, and many of the cells are deposited in the hepatic sinusoids and only some of the cells enter the liver to function. Thirdly, although MSCs possess the capability to migrate towards the liver and differentiate into hepatocytes, either naturally or through in vitro induction, but the efficiency of generating fully functional hepatocytes from MSCs remains relatively low [88]. Fourthly, although BMSC^{ATP7B} increased the expression of *ATP7B*, but the safety profile of the lentiviral vector in human subjects remains uncertain. However, the therapeutic effect of MSCs in treating WD may be primarily attributed to their immunomodulatory and paracrine effects rather than the restoration of the hepatic copper metabolism.

While validating the potential of iPSCs as a stem cell therapy in animal models of WD, the application of iPSCs in the treatment of WD needs further investigation. One concern is the potential risk of malignant transformation due to the unlimited proliferative capacity and tumorigenicity of iPSCs. Additionally, one of the major drawbacks of iPSCs is their genomic instability, which may confer completely different phenotypes to the cells [89]. In comparison, ESCs represent a naturally occurring and pluripotent stem cell population obtained from embryos, thereby avoiding the issue of genomic instability. However, concerns also exist regarding the tumorigenic risk associated with hESCs and the safety of cell products derived from them. Additionally, hESCs are obtained from aborted embryos, which carries ethical considerations, as well as

Table 2
Advantages and disadvantages of stem cell therapies for WD from different sources.

Cell types	Sources	Advantages	Disadvantages
MSCs	Diverse tissue sources	Widely sourced and easily accessible, Low immunogenicity, Reducing inflammation, promoting the regeneration of impaired liver tissues by secreting trophic factors	Lower differentiation efficiency, Unclear mechanism of copper excretion
iPSCs	Reprogramming human epithelial cells	Autologous source without immune rejection, No ethical risks, Individualized treatment	Risk of tumorigenicity, Genome instability, Safety of iPSCs after gene correction is unknown
ESCs	Donated blastocysts	Genome stability, Easy establishment of cell lines	Ethical risks, risk of tumorigenicity, Immunogenicity concerns
LPCs	Adult liver	Low cost, High differentiation efficiency, No immune rejection	Not easily accessible, Safety of HLCs after gene correction is unknown

the risk of immunological rejection in allogeneic transplantation. Therefore, further exploration is needed for the clinical application of ESCs in treating WD. In vitro expansion or genetic correction of patient-derived LPCs into hepatocytes for transfusion back into WD is the preferred option, as there is no immune rejection of autologous LPCs and the efficiency of LPCs induced differentiation into hepatocytes is the highest. However, the acquisition of LPCs increases patient distress and the safety of lentiviral vectors needs to be strictly monitored (Table 2).

Takebe et al. demonstrated that when MSCs were co-cultured with hepatocytes and endothelial cells, the three cell types aggregated and formed liver organoids. These liver organoids were transplanted into mice through mesenteric transplantation. Within 48 h after transplantation, the organoids established vascular connections with the host and successfully rescued a murine model of drug-induced acute liver failure [90]. Nantasanti et al. extracted autologous LPCs from Commd1-deficient dogs, corrected the genetic defect using lentiviral vectors in vitro, and expanded the cells using organoid culture medium. Subsequently, the organoids were reintroduced into the affected dogs through intrahepatic injection, leading to the long-term engraftment and survival of organoid-derived hepatocytes in the liver for a duration of two years [91]. Organoids have the potential to integrate with the host and replace damaged liver tissue, making them a promising new therapy for treating WD.

In conclusion, further investigations are warranted to determine the ideal timing and optimal delivery method for stem cell transplantation in WD patients. Additionally, efforts should be made to enhance the efficiency, maturity, and, most importantly, safety of hepatocyte differentiation.

5. Discussion and future direction

There are currently various induction protocols reported for hepatocytes, the majority of approaches employ recombinant growth factors, including activin A, Wnt3a, hepatocyte growth factor (HGF), Oncostatin M (OSM), and dexamethasone (DEX). However, the high cost, long culture time of cytokines, and sustained consumption pose the biggest challenges for clinical applications. White et al. achieved the reprogramming of mouse fibroblasts into functional HLCs by employing chemical induction of phenotypic plasticity. To induce the cells to differentiate into hepatic lineage, they employed a continuous stimulation approach within the culture medium, utilizing small molecule cocktails to enhance the endogenous expression of key transcription factors (*Hnf4a, Nr1i2, and Nr1h4*) [92]. Chemically induced hepatocytes (CiHeps) demonstrate comparable activity and functionality to primary hepatocytes, particularly in terms of liver regeneration potential, which has been proven to be beneficial for rescuing liver failure in FRG mice. The differentiation of human iPSCs into HLCs using small molecules is a more cost-effective strategy, chemical small molecule induction techniques will be a hot topic in the future. In addition, the treatment of WD requires not only the maturation of hepatic cells derived from stem cells but also need the hepatocytes expressed higher levels of *ATP7B* to rapidly restore the hepatic copper excretion capability. The optimal treatment strategy involves the gene correction of autologous-sourced iPSCs or LPCs, followed by in vitro induction of differentiation into hepatocytes and transplantation back into the patient. Alternatively, a relatively cost-effective treatment approach involves the genetic modification of BMSCs through overexpression of the *ATP7B* gene to compensate for the patient's deficiencies. Despite studies demonstrating the effective amelioration of liver disease induced by WD in animal models through stem cell-derived hepatocytes, further exploration is needed to determine if other symptoms of WD can be relieved and improved.

Although extensive research has showcased the promising potential of stem cell therapy in liver disease treatment, the clinical implementation of this treatment for WD still encounters substantial obstacles. Firstly, the effectiveness of stem cell-derived hepatocytes in replacing damaged liver tissue and restoring hepatic copper excretion in WD remains elusive. Secondly, obtaining highly differentiated and *ATP7B*-expressing hepatocytes without additional genetic modifications remains a challenging task, indicating the immediate necessity to develop an integrated strategy combining stem cell therapy and gene therapy. Lastly, before stem cell therapy can be applied in clinical trials, it must meet stringent requirements for clinical treatment. These requirements include minimizing allograft rejection, exploring the optimal route, timing, and dosage of cell transplantation, as well as rigorous validation of safety and efficacy. In summary, stem cell therapy shows promise in the treatment of WD and holds the potential to permanently correct copper metabolism abnormalities in affected patients.

Authors' contribution

All authors contributed to the conception and the main idea of the work. YS supervised the work and provided comments and additional scientific information. All authors read and approved the final manuscript. XX: writing-original draft preparation. CG, XM, AL and XG: writing-review and editing. XX, CG: visualization. All

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Data availability statement

This is a review article, and no new data were generated or analyzed in this study. Therefore, data sharing does not apply in this article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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